METABOLOMICS SOCIETY

METABOLOMICS2025

PRAGUE, CZECH REPUBLIC JUNE 22-26

21st Annual Conference of the Metabolomics Society



ORAL AND POSTER ABSTRACTS

AGENDA AT A GLANCE

Metabolomics in Health and Disease
Computational Metabolomics, Statistics & Bioinformatics
Technology Advancements
Plants, Food, Environment and Microbes

SUNDAY, JUNE 22

	North Hall	South Hall 1	South Hall 2
11:00 a.m.	REGISTRATION OPEN		
12:00 p.m. – 2:00 p.m.	**W1: Part 1** Web-based Tools for Microbiome Data	**W2:** Metabolome Xchange	
2:15 p.m. – 4:15 p.m.	**W1: Part 2** Web-based Tools for Microbiome Data	**W3:** Reverse Met Big Data Discovery	**W4:** Volatilomics
4:30 p.m. – 6:30 p.m.	**W5:** mzmine Data Analysis	**W6:** Demystifying Stable Isotope Labelling	**W7:** Clinical Implementation
6:45 p.m. – 8:00 p.m.	**Career Night – Roundtable Discussions**		

MONDAY, JUNE 23

	North Hall	South Hall 1	South Hall 2
7:30 a.m.	REGISTRATION / INFO DESK OPEN		
8:15 a.m. – 10:15 a.m.	**W8:** Mass Spectra MS2LDA	**W9:** Met Data Standard for AI	**W10:** EMN Metabo Mentors
10:15 a.m. – 10:30 a.m.	BREAK		
10:30 a.m. – 12:30 p.m.	**W11:** Automated Annotation SIRIUS 6	**W12:** Beyond CID Met/Lipid ID	**W13:** Best Practices MQACC
12:30 p.m. – 1:30 p.m.	LUNCH BREAK *(on your own)*		
1:30 p.m. – 3:00 p.m.	**Opening Ceremony & Plenary Session 1** – Livia Schiavinato Eberlin		
3:00 p.m. – 3:30 p.m.	BREAK		
	Congress Hall	**South Hall 1**	**South Hall 2**
3:30 p.m. – 5:15 p.m.	**1** Cellular Metabolism	**2** Predictive Approaches and Simulations	**3** New Avenues for Lipid Analysis
5:15 p.m. – 6:45 p.m.	**Welcome Reception – Poster Session 1**		
7:00 p.m. – 8:00 p.m.	**Metabolomics Society Town Hall Meeting**		

TUESDAY, JUNE 24

	Congress Hall	South Hall 1	South Hall 2
7:45 a.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 9:30 a.m.	**Plenary Session 2** – Erin Baker		
9:30 a.m. – 10:15 a.m.	BREAK		
9:40 a.m. – 10:10 a.m.	**BioHub 1:** CcpNmr Analysis Metabolomics		
9:45 a.m. – 10:00 a.m.	**Exhibitor Demo Pres:** Beckman Coulter for Life Sciences		
10:15 a.m.– 12:00 p.m.	**4** Clinical Translation of Lipidomics & Met	**5** Quantitative and Statistical Methods	**6** Natural Product Discovery from Plants
12:00 p.m. – 1:30 p.m.	LUNCH BREAK AND SPONSOR PRESENTATIONS		
12:20 p.m. – 1:20 p.m.		**Sponsor Pres:** Thermo Fisher Scientific	**Sponsor Pres:** SCIEX
1:30 p.m. – 3:00 p.m.	**7** Data Repositories and Curation	**8** Mass Spectrometry Imaging	**9** Microbial Metabolomics
3:00 p.m. – 3:30 p.m.	BREAK		
3:00 p.m. – 3:25 p.m.	**BioHub 2:** Metabonaut: LC-MS/MS		
3:30 p.m. – 5:15 p.m.	**10** Vendor Session	**11** Pregnancy, Neonatal, Early Life Metabolism	**12** Agricultural and Animal Products
5:15 p.m. – 6:45 p.m.	**Poster Session 2**		
6:45 p.m. – 8:15 p.m.	**EMN Reception**		

WEDNESDAY, JUNE 25

	Congress Hall	South Hall 1	South Hall 2
8:00 a.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 9:30 a.m.	**Plenary Session 3** – Steffen Neumann		
9:30 a.m. – 10:15 a.m.	BREAK		
9:40 a.m. – 10:10 a.m.	**BioHub 3:** Using PARADISe		
9:45 a.m. – 10:00 a.m.	**Exhibitor Demo Pres:** Metabolon		
10:15 a.m. – 12:00 p.m.	**13** Multi-omics and Integrative Methods	**14** Microbiome and Host-Metabolism Interactions	**15** Natural Products and Plant Defense
12:00 p.m. – 1:30 p.m.	LUNCH BREAK AND SPONSOR PRESENTATIONS		
12:20 p.m. – 1:20 p.m.		**Sponsor Pres:** Waters	**Sponsor Pres:** Bruker
1:30 p.m. – 3:00 p.m.	**16** Age-Related Diseases and Cancer	**17** Biomonitoring and Environmental Metabolomics	**18** Metabolomics Beyond LC-MS: NMR and More
3:00 p.m. – 3:30 p.m.	BREAK		
3:00 p.m. – 3:25 p.m.	**BioHub 4:** Metabox 2.0 and SiMD		
3:10 p.m. – 3:25 p.m.	**Exhibitor Demo Pres:** Thermo Fisher Scientific		
3:30 p.m. – 5:15 p.m.	**19** Metabolite Annotation	**20** Cardiovascular & Endocrine Disease	**21** Going Small – Low Volume and Sample Amount
5:15 p.m. – 6:45 p.m.	**Poster Session 3**		
7:30 p.m. – 10:30 p.m.	**Conference Dinner**		

THURSDAY, JUNE 26

	Congress Hall	South Hall 1	South Hall 2
8:15 a.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 9:30 a.m.	**Plenary Session 4** – Peter Meikle		
9:30 a.m. – 10:30 a.m.	**Poster Session 4**		
9:40 a.m. – 10:10 a.m.	**BioHub 5:** MSlineaR Software		
10:30 a.m. – 12:15 p.m.	**22** Nutrition and Exposome Research	**23** Food, Health, and Sustainability	**24** Data Processing and Cheminformatics
12:15 p.m. – 1:30 p.m.	LUNCH BREAK AND SPONSOR PRESENTATIONS		
12:25 p.m. – 1:25 p.m.			**Sponsor Pres:** Agilent
1:30 p.m. – 3:15 p.m.	**Plenary Session 5** – Clary Clish – **Awards and Closing**		

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PLENARY, VENDOR SESSIONS, SPONSOR AND EXHIBITOR DEMO PRESENTATIONS



PLENARY SESSION 1

Guiding Clinical Decisions with Metabolic information provided by Direct Mass Spectrometry Analysis

Monday, June 23
2:00 p.m. – 3:00 p.m.
Congress Hall

Livia Schiavinato Eberlin, Baylor College Of Medicine – United States

Mass spectrometry techniques that allow direct and fast molecular analysis of clinical samples offer the exciting opportunity to incorporate metabolic data into clinical practice to expedite clinical decision making and thus improve diagnosis and patient care. In this seminar, I will discuss developments and applications of direct mass spectrometry techniques for use in tissue analysis and decision making. I will focus the discussion on two main techniques, Desorption Electrospray Ionization Mass Spectrometry Imaging and the MasSpec Pen technology, and their use for metabolic analysis of clinical samples in cancer research. Key operational principles, depth of molecular data, and the analytical and diagnostic performance metrics achieved with these techniques for disease detection will be discussed to provide a critical assessment of their capabilities and potential uses within the context of routine clinical practice. My presentation will focus on results obtained in ongoing clinical studies employing these technologies to improve pre-operative cancer diagnosis, cancer detection, stratification and treatment planning, and surgical margin evaluation. Challenges and opportunities in implementing the technology into clinical workflows will also be discussed.

PLENARY SESSION 2

You Are What You Breathe and Eat: Linking Chemical Exposure and Molecular Responses

Tuesday, June 24
8:30 a.m. – 9:30 a.m.
Congress Hall

Erin Baker, University of North Carolina at Chapel Hill – United States

CO-AUTHORS: James Dodds, Emily Crawford, Kara Joseph, Ashlee Falls, Greg Kudzin, Anna Boatman, Kaylie Kirkwood-Donelson

Anthropogenic, or human-caused, chemical pollution poses one of the largest threats to the environment and human health. Over the last few decades, the number of chemicals in global commerce has been estimated to range from 25,000 to >350,000 with only a few thousand chemicals having toxicology information. Since total chemical exposure is somewhat unknown from these estimates, we often rely on observed effects such as studies showcasing toxicant links to infertility, diseases, cognitive health, and food security. However, even after concerns have been raised, control measures to limit exposure are often not adopted at the rate needed to avoid chronic and acute effects on human health. An urgent need therefore exists to enable higher throughput and more comprehensive studies on chemical exposure to heighten global awareness about the overall risks posed by chemical usage, dispersal and disposal. This presentation will thus highlight how combining targeted and non-targeted metabolomic, lipidomic and exposomic analyses enable a deeper look into chemical exposure and the ensuing endogenous molecular responses.

PLENARY SESSION 3

Hitchhiker's Guide to Computational Metabolomics

Wednesday, June 25
8:30 a.m. – 9:30 a.m.
Congress Hall

Steffen Neumann, Leibniz Institute of Plant Biochemistry – Germany

Since the 1970s, data processing units and computers have been sold alongside mass spectrometers. Over the past two decades, the concept of computational metabolomics has gained traction in scientific literature, with software capabilities evolving dramatically. These advancements have not only accelerated existing tasks but have also enabled researchers to address entirely new challenges.

In the early days, manual inspection of chromatograms was standard practice. A major turning point for metabolite profiling came with xcms, one of the first open-source tools to implement a complete workflow from feature detection to univariate statistics. Over time, additional algorithms for feature detection, grouping, ion annotation, and many more, were developed. Research groups from all over the world have since contributed to the ever-expanding metaRbolomics ecosystem, with new tools and extensions available via CRAN and Bioconductor.

With robust metabolite profiling workflows in place, the next challenge was metabolite identification and annotation. What once required days or weeks to identify a single metabolite can now be accelerated through computational approaches, enabling the annotation of (almost) all MS/MS spectra, faster than the measurements themselves.

So, what's left to be done? An increasing challenge was, and still is, interpreting the data in a biological and biomedical context. While the above workflow steps have been successfully automated, this final step still relies heavily on human expertise, intuition, and domain knowledge. Let's see what comes next.

PLENARY SESSION 4

Plasma lipidomic profiling of large population cohorts: Development of a precision medicine approach to cardiometabolic disease

Thursday, June 26
8:30 a.m. – 9:30 a.m.
Congress Hall

Peter Meikle, Baker Heart And Diabetes Institute – Australia

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

We have analysed multiple large population and clinical cohorts (>60,000 individuals). These studies have provided an opportunity to investigate new concepts in population lipidomics. These include the use ridge and LASSO regression models for the development of metabolic BMI and metabolic AGE scores, that can provide new, easily interpretable, measures of metabolic health. Using an analogous strategy, we have developed a lipidomic risk score (LRS) for cardiovascular disease. The LRS provided an improvement, over existing risk scores, particularly in the intermediate risk group. When combined with a polygenic risk score the predictive ability of the combined score was further improved. Focusing on people classified as intermediate risk, the combined score was able to reclassify more than half of those who went on to have a cardiovascular event as high risk.

The same analyses have allowed us to identify plasmalogens as a potential therapeutic target to attenuate and mitigate the pathology of multiple cardiometabolic diseases. Genetic models and preclinical studies now support a protective role for plasmalogens in obesity, fatty liver disease and cardiovascular disease.

Combined, these developments support a precision medicine approach to prevent and manage cardiometabolic disease.

PLENARY SESSION 5

Discovery of metabolic indicators of disease, outcomes, and health in human cohorts using nontargeted metabolomics: approaches, challenges, and opportunities.

Thursday, June 26
1:30 p.m. – 2:30 p.m.
Congress Hall

Clary Clish, Broad Institute Of MIT And Harvard – United States

The potential of nontargeted metabolomics to detect and characterize early metabolic changes in subclinical disease has important implications for discovering pathophysiologic mechanisms and disease markers. Our lab has developed robust software tools and hybrid nontargeted metabolomics methods capable of profiling both metabolites of confirmed identity and unknowns, facilitating reproducible large-scale studies of biological specimens from human cohorts. Our work has led to the discovery of plasma metabolic signatures that indicate future risk of developing diabetes, cardiovascular disease, gastrointestinal cancer, and other conditions in longitudinal cohorts; and has included discovery of metabolites associated with the human microbiome, inflammatory bowel disease, and infectious disease in cross-sectional studies. While it remains challenging to identify unknown LC-MS features in nontargeted metabolomics, our field has witnessed exciting discoveries of many new biologically significant metabolites in recent years, such as previously uncharacterized conjugates of canonical metabolites, that further motivate effort in this area. Moreover, existing nontargeted metabolomics datasets from human cohorts and preclinical studies have proven instrumental in elucidating disease associations and origins of novel metabolites. This presentation will highlight recent projects focusing on cardiometabolic and infectious disease.



VENDOR SESSION

SESSION 10: VENDOR SESSION *(Presented by Platinum and Gold Sponsors)* Session Chair: Natasa Giallourou and Aurelia Williams	Tuesday, June 24 3:30 p.m. – 5:15 p.m. Congress Hall

3:30 p.m. – 4:20 p.m.	PLATINUM PRESENTERS

SCIEX

Dr Paul Baker, Senior Staff Scientific Liaison, Lipidomics and Metabolomics, United States

Quantitative and qualitative analysis of bile acids by high-resolution mass spectrometry using electron-based fragmentation

Analyzing bile acids using nominal mass instruments is challenging due to the high chemical background in several precursor ions, which can interfere with multiple reaction monitoring (MRM) transitions. High-resolution mass spectrometry (HRMS), which generates a full product ion spectrum for each targeted bile acid, can reduce background interferences by extracting fragment ions within a narrow mass-to-charge (m/z) window, improving signal-to-noise (S/N) ratios. While detecting individual bile acid isomers relies on chromatographic resolution when using collision-induced dissociation (CID), electron-activated dissociation (EAD) provides structurally diagnostic fragment ions that can differentiate bile acid isomers and reduce isomer resolution time. Here, HRMS was used to sensitively quantify bile acids in human plasma with concurrently acquired qualitative data to ensure specificity. Bile acids were detected and quantified in human plasma using an ultra-sensitive HRMS system capable of both CID- and EAD-based fragmentation. Using the longer chromatographic gradient with CID-based fragmentation resulted in a limit of quantitation (LOQ) approximately 10x lower than previously reported with HRMS analysis, which rivals, if not exceeds, the sensitivity of high-end triple quadrupole instruments. In addition, a high-quality MS/MS spectrum was acquired for each targeted bile acid during analysis, which can improve confidence in the assay's specificity. Using EAD-based fragmentation resulted in identifying unique, diagnostic fragments for some bile acid isomers, and when appropriate, this fragmentation mode was used to allow for faster gradients to improve sample throughput.

Thermo Fisher Scientific

Susan Bird, Vertical Marketing Senior Manager, United States

Orbitrap-based Metabolomics workflows: Discover. Innovate. Exceed.

The field of Metabolomics has been advancing at an impressive rate, inspiring key analytical innovations designed to keep pace with the biological needs of a study. Despite these advances, analytical challenges remain around high-confidence detection and annotation of low abundance compounds in complex biological matrices. Thermo Fisher Scientific is committed to advancing the field of metabolomics through intelligence driven data acquisition, streamlined software processing strategies and key hardware innovations that keep at pace and push beyond the current analytical demands of the field. In this presentation, learn about the enhanced dynamic range (eDR) scanning mode of a novel modified Orbitrap Hybrid MS. eDR is designed to extend the dynamic range of MS full scan measurements by dividing the mass range into narrow windows, in an automated approach, which results in decreasing the saturating impact of high abundant species while improving the signal-to-noise ratios and increasing the quality of low low-abundance metabolites. With this approach, we can extend the range of the metabolites we detect and confidently identify leading to greater insights into important compounds previously undetected using full scan alone.

Bruker

Matt Lewis, Ph.D., VP Metabolomics, United Kingdom

Discover MORE with Bruker´s innovative metabolomics technology portfolio

Metabolomics researchers are pioneers of discovery, combining advanced bioanalytical technologies with rigorous experimental designs and sophisticated data analytics to uncover novel insights to the chemistry of the world around us. However, even the most intrepid explorers face significant challenges on their journey to discovery. Our passion lies in developing innovative technologies and solutions that empower our customers to delve deeper and more confidently into metabolome exploration. Join us for an overview of our diverse portfolio and discover how Bruker technologies can enhance your metabolomics research and help you discover MORE.

VENDOR SESSION

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3:30 p.m. – 4:20 p.m.	PLATINUM PRESENTERS

Waters Corporation
David Heywood, Sr. Manager Omics Market Development, United Kingdom
Advancement of flexible high-resolution workflows for Metabolomics and Lipidomics research

Constant development is needed to address the challenges of analysing complex samples and studies that support the understanding of changes in metabolism due to disease, environmental exposure, or treatment. Instrument vendors must carefully balance advancing technology with supporting established and emerging academic informatics workflows and approaches. In this presentation, we will highlight Waters' efforts to complement academic research in the fields of Metabolomics and Lipidomics.

Agilent
TBD



VENDOR SESSION

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4:20 p.m. – 5:15 p.m.	GOLD PRESENTERS

Cambridge Isotope Laboratories, Inc.
isotope.com

Cambridge Isotope Laboratories

Dr. Andrew Percy, MS 'Omics Product Manager, United States

Stable Isotope-Labeled Tools for QC and/or Quantitation in MS Omics

Metabolite analysis is a growing target of study across research fields in disciplines extending from analytical chemistry to biomedicine. To facilitate improved accuracy/precision of measurement (e.g., in biomarker screening), stable isotope-labeled standards and mixtures are commonly utilized. This presentation will provide an overview of CIL's standard isotopic tools, with extra attention to newly formulated mixes for quantitation and/or benchmarking in MS omics research.

BECKMAN COULTER Life Sciences

Beckman Coulter Life Sciences

Rene Goedde, Market Development Manager, Germany

Enhance Your Metabolomics Research With Automated Liquid Handling

Since the introduction of the Biomek 1000 instrument in 1986, Beckman Coulter Life Sciences has been committed to advancing metabolomics research by streamlining workflows and enhancing efficiency. Our advanced liquid handling solutions are designed to boost throughput while maintaining precision and reproducibility, essential for successful mass spectrometry applications.

In metabolomics, high-quality and reproducible sample preparation is critical, especially when dealing with large sample volumes. Our automated liquid handlers have become invaluable tools for researchers, simplifying complex sample preparation workflows and addressing challenges associated with manual methods. From sample extraction to compound identification, our solutions empower you to focus more on data analysis and discovery, driving innovation in metabolomics research.

In this presentation, we will cover:

- Our collaborations with metabolomics research labs at the University of Birmingham, UK, and Washington University in St. Louis, Missouri, USA, where we are developing the next generation of automated metabolic workflows.

- How our solutions empower researchers to identify metabolic markers associated with various disease indicators, including cancer and infectious diseases.

- Impact of our collaborative projects in assessing the environmental effects of man-made drugs and small molecules.

LECO
EMPOWERING RESULTS

LECO

Christine Loeb-Stubbins, Field Product Manager Sep Sci Europe, United Kingdom

Smarter GC-TOF: Sustainable Strategies for High-Throughput Metabolomics

Advances in non-targeted metabolomics increasingly demand analytical workflows that are not only robust and sensitive but also operationally sustainable. This seminar will highlight practical strategies for optimising laboratory productivity while reducing environmental impact, focusing on innovations in gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS).

We will present how the integration of LECO's StayClean® ion source design minimises instrument downtime and maintenance, ensuring uninterrupted data acquisition for complex biological samples. Additionally, we will explore the use of hydrogen as a sustainable, cost-effective alternative to helium carrier gas, discussing its effects on chromatographic performance and laboratory throughput.

Case examples in biomedical and volatilomics research will illustrate how small workflow adaptations can support faster analyses, lower running costs, and reduce the carbon footprint of metabolomics laboratories — all without compromising data quality or confidence in compound identification.

VENDOR SESSION

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4:20 p.m. – 5:15 p.m.	GOLD PRESENTERS

Shimadzu Europa GmbH

Emily Armitage, Research Scientist, United Kingdom

Flexible workflows to enhance metabolomics and lipidomics research

Metabolomics and lipidomics are popular approaches in the biomarker discovery pipeline, however the detection and identification of potential markers must be precise. To help meet this need, the latest innovations in LC-MS/MS from Shimadzu will be presented along with flexible workflows for processing and analysing targeted and untargeted metabolomics data with high reporting confidence.

To specifically enhance lipidomics workflows, Oxygen Attachment Dissociation (OAD) will be presented as an alternative fragmentation mechanism for the LCMS-9050 QTOF. OAD provides C=C specific fragmentation to structurally characterise lipids, providing more comprehensive identification needed to understand the biological roles of the lipids identified as potential disease markers.

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biocrates life sciences ag

Alice Limonciel, Chief Scientific Officer, Austria

MxP® Quant 1000 kit - Transform medicine with metabolomics

Discover the MxP® Quant 1000 kit — an advanced metabolomics solution that standardizes the quantification of over 1,200 metabolites across 49 biochemical classes on multiple MS platforms. Learn about the small molecules and lipids that will enable tackling chronic disease with metabolomics to support the preventive, predictive, precision, population-based and participatory medicine of tomorrow.

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cmc Instruments GmbH

Rodrigo Aparicio, Head of Brand & Marketing, Germany

Focusing On Your Analysis: Thinking in gas supply systems for a smooth lab operation in MS & GC

A smooth lab operation yields high-quality research and analysis results. The focus of your research is about getting it done, accurately, and reliably; not on worrying whether your instrumentation (especially the back-end) works or not.

Thinking in systems for MS requires going ahead of your on-the-moment status, to where you'll be. On how to think from the start on your research, infrastructure, funding. Thinking in systems will improve your operational efficiencies (simplifying workflows, SOPs, scale reproducibility), and gain certainty in the future for your research.

In this presentation you will find a different way to think about supplying gas for your research instrumentation, so that you get a smooth operation.

Cmbio

Thorsten Gravert, Senior Metabolomics Scientist, Denmark

Solving Complex Biological Questions with Software-Driven Multi-Omics Integration

Where big data meets systems biology to unlock translational insights.

VENDOR SESSION

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4:20 p.m. – 5:15 p.m.	GOLD PRESENTERS

Metabolon
SMALL MOLECULES, BIG INSIGHTS™

Metabolon

Brenan Durainayagam, Product Manager, United Kingdom

Quantify the Exposome: Advancing Precision Health with Metabolomics

The exposome is defined as the totality of environmental exposures over a lifetime, which plays a pivotal role in human health and disease yet remains difficult to quantify and interpret at scale.

Metabolon is driving exposome research by leveraging metabolomics to decode the complex interplay between external exposures, biology, and phenotypic outcomes. In this presentation, we will demonstrate how Metabolon's advanced platform enables broad-spectrum detection of xenobiotics, dietary components, environmental chemicals, and their downstream biological effects. We will highlight case studies that integrate exposomics with multiomics and microbiome profiling to uncover hidden biological signatures, improve mechanistic understanding, and advance biomarker discovery across therapeutic and public health domains. Metabolon's proprietary chemical library and expert-curated databases allow for confident annotation of thousands of compounds, including many previously uncharacterized molecules relevant to real-world exposure.

Attendees will gain insight into how our high-throughput, scalable metabolomics solutions, powered by the Integrated Bioinformatics Platform (IBP) are supporting translational exposome research across academia, industry, and regulatory landscapes. By capturing the chemical fingerprints of life, Metabolon enables researchers to move from exposure to actionable insights.



SPONSOR LUNCH PRESENTATIONS

Tuesday June 24	12:20 p.m. – 1:20 p.m.

Thermo Fisher Scientific

SOUTH HALL 1

The hardware design and functional impact of the Orbitrap Astral in your metabolomics and lipidomics research

Alexander Makarov, Sr. Director Global Research, LSMS, Thermo Fisher Scientific

SQUAD on Orbitrap Astral applied to the study of healthy aging lipidome

Magali Sarafian, Team Lead Metabolomics Proteomics, Bioaster

Simultaneous Quantitation and Discovery (SQUAD) analysis workflows leverage the advantages of Thermo Scientific Orbitrap mass spectrometers. The Thermo Scientific Orbitrap Astral Mass Spectrometer unlocks additional insights into biology and disease mechanisms by utilizing two HRAM detectors, used in parallel, for efficient merging of both targeted and untargeted studies.

SCIEX

SOUTH HALL 2

The portal to multimodal metabolomics and lipidomics

Prof Nicola Zamboni, Institute of Molecular Systems Biology, ETH Zurich

Small-molecule analysis in biological, clinical, and environmental samples has traditionally been constrained by limited dynamic range, insufficient sensitivity, and incomplete structural insights - challenges compounded by collisional-induced dissociation and the lack of comprehensive MS2 data. While recent instrumentation advances such as DIA, alternative fragmentation methods, and ion mobility have moved the field forward, these often necessitate trade-offs in scanning speed or sensitivity.

Now, imagine an instrument that eliminates these compromises entirely. Envision a high-resolution system that combines maximum scanning speed with exceptional sensitivity and a broad dynamic range, capable of delivering electron- and/or collision-induced spectra in under 10 milliseconds - or performing ultra-narrow DIA in mere fractions of a second. With those capabilities at your fingertips, ask yourself: What boundaries would you break? What novel ideas could you implement?

SPONSOR LUNCH PRESENTATIONS

Wednesday June 25	12:20 p.m. – 1:20 p.m.

Waters Corporation

SOUTH HALL 1

Lipid Metabolism in Traumatic Brain Injury – Increased identification confidence whilst extending coverage with the Waters Xevo™ MRT

Dr. Elizabeth Want, Reader in Clinical Mass Spectrometry, Imperial College London

A rapid reversed phase LC-MS analysis showing significant changes in the plasma lipidome related to traumatic brain injury and the effects of Xenon treatment. Data collected in DIA and DDA modes on the Xevo MRT showed excellent mass accuracy, increasing confidence whilst maintaining compatibility with high-throughput methods.

Parallel targeted and untargeted metabolite analysis of mouse plasma samples using a benchtop multi-reflecting time of flight mass spectrometer

Dr. Adam King, Senior Scientist, Waters Corporation

Targeted and untargeted metabolomic data acquired in parallel on a single HRMS platform has become a practical solution for metabolic profiling. Combining both within a single sample injection dramatically reduces the impact on instrument resourcing and sample volume requirements.

Bruker

SOUTH HALL 2

TIMS-enabled Lipidomics: Explore high-sensitivity lipidomics

Dr. Michael Witting, Metabolomics and Proteomics Core, Helmholtz Munich

Dr. Witting is exploring new ways to utilize trapped ion mobility spectrometry (TIMS) to identify lipids and metabolites in C. elegans and human samples.

LC- and NMR-supported Metabolomics: Discover groundbreaking data and insights

Dr. Christoph Trautwein, Director Core Facility Metabolomics, University of Tuebingen

Dr. Trautwein will discuss how NMR and MS enable biomarker discovery and quantitation in cancer and metabolic disorders.

SPONSOR LUNCH PRESENTATIONS

Thursday, June 26	12:25 p.m. – 1:25 p.m.

Agilent Technologies

SOUTH HALL 2

Combining Cellular Bioenergetics with Metabolomics. Take your metabolomics workflow to the next level.

Daniel Cuthbertson, Director, Global Life Science Research Market, Agilent Technologies
David Beale, Senior Research Scientist, Adjunct Professor of RMIT University

We are looking forward to welcoming you to Agilent's lunch seminar where we will provide an overview about a Comprehensive Suite of Next Generation Metabolomics Tools from Agilent and share a study where those tools were applied to investigate the Influence of PFAS on Cellular Metabolism in HeLa and HepG2 Cell Lines Using Seahorse Real-Time Cell Metabolic Analysis and Mass Spectrometry Techniques.



EXHIBITOR DEMO PRESENTATIONS

Tuesday June 24	9:45 a.m. – 10:00 a.m.

GOLD SPONSOR

Beckman Coulter for Life Sciences

Enhance Your Metabolomics Research With Automated Liquid Handling

Rene Goedde, Market Development Manager

Since the introduction of the Biomek 1000 instrument in 1986, Beckman Coulter Life Sciences has been committed to advancing metabolomics research by streamlining workflows and enhancing efficiency. Our advanced liquid handling solutions are designed to boost throughput while maintaining precision and reproducibility, essential for successful mass spectrometry applications.

In metabolomics, high-quality and reproducible sample preparation is critical, especially when dealing with large sample volumes. Our automated liquid handlers have become invaluable tools for researchers, simplifying complex sample preparation workflows and addressing challenges associated with manual methods. From sample extraction to compound identification, our solutions empower you to focus more on data analysis and discovery, driving innovation in metabolomics research.

In this presentation, we will cover:

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- How our solutions empower researchers to identify metabolic markers associated with various disease indicators, including cancer and infectious diseases.
- Impact of our collaborative projects in assessing the environmental effects of man-made drugs and small molecules.



EXHIBITOR DEMO PRESENTATIONS

Wednesday, June 25	9:45 a.m. – 10:00 a.m.

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Microbiome Research Solution Demo

Brenan Durainayagam, Ph.D., Product Manager

The human gut microbiome plays a pivotal role in human health, with growing evidence linking the ecosystem to a broad range of host phenotypes beyond the gut. While metagenomic sequencing has significantly advanced our understanding of the taxonomic and functional potential of the microbiome, it alone cannot fully resolve the dynamic landscape of these microbial communities. Metabolomics, the study of small molecules, provides the ultimate complement to metagenomics by interrogating the interplay within and among organisms.

However, the complexity of integrating multiomics datasets remains a significant barrier to translating these data into actionable strategies for disease prevention and treatment. Incorporating multiple data types demands substantial domain expertise, careful quality control, and data engineering to uncover meaningful biological insights. Metabolon's Integrated Bioinformatics Platform (IBP) has been designed for global collaboration, empowering analytical scientists, experimentalists, and bioinformaticians to simultaneously access and share data in real time across large-scale projects. This secure and unified platform for data analysis facilitates standardization, reproducibility, and accelerates data-driven discovery. Metabolon's IBP accelerates microbiome research and actively supports managed, cohort studies to efficiently translate complex multiomic data into actionable biological understanding.

Wednesday, June 25	3:10 p.m. – 3:25 p.m.

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ORAL ABSTRACTS



★ AWARD WINNERS

SESSION 1. CELLULAR METABOLISM	Monday, June 23	3:30 p.m. – 5:15 p.m.

1.1 KEYNOTE 3:30 p.m. – 4:00 p.m.	168 Hyperacetylated histone H4 is a source of carbon contributing to lipid synthesis

PRESENTING AUTHOR: *Evelina Charidemou, University Of Nicosia, Cyprus*

Histone modifications commonly integrate environmental cues with cellular metabolic outputs by affecting gene expression. However, chromatin modifications such as acetylation do not always correlate with transcription, pointing towards an alternative role of histone modifications in cellular metabolism. Using an approach that integrates mass spectrometry-based histone modification mapping and metabolomics with stable isotope tracers, we demonstrate that elevated lipids in acetyltransferase-depleted hepatocytes result from carbon atoms derived from deacetylation of hyperacetylated histone H4 flowing towards fatty acids. Consistently, enhanced lipid synthesis in acetyltransferase-depleted hepatocytes is dependent on histone deacetylases and acetyl-CoA synthetase ACSS2, but not on the substrate specificity of the acetyltransferases. Furthermore, we show that during diet-induced lipid synthesis the levels of hyperacetylated histone H4 decrease in hepatocytes and in mouse liver. In addition, overexpression of acetyltransferases can reverse diet-induced lipogenesis by blocking lipid droplet accumulation and maintaining the levels of hyperacetylated histone H4. Overall, these findings highlight hyperacetylated histones as a metabolite reservoir that can directly contribute carbon to lipid synthesis, constituting a novel function of chromatin in cellular metabolism.

1.2 4:00 p.m. – 4:20 p.m.	179 Uncovering mechanisms of thiazolidinediones on osteogenesis and adipogenesis using spatial fluxomics

PRESENTING AUTHOR: *Ondrej Kuda, Institute Of Physiology, Czech Academy Of Sciences, Czech Republic*

Objective: Insulin-sensitizing drugs, despite their broad use against type 2 diabetes, can adversely affect bone health, and the mechanisms underlying these side effects remain largely unclear. Here, we investigated the different metabolic effects of a series of thiazolidinediones, including rosiglitazone, pioglitazone, and the second-generation compound MSDC-0602K, on human mesenchymal stem cells (MSCs).

Methods: We developed ^{13}C subcellular metabolomic tracer analysis measuring separate mitochondrial and cytosolic metabolite pools, lipidomic network-based isotopologue models, and bioorthogonal click chemistry, to demonstrate that MSDC-0602K differentially affected bone marrow-derived MSCs (BM-MSCs) and adipose tissue-derived MSCs (AT-MSCs). In BM-MSCs, MSDC-0602K promoted osteoblastic differentiation and suppressed adipogenesis. This effect was clearly distinct from that of the earlier drugs and that on AT-MSCs.

Results: Fluxomic data reveal unexpected differences between this drug's effect on MSCs and provide mechanistic insight into the pharmacologic inhibition of mitochondrial pyruvate carrier 1 (MPC). Our study demonstrates that MSDC-0602K retains the capacity to inhibit MPC, akin to rosiglitazone but unlike pioglitazone, enabling the utilization of alternative metabolic pathways. Notably, MSDC-0602K exhibits a limited lipogenic potential compared to both rosiglitazone and pioglitazone, each of which employs a distinct lipogenic strategy.

Conclusions: These findings indicate that the new-generation drugs do not compromise bone structure, offering a safer alternative for treating insulin resistance. Moreover, these results highlight the ability of cell compartment-specific metabolite labeling by click reactions and tracer metabolomics analysis of complex lipids to discover molecular mechanisms within the intersection of carbohydrate and lipid metabolism.

1.3 4:20 p.m. – 4:40 p.m.	493 Lipidomics, metabolomics and 13C glucose tracing reveal the effects of hyperinsulinemia in sucrose-induced insulin resistance

PRESENTING AUTHOR: ★ *Haoning Howard Cen, Life Sciences Inst, University of British Columbia, Canada*

CO-AUTHORS: *Haoning Howard Cen, Sing-Young Chen, Tom Haoyu Shi, Michael G. Atser, Liam Hall, Armando Alcazar, James D. Johnson*

Hyperinsulinemia has been shown to play a causal role in obesity and insulin resistance during the progression to type 2 diabetes, challenging the long-held dogma that it is solely an adaptive response. Added sucrose in food and beverages has also been shown to influence obesity and diabetes, but the mechanisms are not well defined. We previously found that sucrose-induced weight gain and insulin resistance in mice could be improved by genetically preventing hyperinsulinemia. RNA-seq and proteomics revealed tissue-specific changes in lipid, carbohydrate and amino acid metabolism, with the liver exhibiting the most prominent changes. Therefore, we sought to study the mechanisms of hyperinsulinemia in the liver of these sucrose mice using untargeted lipidomics and metabolomics analysis. Lipids and metabolites were extracted using a biphasic solvent system and analyzed using data-dependent high-resolution mass spectral data acquisition (LC-HRMS/MS; Bruker Impact2). Insulin-stimulated glucose metabolism in the liver was assessed by untargeted metabolomics after intraperitoneal injection of $^{13}C_6$ glucose and insulin. We found that lower fasting and glucose-stimulated insulin levels, but not fasting glucose, were associated with lower fructose levels in the liver. Fructose 6-phosphate had higher ^{13}C-labelled fractions in the insulin-reduced group. Pathway enrichment analysis of the metabolomics suggested altered purine metabolism, proteoglycan biosynthesis, and leukotriene metabolism. In purine metabolism, insulin-reduced mice had lower xanthosine, xanthine and uric acid, which has been shown to associate with improved insulin resistance. Lipid composition analysis revealed upregulated acylcarnitines (CAR) in insulin-reduced group, specifically 3 long-chain CAR species; surprisingly, this occurred without lipid accumulation and with upregulated CPT2 enzyme levels. Glycerol 3-phosphate and GPAT3 enzyme levels were also downregulated, without significant difference in overall triacylglycerol level (TG). Together, we showed that genetically preventing hyperinsulinemia led to profound changes in liver metabolism, which offer new insights into the underlying molecular mechanisms leading to obesity and type 2 diabetes.

★ AWARD WINNERS

SESSION 1. CELLULAR METABOLISM	Monday, June 23	3:30 p.m. – 5:15 p.m.

1.4 4:40 p.m. – 5:00 p.m.	328 Metabolic Reprogramming Drives Immunometabolic and Neuroinflammatory Dysregulation in Treated HIV

PRESENTING AUTHOR: ***Ujjwal Neogi, Karolinska Institutet, Sweden***

Despite viral suppression with combination antiretroviral therapy (cART), people with HIV (PWH) remain at risk for immunometabolic complications and neurocognitive dysfunction. Using a multi-omics approach integrating transcriptomics, proteomics, and metabolomics, we identified key metabolic perturbations in prolonged treated PWH from the Copenhagen Comorbidity in HIV Infection (COCOMO) cohort. Network-based patient stratification revealed that 44% of PWH were at risk of developing immunometabolic complications, with disruptions in central carbon metabolism and tryptophan metabolism (Mikaeloff et al. 2025, Advanced Science). Functional validation in primary cells and cortical forebrain organoids demonstrated persistent monocyte activation and synaptic dysregulation due to a chronic inflammatory plasma microenvironment. As monocytes and macrophages are crucial for immune homeostasis and exhibit impaired function due to metabolic reprogramming, we further performed single-cell RNA sequencing and immune phenotyping. Our data revealed altered chemokine signaling and metabolic flux in monocytes from PWH, leading to decreased migration and increased inflammatory signaling. Plasma metabolomics identified elevated α-ketoglutarate levels, which promoted an M2-like macrophage state, increased CCR5 expression, and enhanced susceptibility to HIV-1 infection. Further, to investigate neuroinflammatory consequences, we developed HIV-infected microglia-infused brain organoids. HIV-1 infects microglia and astrocytes but not neurons. Transcriptomic and metabolic modeling revealed dysregulation in polyamine and tryptophan metabolism, with elevated spermidine linked to neuronal dysfunction. Our findings highlight metabolic reprogramming as a driver of chronic inflammation, immune dysfunction, and neuroinflammation in treated PWH. Identifying metabolic biomarkers enables early risk stratification and targeted interventions to mitigate long-term complications associated with HIV.

1.5 5:00 p.m. – 5:15 p.m.	180 Space Metabolomics: Investigating Neural Stem Cell Adaptations to Microgravity

PRESENTING AUTHOR: ★ ***Lucie Davidová, Uppsala University, Sweden***

CO-AUTHORS: ***Lucie Davidová, Lukas Zeger, Yilin Han, Elena Kozlova, Ingela Lanekoff***

Long-duration space missions expose human cells to microgravity, leading to physiological changes that impact astronaut health, including cardiovascular slowing, muscle atrophy, osteopenia, and even brain structure alterations. Thus, understanding cellular responses to microgravity is crucial for developing countermeasures. Boundary cap neural crest stem cells (BCs) are essential for neural development, tissue repair, and regeneration, making them relevant for studying the effects of spaceflight on neurobiology.

In this study, we investigated the metabolic response of BCs to microgravity by sending them aboard the International Space Station (ISS) for 25 days. A ground-based control group was maintained under identical conditions for direct comparison. Following their return, cells were cultured and harvested for metabolomics with direct infusion mass spectrometry. Cells were washed with ammonium formate and lysed with a 9:1 MeOH:H2O solution. Final cell extracts (~600 cells/µL) were analyzed using an in-house developed direct infusion probe (DIP) coupled to Orbitrap IQ-X Tribrid mass spectrometer. DIP-MS provides rapid analysis of minute sample volumes with minimal carryover effects, making it ideal for precious space-exposed cell samples. Data were acquired for 30 seconds and further processed using custom MATLAB scripts. The most significantly altered features were targeted for annotation with MS2.

Our results reveal significant differences in metabolite profiles between the space-exposed and control BCs, suggesting a shift in energy metabolism and cellular adaptations to microgravity. Non-targeted multivariate analysis showed separation of control and space cells in PCA and PLS-DA, while targeted analysis focusing on glycolysis and TCA cycle showed an increase in glucose levels alongside decreased lactate, citrate, and fumarate. Notably, space-exposed BCs exhibited enhanced cell proliferation, further suggesting metabolic reprogramming in response to microgravity.

Overall, these findings provide insights into the effects of spaceflight on neural cell function, offering potential implications for regenerative medicine and further understanding of space-related cellular adaptations.

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SESSION 2. PREDICTIVE APPROACHES AND SIMULATIONS | Monday, June 23 | 3:30 p.m. – 5:15 p.m.

2.1 KEYNOTE 3:30 p.m. – 4:00 p.m. **546 Simulated metabolic profiles reveal biases in pathway analysis methods**

PRESENTING AUTHOR: *Fabien Jourdan, INRAE-MetaboHUB, France*

Initially developed for transcriptomics data, pathway analysis (PA) methods are broadly used in metabolomics. Despite the fact that they are convenient tools for a first functional interpretation of data, they can introduce biases, especially if input parameters are not chosen with care. This is particularly true for metabolites measured in biofluids (exometabolomics data), where exported metabolites may be far from internal disruptions in the organism.

Experimentally evaluating PA methods is practically impossible when the sample's "true" metabolic disruption is unknown. Using in silico metabolic modelling, we simulated metabolic profiles for entire pathway knockouts, providing both a known disruption site as well as a simulated metabolic profile for use in PA methods. PA should be able to detect the known disrupted pathway among the significantly enriched pathways for that profile. Through network-level statistics, visualisation, and graph-based metrics, we show that even when a given pathway is completely blocked, it may not be detected as significantly enriched with PA. This work highlights how some metabolomics data may not be suited to typical PA methods, and serves as a benchmark for analysing, improving and developing new PA tools.

2.2 4:00 p.m. – 4:20 p.m. **390 Topology-Aware Single Sample Pathway Analysis using the Graph Fourier Transform**

PRESENTING AUTHOR: *Tom Rix, Imperial College London, United Kingdom*

CO-AUTHORS: *Tom Rix, Juliette Cooke, Cecilia Wieder, Sandra Costa Gonzalez, Nathalie Poupin, Clément Frainay, Fabian Jourdan, Elena Chekmeneva, Elizabeth Want, Timothy Ebbels*

Pathway analysis is a valuable and popular approach for interpreting omics datasets in terms of biological function. In metabolomics, an emerging method is single sample pathway analysis (ssPA) which computes pathway scores for individual samples that enable downstream analyses at the pathway level that are often not possible with conventional methods, such as multi-group comparisons, survival analysis and disease-subtyping. However, for metabolomic datasets, these methods are often hindered by the small number of assayed metabolites, the sparseness of pathway annotations, and the resulting low pathway coverage. Consequently, there is a need for methods that can extract meaningful insights from limited metabolomic data.

To enhance the detection of altered pathways in metabolomics data, even when the pathway coverage is low, we developed "topo-ssPA": a new method for integrating network topology into an ssPA framework. Our approach exploits network structure by applying the Graph Fourier Transform to pathway-level networks constructed from intensity correlation structure, chemical similarity and pathway connectivity. Here, we first benchmarked topological-ssPA approaches on synthetic datasets, including simulated metabolic profiles for inborn errors of metabolism generated using the SAMpling Biomarker Analysis (SAMBA) software. We then applied topo-ssPA to several publicly available LC-MS datasets, spanning 42-81 pathway mapping metabolites and 60-356 samples. Although the true altered pathways are unknown, comparing the predictive ability of pathway scores in classifying samples revealed broadly consistent results between SVD-ssPA and topo-ssPA. Notably, topological scores demonstrated greater robustness to reduced metabolite sets in dropout studies on LC-MS datasets, consistently outperforming SVD-ssPA in ROC-AUC for dropout rates ranging from 5-50%. Furthermore, analysis of simulated datasets showed improved ability to detect known altered pathways under both additive and multiplicative noise. In summary, our work for the first time introduces topological information into metabolomic ssPA and illustrates how robustness may be a key benefit of this novel technique.

2.3 4:20 p.m. – 4:40 p.m. **377 Data-driven 13C-fluxomics towards ab initio reconstruction of metabolic networks.**

PRESENTING AUTHOR: *Noémie Butin, MetaboHub-MetaToul, France*

CO-AUTHORS: *Noémie, Pierre Millard, Clément Frainay, Loïc Le Grégam, Sergueï Sokol, Fabien Jourdan, Uwe Schmitt, Floriant Bellvert, Patrick Kiefer, Jean-Charles Portais*

Metabolic networks reconstruction is a valuable tool for understanding biological systems, by identifying the link between the structure of the network, its organization and its functional properties. The reconstruction process, usually based on the genome, provide a comprehensive model that represents an organism's entire set of biochemical reactions. Alternative experimental reconstruction approaches, aim at reconstructing the active metabolic networks directly from metabolome observation, in a way that is orthogonal and complementary to in silico approaches. In this work, we have developed a new data-driven approach for ab initio metabolic reconstruction based on isotopic tracing experiments. Isotopic labeling, especially with ^{13}C isotopic tracers, has long been used to study the structure and the activity of metabolic pathways. Our approach, IsoMet for Isotopic driven Metabolic reconstruction, is based on the interpretation of untargeted metabolomics data collected in ^{13}C-labeling dynamic experiments. It aims at providing direct access to the active metabolic network of an organism, specific to a cell type in a given context, without prior considerations. IsoMet shares concepts with non-stationary ^{13}C-fluxomics approaches, such as label propagation simulation and flux calculation. Its basic principle consists to construct and test the ability of different network topologies to explain observed labeling dynamic data, leading to the construction of active metabolic subnetworks. IsoMet is generic and can be applied to various stable isotope tracers (^{13}C, ^{15}N, etc) and different type of isotopic measurement (MS, MS/MS, NMR, etc). It is intended to be applicable to various biological models, ranging from poorly known organisms to complex systems.

★ AWARD WINNERS

SESSION 2. PREDICTIVE APPROACHES AND SIMULATIONS	Monday, June 23	3:30 p.m. – 5:15 p.m.

2.4 4:40 p.m. – 5:00 p.m.	170	Times are changing but order matters: Transferable prediction of small molecule liquid chromatography retention times

PRESENTING AUTHOR: *Fleming Kretschmer, Friedrich Schiller University Jena, Germany*

CO-AUTHORS: *Fleming, Eva-Maria Harrieder, Michael Witting, Sebastian Böcker*

The prediction of retention times in liquid chromatography has been studied for decades, with thousands of papers published on the subject. Potential applications range from aiding chromatographic method development to improving metabolite annotation. The ultimate goal is undoubtedly transferable prediction: A model trained on a certain set of compounds from one dataset should be able to predict retention times for a different dataset, using a different chromatographic system. However, retention times may change massively, even for nominally identical chromatographic conditions. Retention order is much more conserved for identical conditions, yet can also change if the conditions vary.

Here, we first systematically investigate the influence of chromatographic parameters on retention order and determine which of them are most important for modeling retention behavior. Instead of directly predicting retention time, we propose to disentangle the problem using a two-step approach: We train a Siamese graph neural network to predict retention order, or more precisely, a retention order index, using important chromatographic parameters as additional features besides compound structure. The model is trained on a large number of curated datasets, covering a wide compound space. We show that this allows generalization to unseen compounds and chromatographic conditions. As the second step, we introduce a robust method for mapping from retention order index to retention time, resulting in accurate predictions even for novel datasets, while requiring only a small number of anchor compounds. Disentangling the two tasks finally enables transferable retention time prediction across chromatographic conditions and compound classes.

2.5 5:00 p.m. – 5:15 p.m.	31	Bridging polarities in metabolomics: Cross-ionization mode chemical similarity prediction between tandem mass spectra

PRESENTING AUTHOR: ★ *Niek de Jonge, Wageningen Univ. & Res., Netherlands*

CO-AUTHORS: *Niek de Jonge, Elena Chekmeneva, Robin Schmid, David Joas, Lem-Joe Truong, Justin J.J. van der Hooft, Florian Huber*

Mass spectrometry is a cornerstone of untargeted metabolomics, enabling the characterization of metabolites in both positive and negative ionization modes. However, comparing mass spectra across ionization modes remains challenging due to distinct fragmentation patterns produced by each polarity. To overcome this barrier, we present MS2DeepScore 2.0, a machine learning-based model to predict chemical similarity between mass fragmentation spectra, capable of functioning both between and across ionization modes.

We demonstrate high prediction accuracy both between and across ionization modes and showcase the utility of MS2DeepScore 2.0 in a human urine case study. In this study, MS2DeepScore 2.0 enabled cross-ionization mode molecular networking, successfully linking positive and negative ionization mode spectra that classical approaches, such as the modified cosine score, could not connect. Expert annotation of 37 mass spectra confirmed the accurate cross ionization mode connections between these mass spectra.

MS2DeepScore 2.0 utilizes a Siamese Neural Network trained to predict chemical similarity. Key improvements in MS2DeepScore 2.0 include a new training sampling algorithm and the incorporation of spectrum metadata as model inputs. Additionally, we implemented a quality estimation method that flags spectra with low information content or those dissimilar to the training data, thereby minimizing false predictions.

MS2DeepScore 2.0 is now also integrated into mzmine, a widely used mass spectrometry data analysis platform with a user-friendly graphical interface, ensuring accessibility for researchers without bioinformatics expertise. Altogether, MS2DeepScore 2.0 substantially extends our current capabilities in organizing, exploring, and annotating untargeted metabolomics profiles. Further details are available in our preprint: https://www.biorxiv.org/content/10.1101/2024.03.25.586580v4.

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SESSION 3. NEW AVENUES FOR LIPID ANALYSIS	Monday, June 23	3:30 p.m. – 5:15 p.m.

3.1 KEYNOTE 3:30 p.m. – 4:00 p.m. **193 ZT Scan DIA with Two-Dimensional Deconvolutions for Untargeted Metabolomics**

PRESENTING AUTHOR: ★ ***Hiroshi Tsugawa, TUAT, Japan***

Data-independent MS/MS acquisition (DIA) can produce contaminated spectra from multiple molecules, even with narrow precursor windows, causing decreased annotation scores and potential false negatives in metabolomics. While MS-DIAL's retention time (RT)-axis deconvolution (RDec) helps isolate single metabolite spectra, it struggles when peaks share identical RTs. We propose a new workflow using ZT Scan DIA, which continuously manipulates precursor windows to enable simultaneous deconvolution along both RT and Q1 axes. To validate this method, we analyzed human SRM 1950 plasma and SRM 1951C serum in HILIC and reversed-phase LC modes, comparing data-dependent acquisition (DDA), Zeno SWATH-DIA, and ZT Scan DIA.

Four novel MS-DIAL algorithms fully leverage ZT Scan DIA data: (1) a dynamic MS2 reader that retrieves raw MS2 information on demand, (2) Q1-axis deconvolution (QDec) to detect co-eluting metabolites by identifying distinct peak tops in product ion traces, (3) sample-axis deconvolution (SDec) to separate components with identical RT–m/z across different samples, and (4) MS2 chromatogram-based quantification for robust measurement of fully co-eluting peaks. With ZT Scan DIA data sizes often exceeding 2 GB, the dynamic MS2 reader reduced preprocessing time from over two hours to under a minute and slashed RAM usage from 20–30 GB to about 3 GB. QDec successfully distinguished triacylglycerol [M+NH4]+ from [M+Na]+ species, improving spectral matching and annotation confidence. SDec further refined spectral purity across samples, and MS2 chromatogram-based quantification enabled precise peak separation even under severe co-elution.

Overall, this pipeline boosted annotation rates by more than 20% and significantly enhanced reproducibility compared to conventional workflows. We applied it to untargeted lipidomics of mouse brain and liver, constructing a comprehensive lipidome map to reveal age- and diet-dependent changes. These advances underscore ZT Scan DIA's potential to deliver high-quality, large-scale metabolomics datasets and facilitate more accurate metabolite annotation. It also enables rapid data processing in resource-limited settings.

3.2 4:00 p.m. – 4:20 p.m. **315 Expanding the Annotation of Esterified Oxylipins Using Multidimensional Databases and LC-MS/MS-based Ion Identity Molecular Networking Analysis**

PRESENTING AUTHOR: ***Sandra M. Camunas-Alberca, Centro de Metabolómica y Bioanálisis (CEMBIO), Spain***

CO-AUTHORS: ***Sandra M. Camunas-Alberca, Francesco Bartolini, Hana Cermakova, David Perez-Guaita, Ángel Sánchez-Illana, Jean-Marie Galano, Thierry Durand, Georgios Chamilos, Coral Barbas, Ana Gradillas***

The analysis of enzymatic and non-enzymatic oxylipins in biological samples, both in their free and esterified forms, has garnered significant attention due to their crucial biological roles. However, esterified oxylipins remain underrepresented in lipidomic databases, posing a challenge for their comprehensive characterization in LC-MS-based lipidomic studies.

Recently, feature-based molecular networking (FBMN) —a method that maps the similarity among tandem mass (MS/MS) spectra of LC features— has been applied to non-targeted oxylipin analysis using libraries of free oxylipins, demonstrating its potential to expand oxylipin annotations. However, esterified oxylipins typically generate multiple ion species with distinct fragmentation behaviors, complicating the interpretation of network topology. To address this limitation, we propose the application of ion identity molecular networking (IIMN), a complementary approach that integrates ion correlation data into molecular networks, in combination with a novel esterified oxylipin library.

In this study, we present a multidimensional database (i.e. lipid names, retention times (RT), accurate mass values (m/z), adduct profiles, and MS/MS) of oxidation products of 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (oxPAPC) that includes 34 oxidized lipids fully characterized using UHPLC-ESI-QTOF-MS (Agilent 6545 QTOF), including truncated, full-length, and cyclic variants.

We developed a comprehensive workflow combining our database with IIMN using mzmine and GNPS2. This approach enables a multilevel annotation strategy, improving oxidized lipid profiling by enhancing coverage, accuracy, and confidence in annotation. Quality assurance procedures, including adduct formation assessment, elution order evaluation and fragmentation pattern consistency were incorporated to enhance annotation robustness. Special attention was paid to neutral losses and diagnostic ions by studying the fragmentation mechanisms involved.

We applied this methodology to serum samples from patients with mucormycosis, a fungal infection characterized by severe oxidative stress. Our findings provide new insights into oxylipin dynamics, revealing possible molecular mechanisms linked to oxidative stress-related diseases.

SESSION 3. NEW AVENUES FOR LIPID ANALYSIS	Monday, June 23	3:30 p.m. – 5:15 p.m.

3.3 4:20 p.m. – 4:40 p.m.	58 A metabolite array for 10-second classification of brain cancers with ambient mass spectrometry: towards a pilot database using 1,300 patients

PRESENTING AUTHOR: ***Arash Zarrine-Afsar, University of Toronto, Canada***

CO-AUTHORS: ***Alexa Fiorante, Michael Woolman, Howard Ginsberg, Sunit Das, Gelareh Zadeh, Ahmed Aman, Taira Kiyota, David Munoz, Arash Zarrine-Afsar***

Surveys of neurosurgical outcome data have suggested suitable extents (aggressiveness) of resection to improve survival for many brain cancer types. Here, personalization of the extent of resection relies on highly subjective intraoperative diagnoses wherein the depth of the diagnostic information depends on the level of experience of the pathologist.

Picosecond InfraRed Laser Mass Spectrometry (PIRL-MS) uses picosecond bursts of mid- infrared laser radiation to extract, in a non-thermal manner, tissue lipids (1) present in cellular membranes, giving cells and nuclei their unique shapes (utilized in morphometric pathology with staining & microscopy) and (2) altered in tumorigenesis due to cross-talk with cellular signaling in metabolism (potentially also revealing cancer molecular subtypes in 10 seconds as shown) for real-time profiling of molecular content unique to each tumour type.

Close to 1,300 frozen brain cancer specimens over 30 different classes of adult and pediatric cancers are being subjected PIRL-MS to build a comprehensive molecular signature library using various supervised and unsupervised dimensionality reduction and data analysis methods. The sensitivity and specificity of the integrated morphometric and molecular diagnosis for pediatric brain cancers with 10-second PIRL-MS was > 96%.

Mining the tissue-classifying mass-to-charge (m/z) with chromatography and tandem mass spectrometry has resulted in 'molecular arrays' for 10-second pathology determination with PIRL-MS, utilizing between 20-40 metabolic lipids. Greater than 90% sensitivity and specificity have been obtained for adult brain cancer classifications with 10-second PIRL-MS analysis utilizing lipids spanning fatty acids, phospholipids and ceramides among others.

PIRL-MS can thus provide non-subjective differentiation between various brain cancer types with as little as 10 seconds of sampling and analysis time. Integration with surgical navigation and positioning technologies has allowed real-time display of spatially encoded mass spectrometry classifiers based on identified cancer-type classifying molecular arrays via augmented reality to expedite surgical decisions faster and more accurately than morphometric methods.

3.4 4:40 p.m. – 5:00 p.m.	152 Innovations in Skin Ceramide Profiling: Leveraging Supercritical Fluid Chromatography and High-Resolution Mass Spectrometry (Q-Tof) for Advanced Analysis

PRESENTING AUTHOR: ***Justine Bertrand-Michel, MetaboHUB-metatoul, France***

CO-AUTHORS: ***Cyrielle Clément, Julia Soullier, Nuria Pell Vidal, Nathalie Jonca, Lukas Opalka, Pauline Le Faouder, Justine Bertrand-Michel***

Lipids play a crucial role in the structure and function of the skin. They are primarily composed of ceramides, fatty acids, and cholesterol. In a health context, a comprehensive characterization of the skin lipid composition is essential, as it varies in relation to skin diseases such as ichthyosis and psoriasis. The improvement of skin sample collection is a growing concern, as current methods are invasive (skin scraping), which has led to the development of new analytical methods, particularly in the extraction of these new matrices (strips, reconstructed epidermis, etc.).

However, the diversity of epidermal ceramides, combined with the complexity of their structure and the lack of reference standards, makes their analysis particularly challenging. An innovative profiling of epidermal ceramides has been implemented through the combination of Supercritical Fluid Chromatography (UPC^2, Waters), which is particularly suitable for non-polar metabolites, and high-resolution Q-TOF mass spectrometry (Xevo, Waters).

This analytical development will be presented, it has enabled the MS characterization and the relative quantification of 15 lipid sub-classes of skin ceramides. This method allows the fast profiling (10 minutes) of 300 molecular species, facilitating high-throughput applications in clinical research. The extraction method has also been improved to be adapted to various matrices (tissues, sebum from strips) while being rapid and suitable for all ceramide subfamilies, despite their differing chemical properties. Moreover, we will demonstrate that this novel method enables the analysis of reconstructed epidermises and strips, confirming the role of the protein PNPLA1 (patatin-like phospholipase domain-containing in the synthesis of specific epidermal ceramides, which is implicated in autosomal recessive congenital ichthyosis.

This approach offers a new and efficient way to analyze epidermal ceramides, providing valuable insights into skin health and disease management.

SESSION 3. NEW AVENUES FOR LIPID ANALYSIS	Monday, June 23	3:30 p.m. – 5:15 p.m.

3.5 5:00 p.m. – 5:15 p.m.	127 Development of a Novel High-Throughput Acoustic Droplet Ejection Mass Spectrometry Method with Signal Durations for Lipidomics

PRESENTING AUTHOR: *Yu Zhang, Leiden University, Netherlands*

CO-AUTHORS: *Yu Zhang, Nicolas Drouin, Amy Harms, Thomas Hankemeier*

Lipidomics, the comprehensive study of lipids within biological systems, is essential for understanding the mechanisms underlying health and cellular functions. Mass spectrometry-based lipid analysis is a powerful tool, with LC-MS as the gold standard for identifying and quantifying thousands of lipid species. However, its relatively slow throughput limits large-scale applications in personalized medicine. Shotgun Lipidomics offers an alternative by directly introducing samples into the mass spectrometer but faces challenges such as carry-over, sample loading inefficiencies, and matrix effects. Acoustic droplet ejection mass spectrometry (ADE-MS) presents a novel approach, enabling touchless nanoliter-scale sample introduction at high speed, precision, and accuracy. Initially designed for single droplet injection, ADE-MS can be adapted for direct infusion with stable signals. In this study, we aimed to develop a high-throughput method for the precise detection and quantification of a wide range of lipids using ADE-MS and the Zeno TOF system operating in Parallel reaction monitoring (PRM) mode while ensuring high signal reproducibility.

We optimized various operational parameters (e.g., flow rate, injection solvent and volume, carrier solvent). By successively injecting thousands of droplets at a high frequency (10 Hz), we were able to simulate a direct injection profile. Taking advantage of the low sample consumption and extended scan time, our method successfully identified and quantified over 500 lipid species across 15 lipid (sub)classes, covering both polar and nonpolar lipids, with a total run time of approximately four minutes. The method was fully validated, demonstrating robust analytical performance in terms of linearity, precision, reproducibility and recovery in 384 microplates. We then benchmarked our approach against the gold-standard LC-MRM method by analyzing plasma samples, highlighting the advantages of our method, reinforcing its potential for high-throughput lipidomic studies. Finally, we applied this method successfully to analyze plasma lipids in 200 human subjects exposed to cold for two hours.



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SESSION 4. CLINICAL TRANSLATION OF LIPIDOMICS & METABOLOMICS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

4.1 SESSION KEYNOTE
10:15 a.m. – 10:45 a.m.

500 Comprehensive, quantitative analysis of SRM 1950: the NIST human plasma reference material

PRESENTING AUTHOR: ***Rupasri Mandal, University Of Alberta, Canada***

CO-AUTHORS: ***Rupasri Mandal, Jiamin Zheng, Lun Zhang, Eponine Oler, David Wishart***

Many analytical methods have been developed for performing targeted metabolomics. By combining multiple analytical techniques, comprehensive coverage of the metabolome can be achieved. We combined multiple analytical techniques to comprehensively and quantitatively characterize the widely studied NIST human plasma reference material, SRM 1950. Our goal was to pro-vide a large, well-validated list of confident metabolite concentration values (i.e., benchmarks) to assist the metabolomics community in its calibration and comparison efforts. We used four analytical platforms: high resolution NMR spectroscopy, direct injection tandem MS (DI-MS/MS), liquid chromatography tandem MS (LC-MS/MS) and inductively coupled plasma MS (ICP-MS). Eight validated analytical assays were run, yielding accurate quantitative measurements for 728 unique metabolites or metabolite species. Through computer-aided literature mining, we identified another 330 unique metabolites previously quantified in SRM 1950. We compared NIST certified values along with literature-derived concentrations/ranges to the metabolite concentrations measured by our four platforms and eight assays. From these assays/platforms, we generated a list of high-confidence concentration values of 1058 metabolites or metabolite species in SRM 1950 including data for 60 amino acids/ related compounds, 48 bile acids, 72 amines/sugars/alcohols, 21 metals, 8 catecholamines, 11 vitamins, 92 organic acids, 40 fatty ac-ids/steroids/nucleobases/indole derivatives, 5 polyfluorinated compounds, 7 carotenoids, 39 acylcarnitines, 76 oxylipins, 13 sterols and 566 lipids/ lipid species. This dataset represents the most complete quantitative characterization of SRM 1950. An online database (SRM1950-DB) containing 1058 plasma metabolites/ metabolite species in SRM 1950, their structures, HMDB IDs, mass, chemical class, concentrations, references and reliability, is freely available at https://srm1950-data.wishartlab.com.

4.2 10:45 a.m. – 11:05 a.m.

407 Bridging the Gap: from Research-Grade Methods to Routine Clinical Applications

PRESENTING AUTHOR: ***Anne Bendt, National University of Singapore, Singapore***

CO-AUTHORS: ***Anne Bendt, Alicia Chan, Yichen Yao***

Introduction:

Like many research laboratories worldwide, SLING has worked for years to establish robust workflows for metabolite analyses, primarily focusing on LC-MS-based lipidomics. Through interactions with leading clinical laboratories and diagnostic service providers, we have come to appreciate the significant differences between research-grade methods versus assays suitable for routine clinical applications. While mass spectrometry-based metabolite analysis holds great potential for future diagnostic applications, advancing on this path requires rigorous standardization (Vogeser & Bendt, Clin Chem Lab Med 2022).

Aim:

To bridge the gap between research and routine diagnostics, we decided to establish an internationally accepted framework to ensure reliable, reproducible, and accurate liquid chromatography–mass spectrometry (LC-MS) analyses of clinically and commercially relevant analytes.

Methods:

To meet the vigorous medical laboratory standards, we implemented an ISO 15189-compliant quality management system (QMS). As a first step, we validated a commercial LC-MS assay steroid hormone according to international guidelines (CLSI, FDA), enabling metrologically traceable quantification. This approach encompasses method standardization, internal and external quality assurance, competent staff training, and extensive documentation.

Results:

Over nearly two years, a multidisciplinary team worked to establish the QMS, requiring significant investment in expertise and infrastructure. The steroid hormone assay is now nearing readiness for routine application. The next phase includes expanding to a multi-analyte panel, with a laboratory-developed test (LDTs) for per- and polyfluoroalkyl substances (PFAS) and ceramide species. Future efforts will extend to broader omics-based analyses.

Conclusions:

With the increasing interest in clinical metabolomics, workflow standardization to accepted guidelines is crucial—not only for ensuring high-quality, reproducible clinical research results but also for facilitating the potential transition and adoption of metabolite panels into routine diagnostics. Developing LC-MS assays in compliance with medical diagnostics standards requires major investment, dedication, and expertise not commonly found in research labs, but it is an essential step toward enabling future diagnostic applications.

SESSION 4. CLINICAL TRANSLATION OF LIPIDOMICS & METABOLOMICS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

4.3 11:05 a.m. – 11:25 a.m.	40 Pharmacometabolomics reveals notable differences between real-world and expected drug metabolism patterns

PRESENTING AUTHOR: ***Frank Klont, University Of Groningen, Netherlands***

Background: Drug potency depends on the intrinsic activity of the substance administered and the body's ability to transform it into (in)active metabolites. Knowledge of this metabolism is typically derived from early-phase clinical trials conducted in young, healthy males receiving single doses. These findings likely have limited generalizability to the intended drug users, who represent a considerably more heterogeneous group. This suggests a need for larger-scale drug metabolism studies in real-world patient populations, where we aim to confirm expected metabolites and (potentially) detect previously unreported ones.

Methods: To address this need, we employed untargeted pharmacometabolomics, combining reversed-phase liquid chromatography and 'SWATH' data-independent acquisition mass spectrometry. Our method was applied to 24-hour urine samples of 1,421 kidney transplant recipients and 283 (potential) living kidney donors from the TransplantLines studies (NCT03272841/02811835). Data analysis was performed to assess the alignment of observed metabolite profiles with established consensus metabolic patterns of various immunosuppressants, cardiovascular drugs, and antibiotics.

Results: Real-world metabolic patterns frequently diverged from consensus expectations, with generally more metabolites identified than expected. For example, for metoprolol, 9 metabolites were anticipated, 7 were confirmed, and 10 unreported metabolites were discovered. We occasionally failed to confirm expected metabolites, mostly intermediate metabolites for which downstream variants were detected. Interestingly, several studied drugs are prodrugs requiring activation by carboxylesterases, which are assumed to enable rapid and full conversions. Our findings, however, contradicted this assumption, as the prodrugs themselves and their glucuronide counterparts were detected in most users, suggesting impaired prodrug activation.

Conclusions: Our study offers new insights into the metabolic fate of various drugs in kidney transplant recipients and (potential) living kidney donors. Thereby, it confirms the limited generalizability of clinical trial-derived metabolic patterns. Moreover, it underscores the need for real-world drug metabolism studies that could contribute to improved drug therapies, for which untargeted pharmacometabolomics holds significant potential.

4.4 11:25 a.m. – 11:45 a.m.	273 Metabolomics and clinical chemistry insights from 20,000 individuals

PRESENTING AUTHOR: ***Natasa Giallourou, Metabolon Inc., United States***

CO-AUTHORS: ***Natasa Giallourou, Matthew W. Mitchell, Cynthia Balion, Parminder Raina, HELIOS Study Team, John Chambers, Nilanjana Sadhu, Hong Kiat Ng, Theresia Mina, Alain Bertoni, Yii-Der Ida Chen, Mark O. Goodarzi, Janice C. Jones***

Background

Clinical chemistry measurements derived from blood samples are essential tools for assessing health status, disease progression, and therapeutic responses. However, traditional clinical biomarkers capture only a fraction of an individual's metabolic state. The work presented here expands the biochemical insights available from blood chemistry by correlating over 1,000 metabolites with routine clinical chemistry and hematological parameters from three geographically distinct cohorts. This study integrates metabolomics with large, diverse population cohorts to identify novel biomarker relationships that can enhance our understanding of disease mechanisms and physiological health states.

Methodology

Metabolomic profiling was performed on plasma samples from three well-phenotyped population studies: CLSA (Canadian Longitudinal Study on Aging, n=9,992), HELIOS (Health for Life in Singapore, n=9,993), and MILES (Microbiome and Insulin Longitudinal Evaluation Study, n=353). Metabolite profiles were generated using Metabolon's Global Discovery Panel. These profiles were correlated with 45 clinical chemistry and hematological parameters covering cardiovascular, metabolic, and organ function markers (Number of parameters analyzed per cohort: MILES=7, HELIOS=20, CLSA=35). Partial correlation analysis was used to control for confounding variables, including BMI, age, race, sex, and ethnicity.

Results

Significant correlations between clinical chemistry markers and metabolites were identified, including expected relationships such as cholesterol with LDL, metformin with fasting glucose, and gamma-glutamyl transferase with glutamate-derived metabolites. Additionally, novel correlations were observed, revealing potential new markers for clinical assessment. For example, N-lactoyl amino acids correlated inversely with insulin sensitivity index, supporting their role in metabolic dysregulation. Importantly, ferritin significantly correlated with perfluoroalkyl substances (PFAS) (perfluorooctanesulfonate rp=0.24, p-val<0.001, perfluorooctanoate rp=0.25, p-val<0.001), suggesting a potential link between iron metabolism and exposure to PFAS.

Significance

This study showcases the integration of untargeted metabolomics with routine blood chemistry to enhance biomarker discovery. By identifying novel metabolite-clinical correlations across cohorts, we propose a framework to improve metabolic health assessment and connect clinical chemistry with metabolomics-driven insights.

SESSION 4. CLINICAL TRANSLATION OF LIPIDOMICS & METABOLOMICS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

4.5 11:45 a.m. – 12:00 p.m.	74	Infant formula composition affects tetraglucoside (Glc4) excretion: the impact on the diagnostics of inborn errors of metabolism

PRESENTING AUTHOR: ***Silvia Radenkovic, UMC Utrecht, Netherlands***

CO-AUTHORS: ***Silvia Radenkovic, Birgit Schiebergen-Bronkhorst, Daan Panneman, Judith Jans, Monique de Sain-van der Velden, Hubertus Prinsen***

Background: Tetraglucoside (Glc4) is a breakdown product of glycogen and is generally increased in glycogen storage disorders (GSD)-II, III, VI, and IX. Glc4 is therefore, frequently used in clinical settings to aid GSD diagnosis. Since glycogen is a polysaccharide, the influence of the polysaccharide-rich diet on Glc4 excretion was speculated. However, no clear relationship has been found between polysaccharide ingestion and Glc4 excretion so far. Recently, a patient at our hospital presented with extremely elevated urinary Glc4, without GSD. He received fructose-oligosaccharides (FOS)-rich formula at the time. As many patients receive formula, we investigated the relationship between different formulas and Glc4 excretion, and the possible impact on results interpretation.

Methods: Glc4 measurements (n=973) were performed using UPLC-MS/MS (negative ESI mode in MRM). Briefly, 10 µL of patient urine, internal standard, diluted urine pool, and 70 µL of 55% ACN were vortexed together, and the supernatant was transferred to a 96-well plate. Five µL injection volume was used. Absolute quantification was performed. Patient demographic, clinical, genetic, and dietary information was collected retrospectively. Only individuals without genetic underlying causes and with clear dietary information were included (n=200). GSD patients were used as positive controls. Glc4 excretion was investigated before and after ingesting formula. Glc4 content was determined in the formulas containing FOS, galactose-oligosaccharides (GOS), or maltodextrin.

Results: A thorough investigation of historic Glc4 measurements revealed significantly increased Glc4 excretion in individuals receiving formula containing FOS/GOS. The Glc4 excretion under FOS/GOS was comparable to the Glc4 excretion in GSD individuals, hence, resulting in false positives. Glc4 excretion normalized in non-GSD patients after stopping FOS/GOS supplementation.

Conclusion: For the first time, we show a relationship between FOS/GOS-containing formula and Glc4 excretion. The dietary status is therefore crucial when interpreting the Glc4 results and considering GSD diagnosis, as these can lead to false positive results.



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SESSION 5. QUANTITATIVE AND STATISTICAL METHODS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

5.1 KEYNOTE **10:15 a.m. – 10:45 a.m.**	**520 Overlooked Challenges in Quantitative Metabolomics**

PRESENTING AUTHOR: ★ ***Tao Huan, University of British Columbia, Canada***

Metabolomics has emerged as an indispensable technology with wide-ranging applications in biological and clinical research. However, despite its great potential, several challenges continue to hinder its broader implementation. This talk will highlight critical stages in the metabolomics pipeline that are particularly susceptible to errors and misinterpretations. Key areas of focus include sample preparation, data acquisition, data processing, statistical analysis, and biological interpretation. Additionally, it will introduce analytical and bioinformatics tools developed by the Huan lab at the University of British Columbia in Canada to address these challenges and improve the accuracy and reliability of mass spectrometry-based quantitative metabolomics in biological and environmental applications.

5.2 10:45 a.m. – 11:05 a.m.	**400 Enhancing biomarker discovery in metabolomics through cooperative learning-based vertical data integration**

PRESENTING AUTHOR: ★ ***Elfried B. M. Salanon, INRAE, France***

CO-AUTHORS: ***Elfried B. M. Salanon, Etienne Jules, Blandine Comte, Julien Boccard, Estelle Pujos-Guillot***

INTRODUCTION AND AIMS: Integrating metabolomic data from multiple studies/cohorts (vertical integration) offers a powerful strategy to enhance statistical power and identify robust biomarkers. However, challenges associated with batch effects and dataset heterogeneity hinder results reproducibility and generalizability. Limitations were reported in existing vertical integration approaches: early fusion (at the extracted feature level) strategies struggle with inter-study variability, while late fusion (at the model output level) may overlook crucial inter-dataset relationships. Here, we propose a novel cooperative learning framework for vertical data integration in metabolomics, designed to improve biomarker discovery by balancing integration across datasets, while mitigating study-specific confounders.

METHODS: The proposed cooperative learning approach consists in leveraging multivariate and univariate analysis, alongside dataset-specific adjustment factors and an optimized loss function. To improve robustness, early-stage integration based on a multiblock method, MINT-PLS-DA, was combined with a generalized propensity score built on principal components controlled for confounding effects. Biomarker selection was refined using univariate analysis. Validation was performed by: 1-stability assessment using bootstrap resampling to ensure consistency across different sub-datasets, 2-benchmarking with traditional early and late fusion methods. The approach was tested on synthetic data before being applied to two existing untargeted LC/MS datasets (n=168 & 120 subjects) from human cohort studies on metabolic syndrome to illustrate the proposed framework value.

RESULTS: While early fusion was strongly affected by batch effects, late fusion led to a lack of convergence in the candidate biomarkers. The proposed framework outperformed traditional integration approaches, with a mean decrease in error rate of almost 7%, indicating an improved estimate of the outcome. Those results showed the ability of the proposed approach to capture relevant signatures of different phenotypes.

CONCLUSION:

The developed strategy based on cooperative learning leverages the complementary strengths of early and late fusion approaches, improving metabolomics data integration to enhance the reliability of biomarker discovery.

SESSION 5. QUANTITATIVE AND STATISTICAL METHODS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

5.3 11:05 a.m. – 11:25 a.m. **427 Towards enhanced reproducibility and performance of quality control sample-based normalization: key issues and new ideas**

PRESENTING AUTHOR: ***Matthijs Vynck, Ghent University, Belgium***

CO-AUTHORS: ***Matthijs Vynck, Pablo Vangeenderhuysen, Ellen De Paepe, Lieselot Hemeryck, Vera Plekhova, Lynn Vanhaecke***

Introduction: As the field of metabolomics increasingly shifts towards the analysis of large sample series, maintaining reproducibility has become both more critical and more challenging. In both targeted and untargeted large-scale experiments, it is now well-established that including quality control (QC) samples interspersed between biological samples is important for studying and mitigating technical variation. The latter can be achieved by proper (QC sample-based) data normalization.

While several popular normalization strategies are available in various software packages, the variety in implementations, normalization performance, and reporting pose threats.

Aim: To conduct a holistic evaluation of data normalization using QC samples, and subsequently (i) introduce novel, expectedly more robust normalization strategies, (ii) benchmark them against current approaches, and (iii) provide guidelines towards improved reproducibility and data quality post-normalization.

Methods: Through literature and code review, we investigate key reporting and reproducibility challenges. Further, we qualitatively and quantitatively study the impact of normalization strategies. This is done through benchmarking of existing QC sample-based normalization approaches using (i) in silico simulations where we vary signal drift severity and outlier frequency, (ii) empirical datasets, i.e., human saliva, plasma and urine metabolomics studies with 618, 1002 and 396 samples respectively. We introduce and similarly benchmark three novel normalization approaches.

Results:

We observe major differences in reporting quality and software implementations, posing significant reproducibility issues. Further, common evaluation strategies for post-normalization data quality aren't always informative, and alternatives are proposed and evaluated. We show that cross-validation does not always prevent overfitting and introduce three novel approaches less prone to overfitting and outliers, both in simulation studies and on empirical data, e.g., up to 50-90% reduction in mean squared error and between-replicate variance.

Conclusion: We highlight key issues concerning data normalization, laying the foundation for improved reporting and reproducibility. Enhanced data normalization strategies will support information extraction and strengthen interpretation.

5.4 11:25 a.m. – 11:45 a.m. **69 A Bayesian Framework for Integrating Multiple Variable Selection Methods in Metabolomics: A Case study on pneumonia-biomarker detection**

PRESENTING AUTHOR: ***Ioanna Barla, National & Kapodistrian Univ. Of Athens, Greece***

CO-AUTHORS: ***Ioanna Barla, Ioannis Papoutsidakis, Aristides Eliopoulos, Nikolaos Thomaidis, Evangelos Gikas***

The discovery of reliable biomarkers remains a challenge in metabolomics, as different statistical methods, due to their underlying principles, often identify different biomarkers or assign varying statistical significance to the same metabolite. A common strategy is to combine results from univariate (e.g., t-tests, ROC curve analysis) and multivariate (e.g., PLS-DA, HCA) methods, selecting only the overlapping metabolites as important. However, this approach is largely empirical and lacks a quantifiable measure of reliability.

Does a metabolite with a low p-value and high VIP have a higher probability of being a true biomarker compared to another with low p-value and moderate VIP? This question remains unanswered.

To address this, we propose a Bayesian framework that mathematically quantifies how the probability of a metabolite being truly important increases as more independent selection methods confirm its relevance. We integrate four feature selection methods, each designed to identify all relevant variables while minimizing overfitting:

•BORUTA: Identifies all relevant variables using a random forest-based shadow feature approach.

•MUVR: Multi-validation recursive feature elimination optimizing predictive performance.

•VSURF: Random forest-based ranking of variable importance while filtering noise.

•VIAVC: Accounts for multicollinearity, ensuring the selection of the most meaningful variables.

These methods applied independently to an untargeted metabolomics dataset comparing patients of pneumonia and healthy controls, extracting a consensus biomarker set from each method. Our Bayesian approach then:

1.Integrated the results from the four feature selection techniques.

2.Computed probabilistic scores for each metabolite, reflecting its reliability across the different methods.

3.Evaluated how statistical confidence increases as additional methods confirm the relevance of a metabolite.

By assigning probabilistic confidence scores, our framework ensured the selection of statistically robust metabolites, reducing false positives. This is a crucial step, as it prioritizes high-confidence biomarkers for further validation, saving time and resources in downstream experimental studies.

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SESSION 5. QUANTITATIVE AND STATISTICAL METHODS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

5.5 11:45 a.m. – 12:00 p.m. | **414** | **GcDUO: A Batch Processing Framework Integrating PARAFAC and PARAFAC2 for GC×GC-MS Data Analysis**

PRESENTING AUTHOR: ★ ***Maria Llambrich, Fun. Ins. Inv. San. Pere Virgili (IISPV), Spain***

CO-AUTHORS: ***Maria Llambrich, Frans M. van der Kloet, Lluc Sementé, Anaïs Rodrigues, Saer Samanipour, Pierre-Hugues Stefanuto, Johan A. Westerhuis, Raquel Cumeras, Jesús Brezmes***

Multidimensional chromatography-mass spectrometry (MS) is a powerful analytical technique that integrates two or more chromatographic separations with MS, offering superior resolution, increased signal-to-noise, and selectivity for complex sample analysis. Despite its potential, its adoption remains limited in fields such as metabolomics, environmental analysis, and food science due to data complexity and processing challenges. Chemometric approaches, particularly multiway models like Parallel Factor Analysis (PARAFAC), have proven effective in addressing these challenges by enabling the extraction of meaningful chemical information from multi-dimensional datasets. However, traditional PARAFAC is constrained by its assumption of data tri-linearity, which may not be valid in all cases, where data have misalignments. To overcome these limitations, we present GcDUO, an open-source data processing software that enables annotation, deconvolution, and analysis of batch GC×GC-MS data (Llambrich et al., Briefings in Bioinformatics 2025, doi: 10.1093/bib/bbaf080, https://github.com/mariallr/GcDuo). GcDUO, implemented in R, accepts non-vendor-specific standardized CDF files, and rearranges the data into four-dimensional tensor structures, preserving the GC×GC-MS data structure in every dataset. GcDUO integrates advanced chemometric methods, including PARAFAC and PARAFAC2, for a more accurate and comprehensive analysis. PARAFAC is particularly useful for deconvoluting overlapping peaks and extracting pure chemical signals, while PARAFAC2 relaxes de tri-linearity constraint, allowing the batch analysis for samples. By applying PARAFAC first for deconvolution at the individual sample level and then PARAFAC2 for batch-wide alignment and integration, GcDUO achieves both high-resolution peak detection and robust quantification across complex GC×GC-MS datasets. This sequential approach ensures that the software maintains accuracy while handling real-world experimental variations effectively. GcDUO was validated against the gold-standard software for comprehensive GC, demonstrating a high correlation (R^2 = 0.9) in peak area measurements, confirming its effectiveness and reliability. GcDUO provides a valuable, open-source platform for researchers in metabolomics and related fields, enabling more accessible and customizable GC×GC-MS data analysis.



SESSION 6. NATURAL PRODUCT DISCOVERY FROM PLANTS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

6.1 KEYNOTE 10:15 a.m. – 10:45 a.m.	543 Integrated Multi-omics Approaches for Discovering Plant Biosynthetic Pathways

PRESENTING AUTHOR: ***Jing-Ke Weng, Northeastern University, United States***

Elucidating plant biosynthetic pathways is central to unlocking nature's chemical repertoire and advancing a sustainable bioeconomy. In this talk, I will present an integrated multi-omics framework for discovering biosynthetic pathways in plants, drawing from recent advances in genomics, transcriptomics, metabolomics, and emerging AI-powered tools. While vast amounts of omics data have been generated, much of it remains underutilized due to challenges in data integration and interpretation. I will highlight computational strategies—including molecular networking, reaction pair analysis, and co-expression profiling—that bridge the gap between data and discovery. Emphasis will be placed on how these approaches can be combined into a cohesive workflow to accelerate the identification of gene clusters and enzymes underlying specialized metabolism. Case studies from recent work on diverse medicinal plants will illustrate the power and versatility of these methods in uncovering previously inaccessible pathways and natural products. Together, these advances point toward a future where plant biosynthetic potential can be systematically mined and engineered for pharmaceutical and agricultural innovation.

6.2 10:45 a.m. – 11:05 a.m.	402 Charting the chemical space of South African medicinal plants with computational metabolomics

PRESENTING AUTHOR: ***Fidele Tugizimana, University Of Johanesberg & Omnia Group Ltd, South Africa***

South Africa is a treasure trove of a wide range of medicinal plants. The latter still represent a resource that can be further explored for potential "hit" compounds with significant biological activity, i.e., drug leads. These hit compounds are typically found in biochemically complex extracts, and their identification can be equivalent to searching for a "needle in a haystack". Thus, reported herein are computational metabolomics studies to comprehensively characterize chemical landscapes of different medicinal plants occurring in South Africa, particularly Momordica, Artemisia and Cannabis species. Mass spectrometry-based computational strategies in GNPS such as molecular networking methods and related workflows (MS2LDA, network annotation propagation and MolNetEnhancer) were used to mine and evaluate the methanolic extracts of these plants. The results revealed, for instance, that different Momordica species (M. cardiospermoides, M. balsamina, M. charantia and M. foetida) are phytochemically rich plants consisting of many biologically active metabolites differentially distributed within the different species, with the metabolome of M. cardiospermoides dereplicated for the first time. Furthermore, findings from these studies highlight distinctive chemical profiles beyond cannabinoids in Cannabis strains. This novel mapping of the metabolomic landscape of Cannabis provides actionable insights into plant biochemistry and justifies selecting certain varieties for medicinal use. Decoding the chemical space of three Artemisia plants, viz: A. afra, A. annua, and A. absinthium revealed their differentially metabolomic charts comprising alkaloids, carboxylic acids, phenolics, fatty acyls, flavonoids, and terpenes. Thus, work reported here in, computational metabolomics studies, provided actionable insights into the 'dark matter' of the phytochemical maps of these medicinal plants, highlighting also compounds with pharmaceutical potential.

6.3 11:05 a.m. – 11:25 a.m.	218 Feature-Based Molecular Networking Guided Discovery and Isolation of Novel Capsaicinoids from the chili pepper (Capsicum sp.) and their TRPV1 activation

PRESENTING AUTHOR: ***Joshua Smith, Iocb, Czech Republic***

CO-AUTHORS: ***Joshua Smith, Vendula Stillerová, Martin Dračinský, Martin Popr, Hannah Gaustad, Quentin Lorenzi, Helena Smrčková, Jakob Reinhardt, Marjorie Lienard, Pavel Šácha, Tomáš Pluskal***

Chilis are known for their pain-causing molecule, capsaicin, which agonizes the membrane protein transient receptor potential cation channel subfamily V member 1 (TRPV1). However, molecules with similar structures to capsaicin, called capsaicinoids, are also found in chilis and activate TRPV1. Most capsaicinoid studies rely on targeted analysis of capsaicin and one other capsaicinoid, dihydrocapsaicin. Due to previous studies restricting their analysis to only a couple compounds, we speculated that a more potent agonist may exist amongst the spicier varieties of chilies. Therefore, to answer this question, we analyzed 40 different chili varieties using high-resolution tandem mass spectrometry and applied an untargeted metabolomics pipeline. Our pipeline leverages feature-based molecular networking, SIRIUS compound prediction and data-mining compound dereplication, which led us to the isolation of 18 capsaicinoids, with 5 novel capsaicin molecular structures. Of these 18 compounds, 16 were confirmed with NMR and the other two with MS/MS in combination with NMR. We tested the isolated compounds, along with eight other related analogs, on a TRPV1 calcium influx activation assay. Our findings revealed that the slightest deviation from the capsaicin structure reduces potency to TRPV1. Additionally, we found that capsaicinoids additively agonize TRPV1 and non-activating analogs act as positive modulators. Ultimately, our pipeline highlights how untargeted metabolomics can be utilized in the discovery of novel, biologically relevant molecules.

★ AWARD WINNERS

SESSION 6. NATURAL PRODUCT DISCOVERY FROM PLANTS	Tuesday, June 24	10:15 a.m. – 12:00 p.m.

6.4 11:25 a.m. – 11:45 a.m.	405 Integrating Spatial Mapping and Metabolomics: A Novel Platform for Bioactive Discovery and Saline Land Reclamation

PRESENTING AUTHOR: ***Rossarin Tansawat, Chulalongkorn University, Thailand***

CO-AUTHORS: ***Rossarin Tansawat, Tushar Andriyas, Pichaya Pongchaidacha, Arashaporn Uthairangsee, Nisa Leksungnoen***

Saline land poses a significant environmental and agricultural challenge, as high soil salinity adversely affects plant growth by disrupting water uptake and causing ionic imbalances. This hostile environment limits the cultivation of conventional crops, leading to reduced agricultural productivity. However, certain endemic plant species have adapted to thrive in saline soils, offering a unique reservoir of bioactive compounds. Despite their potential, these species remain underexplored, highlighting the need for innovative approaches to unlock their ecological and pharmaceutical value while addressing saline land restoration. This study proposes a novel platform that integrates species distribution modeling (SDM) and advanced metabolomic analysis to screen for bioactive secondary metabolites, using Buchanania siamensis, a rare native species, as a case study. An ensemble SDM model incorporating environmental and soil parameters identified salinity as a critical factor influencing the distribution of this species. Leaf samples were collected from naturally growing trees based on spatial mapping results in both saline (SS) and non-saline (NS) locations. LC-QTOF metabolomic analysis annotated a total of 1,106 metabolites across the leaf samples, with 175 found to be significantly different between the groups. Among them, 108 metabolites exhibited higher abundance in the SS group. Additionally, antioxidant assays, including DPPH, FRAP, and Total Phenolic Content (TPC) tests, were conducted. Data were further analyzed using O-PLSR models to identify key metabolites most relevant to antioxidant properties. The results indicated that Afzelin was the key metabolite responsible for the antioxidant properties of B. siamensis, with significantly higher levels in SS compared to NS location ($p < 0.05$), as determined by peak area. By leveraging this multidisciplinary approach, we establish a novel framework that advances both bioactive compound discovery and saline land reclamation, demonstrating dual environmental and pharmaceutical benefits. This integrative platform holds significant potential for applications in the pharmaceutical industry, particularly in drug discovery initiatives.

6.5 11:45 a.m. – 12:00 p.m.	283 Integrating omics for untargeted discovery of plant specialized metabolites

PRESENTING AUTHOR: ★ ***Elena Del Pup, Wageningen University & Research, Netherlands***

CO-AUTHORS: ***Elena Del Pup, Hannah E. Augustijn, Kumar Saurabh Singh, Justin J.J. van der Hooft, Marnix H. Medema***

Plant specialized metabolism represents nature's most extensive library of bioactive compounds. However, the limited structural and functional elucidation of plant specialized metabolites remains a major bottleneck in harnessing these compounds as pharmaceuticals, agrochemicals, and bio-based products. Untargeted metabolomics alone struggles to resolve the complexity of plant chemical and functional diversity. Thus, integrative approaches can aid metabolic network reconstruction and structural annotation by incorporating biosynthetic, regulatory, and genomic signatures.

Here, we present a workflow that leverages functional annotations from biosynthetic pathways and multi-omics data to prioritize and elucidate plant specialized metabolites from untargeted metabolomics datasets. To achieve this, we rely on gene clustering, gene co-expression, and transcriptional co-regulation to predict functional associations between genes and integrate them with metabolomics datasets via predicted bio-transformations and mass signatures.

We highlight the value of publicly available datasets to annotate molecular networks by relying on a manually curated set of plant specialized pathways from the literature, public transcriptomics datasets, and plant motif databases. Functional biosynthetic gene clusters are identified across 368 plant species with plantiSMASH including co-occurring regulatory binding sites for their prioritization. Linking transcriptomics and metabolomic datasets in tomato via MEANtools, we identified key steps in the alfa-tomatine pathway and the hydroxycinnamic acid amide (HCAA) pathway, candidate enzymes, and intermediates. Additionally, cross-species analysis pinpoints conserved functional associations between genes to support the annotation of molecular networks in related plant species.

These findings demonstrate that integrating functional and biosynthetic signatures with untargeted metabolomics can help illuminate metabolomic 'dark matter' and prioritize gene-metabolite pairs for further experimental validation. Using publicly available datasets to extract functional insights supports further collaborative efforts for natural product discovery in plant multi-omics research. By aiding the interpretation of the untargeted plant metabolome, our workflow will accelerate natural product discovery and facilitate plant metabolic engineering and targeted plant breeding.



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SESSION 7. DATA REPOSITORIES AND CURATION	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

7.1 1:30 p.m. – 1:50 p.m. **121 Three Years of MDCC Efforts in Korea MetAbolomics rePository (KMAP): Advancing Metabolomics Data Curation and Global Harmonization**

PRESENTING AUTHOR: ★ ***Woori Chae, Seoul National University, South Korea***

CO-AUTHORS: ***Woori Chae, Joo-Youn Cho***

*This presentation is given on behalf of the Metabolomics Data Curation Center (MDCC).

Ensuring FAIR (Findable, Accessible, Interoperable, and Reusable) metabolomics data is essential for advancing research across diverse fields. The MDCC has been leading efforts in Korea to establish quality control (QC) guidelines and enhance data curation since July 2022. While the Korea Bioinformation Center (KOBIC) develops the infrastructure for the Korea MetAbolomics rePository (KMAP), MDCC focuses on data curation, functional enhancement, and quality management. This presentation highlights the achievements of MDCC and KMAP over the past three years and discusses its future expansion as a globally harmonized data repository.

To ensure high-quality metabolomics data, MDCC has developed 16 QC guidelines, covering sample preparation, instrumental analysis, and data processing. Additionally, MDCC has trained and deployed data curators who review submitted data and ensure specialized data collection in KMAP. To facilitate the consistency and integrity of datasets, a curation manual for the curators was also developed. As part of international harmonization efforts, MDCC has introduced Universal Spectrum Identifiers (USI) for mass spectrometry data in KMAP, enabling seamless integration with external databases. Multiple APIs are under development to enhance accessibility and data retrieval for global researchers.

Over the next two years (2025-2026), MDCC will refine QC indices, enhance existing guidelines, and validate them across diverse metabolomics applications. Additionally, MDCC plans to generate data to develop a reference metabolomics profile of healthy Koreans, contributing to standardized datasets for biomedical and clinical research. MDCC also intends to expand collaborations with initiatives such as the Pan-ReDU project to promote global metadata standardization while continuing data curator training to strengthen expertise and ensure sustainable data quality. Ultimately, MDCC strives to position KMAP as a FAIR-compliant, internationally recognized metabolomics repository, setting new benchmarks for data management and scientific applications.

7.2 1:50 p.m. – 2:05 p.m. **311 MSnLib: High-Throughput Library Generation of 60,000 Compounds in 33 Days**

PRESENTING AUTHOR: ★ ***Corinna Brungs, University of Vienna, Austria***

CO-AUTHORS: ***Corinna Brungs, Robin Schmid, Steffen Heuckeroth, Tomáš Pluskal***

The discovery of novel natural products is mainly driven by untargeted high-resolution mass spectrometry (HRMS) analysis, with compound annotation remaining a major bottleneck. So far, MS2 fragmentation and spectral library matching are the standard for confident annotation. However, multi-stage fragmentation (MSn) provides more insights into structural information. Considering GNPS was the largest open spectral library with ~500,000 spectra and ~25,000 unique structures, the coverage of the chemical space remains sparse. Another limitation is the unknown source of and processing applied to library entries. We developed a high-throughput flow injection-MSn method and algorithms within mzmine for fully automatic MSn library generation, following the FAIR principles.

We developed Python scripts for metadata curation and structure standardization, automating the planning of compound mixing and experiments. The dual-pump flow injection method required 3 min per sample, achieving a 1.5 min rectangular shape elution profile and fast washout. This was enough time to acquire MS2-5 trees for multiple ions of 10 mixed compounds at three fragmentation energies on the Orbitrap ID-X. The automatic library generation workflow within the open-source project mzmine combines spectral processing, quality assessment, and compound annotation based on the curated metadata.

The automatic workflow enabled the analysis of 60,000 reference compounds in 33 days in both ionization modes, totaling 13,496 injections. After automated metadata curation, around 87% of all compounds were annotated by the mzmine workflow, yielding 3,975,562 MSn library spectra with ≥2 signals. The high coverage was only possible by combining both ionization modes and annotating multiple ion adducts and in-source fragments. The spectral qualities were high, with an average of 18 signals above the noise level across all MSn. The data processing took ~0.5 s per sample, including optional spectral cleanup, merging, and quality scoring. This unique MSn resource already accelerated the development of annotation tools and machine learning-based methods.

SESSION 7. DATA REPOSITORIES AND CURATION	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

7.3 2:05 p.m. – 2:25 p.m. **330 Lessons from Mass Spectral Library Integration: Addressing Metadata Gaps and Expanding Chemodiversity**

PRESENTING AUTHOR: ***Guillaume Marti, Metabohub, France***

CO-AUTHORS: ***Guillaume Marti, Dablanc Axel, Chassagne François, Fraisier-Vannier Ophélie, Cabanac Guillaume***

Open mass spectral libraries (OMSLs) are indispensable for metabolite annotation and advancing machine learning applications in metabolomics, especially given the rising volume of untargeted metabolomic studies and the development of annotation pipelines. However, their applicability to broader untargeted workflows is significantly hindered by thematic limitations (e.g., natural products, lipids) and poor standardization — inconsistent file formats, heterogeneous metadata fields, or incomplete ontologies. To address these challenges, we developed FragHub, a computational workflow that integrates diverse OMSLs into a harmonized, comprehensive database [1]. By reconciling metadata discrepancies and supporting multiple data formats (e.g., .MSP, .MGF, JSON), FragHub enhances interoperability and annotation pipelines. The latest iteration of FragHub (https://zenodo.org/records/14761050) consolidates 1.5 million spectra representing 220,000 unique compounds (InChIKeys), enabling large-scale analysis of spectral library records.

Our work revealed critical gaps in metadata standardization, particularly in collision energy reporting, which impair spectral reproducibility and interoperability. Furthermore, disparities in chemical space coverage emerged when comparing OMSLs to reference catalogs [2] such as HMDB, the Dictionary of Natural Products®, and COCONUT. Experimental libraries overrepresent chemical classes like alkaloids, while underrepresenting scaffolds such as terpenoids and polyketides prevalent in natural product databases. These findings highlight opportunities to prioritize the expansion of OMSLs by targeting gaps between experimental MS/MS data and chemical catalogs.

By mapping these trends, FragHub advances spectral resource utility and provides actionable strategies to address chemodiversity biases in metabolomics. This work underscores the need for collaborative efforts to harmonize spectral metadata and expand OMSL coverage to underrepresented chemical classes, particularly those with ecological or pharmacological relevance.

1. Dablanc, A.; et al. FragHub: A Mass Spectral Library Data Integration Workflow. Anal. Chem.2024, acs.analchem.4c02219.

2. Chassagne, F. et al. The Landscape of Natural Product Diversity and Their Pharmacological Relevance from a Focus on the Dictionary of Natural Products®. Phytochem. Rev. 2019, 18(3), 601–622.

7.4 2:25 p.m. – 2:40 p.m. **177 Expanding Reference Data-Driven Metabolomics: A Framework for Enhanced Untargeted Metabolomics Annotation and Interpretability**

PRESENTING AUTHOR: ***Alejandro Mendoza Cantu, University Of Antwerp, Belgium***

CO-AUTHORS: ***Alejandro Mendoza Cantu, Julia Gauglitz, Wout Bittremieux, Pieter Dorrestein, Julius Agongo, Vincent Charron-Lamoureux, Harsha Gouda***

Untargeted tandem mass spectrometry (MS/MS) metabolomics often faces low annotation rates, limiting biological insight. Reference data-driven (RDD) metabolomics addresses this challenge by matching MS/MS spectra to a metadata-enriched reference database rather than relying solely on spectral libraries. This approach boosts annotation rates by 5.1±3.3-fold over traditional methods while leveraging curated metadata to enhance interpretability.

RDD metabolomics was initially validated using the Global Foodomics Project (GFOP), one of the largest foodomics datasets encompassing 3,579 foods and 107,968 unique spectra, enabling compositional insights across dietary groups.

We have expanded RDD metabolomics by introducing a computational framework to automate matching and visualization. It includes a Python package and user-friendly web platform for metadata-driven spectral matching and interactive visualization (e.g., principal component analysis and Sankey diagrams). Advanced users can access its functionalities via the Python package, while the web-based application accommodates researchers with minimal programming experience, ensuring that a broad scientific community can benefit from the platform.

Additionally, we demonstrate a generalized RDD approach beyond foodomics by adopting microbial MS/MS reference data from microbeMASST, one of the largest available MS/MS microbial databases. Using this reference data, we extend RDD to the microbiome domain, successfully interpreting spectra from 96 American Gut Project samples. This approach allowed us to identify matches to known gut microorganisms, demonstrating its potential to improve microbial metabolite characterization and microbiome analysis.

By providing a flexible and user-friendly RDD framework, this research enables broader adoption of metadata-driven metabolomics for comprehensive MS/MS interpretation. The ability to match against known gut microorganisms demonstrates its general performance in interpreting MS/MS data compared to reference datasets and presents a promising new approach for microbiome research.

SESSION 7. DATA REPOSITORIES AND CURATION	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

7.5 2:40 p.m. – 3:00 p.m. **359 IntegMET: An integrated database for knowledge discovery through differential expression re-analysis of existing databases.**

PRESENTING AUTHOR: ***Kozo Nishida, Riken, Japan***

CO-AUTHORS: ***Kozo Nishida, Mikiko Takahashi, Rira Matsuta, Hiroyuki Yamamoto, Shin Kawano, Takaki Oka, Hiroshi Tsugawa, Eisuke Hayakawa***

"The amount of publicly available metabolomics data is constantly increasing, with datasets covering a wide range of research areas being stored and shared in databases and repositories. However, data mining of these databases has not progressed effectively, especially in terms of integration and comparative analysis across heterogeneous studies. This is mainly due to the fact that mass spectrometry datasets from different studies cannot be directly compared due to differences in analytical settings.
To address these challenges, we are developing a comprehensive data integration and re-analysis platform to enable cross-study metabolomics data mining. We used a data integration method called iDMET (Matsuta et al., BMC Bioinformatics, 23, 20–22), which quantifies the similarity in metabolic changes between different metabolomics study results. By using this approach, we constructed a large-scale network database incorporating data from two major metabolomics databases, MetaboLights and Metabolomics Workbench. Our platform combines network-based analysis methods with automated data standardization and metadata curation to facilitate the seamless integration of heterogeneous metabolomics datasets.
In this presentation, we demonstrate our comprehensive approach to metabolomics data integration. First, we used an LLM-assisted workflow to process studies from public repositories. This workflow automatically organizes metabolite and sample information and assigns standardized metadata using identifiers and ontologies such as InChIKey, Gene Ontology, PubMed, and MeSH. Based on these processed data, we construct a metabolic change network in which nodes represent studies and edges indicate similarities in metabolic patterns. The metabolic change network, based on the iDMET approach, allows researchers to discover studies with similar metabolic responses, while the metadata network reveals hidden relationships between different research fields.
To make this integrated network-based database accessible to the metabolomics community, we are developing a web-based analysis interface and plan to provide embeddings that will enable researchers to explore and use these comprehensive metabolomics resources."



SESSION 8. MASS SPECTROMETRY IMAGING	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

8.1 1:30 p.m. – 1:50 p.m. **167 Dysregulated sphingolipid metabolism in liver fibrosis: insights from mass spectrometry imaging**

PRESENTING AUTHOR: ***Zoe Hall, Imperial College London, United Kingdom***

CO-AUTHORS: ***Zoe Hall, Aleksandra Gruevska, Fiona Oakley, Jack Leslie, Niloufar Safinia, Elena Perpinan, Hannah Maude, Ines Cebola, Joram Posma, Matthew Hoare, Mark Thursz***

During steatotic liver disease (SLD), lipid deposition, inflammation and fibrosis progress heterogeneously across the hepatic tissue. It is therefore crucial to employ approaches that retain spatial information, such as mass spectrometry imaging (MSI). The aim of this study was to characterize the metabolic microenvironment across fibrotic liver, and to establish a link between key lipids and different cell populations within liver fibrosis. We analysed liver tissue from patients with SLD-cirrhosis and 3 different murine models of fibrosis (bile duct ligation, chronic CCl4 treatment and western diet + CCl4). We performed spatial lipidomics using matrix-assisted laser desorption ionisation (MALDI) MSI, bulk transcriptomics, imaging mass cytometry and analysis of published spatial and single-cell RNA sequencing data to identify potential cell type-specific mechanisms of lipid dysregulation in hepatic fibrosis. To explore whether manipulating lipid metabolism could serve as a potential anti-fibrotic strategy, we targeted sphingolipid metabolic pathways in activated hepatic stellate cells (HSCs) and stimulated human precision cut liver slices (hPCLSs). Both in human and murine liver tissue, the spatial distribution of sphingolipids, particularly sphingomyelin, was significantly enriched in the fibrotic tissue. Through the integration of the different multi-omic methodologies and spatial analysis, our research revealed that disrupted sphingolipid metabolism in non-parenchymal cells is linked to fibrosis. Furthermore, treatment with inhibitors targeting sphingolipid metabolism demonstrated anti-fibrotic effects in activated HSCs and in hPCLS model of fibrosis. Overall, these results have identified key lipid markers for liver fibrosis and suggest that altered sphingolipid metabolism plays a role in driving fibrogenesis. By employing spatial multi-omics, we show that the mechanisms of fibrogenesis involving sphingolipid metabolic pathways are cell type-specific. Furthermore, sphingolipid metabolic pathways can be targeted and modified, offering potential as a therapeutic strategy to combat fibrosis.

8.2 1:50 p.m. – 2:05 p.m. **162 Visualizing the Spatial Landscape of the Brain Endocannabinoidome by MALDI-2-MS Imaging**

PRESENTING AUTHOR: ***Emanuela Salviati, University of Salerno, Italy***

CO-AUTHORS: ***Emanuela Salviati, Eduardo Sommella, Fabiana Piscitelli, Pietro Campiglia***

Endocannabinoids (eCBs) are endogenous lipid messengers that interact with cannabinoid receptors CB1/CB2 and play a critical role in the central nervous system, often altered in neurological disorders. However, spatial mapping of eCBs using Mass Spectrometry Imaging (MSI) is challenging due to their low concentration and tissue-dependent ion suppression. This study addresses these challenges by optimizing and applying a laser-induced post-ionization (MALDI-2) approach. Spatial analysis of eCBs was conducted on coronal mouse brain sections at bregma levels -2.06 mm and -2.92 mm using a timsTOF fleX equipped with MALDI-2. A comparison between MALDI and MALDI-2 was performed by spotting dilution series of authentic and deuterated internal standards (I.S.) of 2-arachidonylglycerol (2-AG) and N-acylethanolamines (AEA, OEA, and PEA) on Wild Type mouse brain slices. Ion transmission parameters and matrices were assessed to improve eCB response. The regio-specific distribution of eCBs in the 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. MALDI-2 boosted the detection of eCBs, including 2-AG, AEA, PEA, OEA, SEA, and LEA, allowing visualization of eCBs at endogenous concentrations, which was not possible with traditional MALDI. MALDI-2 provided higher sensitivity, with a 21-fold improvement in LOD/LOQ gain (MALDI/MALDI-2). Several normalization approaches were evaluated, with isotopically labeled internal standards (I.S.) providing the best results, showing improved pixel-to-pixel variation and uniform distribution, especially for 2-AG and PEA. The study also demonstrated the potential for semi-quantitative analysis by correlating MALDI-2 results with LC-MS/MS data. High spatial resolution (down to 5 µm) enabled detection of all eCBs despite minimal tissue ablation. Finally, the method was applied to a mouse model of mild traumatic brain injury (mTBI), revealing differences in eCB modulation in the Cortex, Hippocampus, and Hypothalamus, providing insights for neuropharmacology.

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SESSION 8. MASS SPECTROMETRY IMAGING	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

8.3 2:05 p.m. – 2:25 p.m.	220 The effect of the solvent and tissue type on Desorption Electrospray Ionization Mass Spectrometry Imaging of metabolites

PRESENTING AUTHOR: ***Marco Giampà, Vib/ku Leuven, Belgium***

CO-AUTHORS: ***Marco Giampà, Emmanuelle Claude, Emry Jones, Jan Claereboudt, Alex Muck, Nina Ravoet, Jakub Idkowiak, Peter Verhaert, Johann Swinnen, Bart Ghesquière***

The application of Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI MSI) is becoming essential for spatial molecular elucidation of tissues, offering more accessible multimodal integration with other imaging techniques due to its minimal sample preparation requirements. However, DESI MSI results depend on unique ionization efficiency of metabolites, ionization suppression, and varying solvent stream-driven extraction efficiency of analytes in heterogenous tissue areas. Understanding these factors is essential for a correct interpretation of imaging results.

In this study, we first evaluated the efficiency of the solvent in overall tissue extraction and ionization of analytes of interest (e.g., metabolites) during the DESI process. A comparative DESI MSI analysis was conducted on cryosections of different tissues from mice (liver, brain, heart and kidney) evaluating 90% acetonitrile (ACN) and 90% methanol (MeOH) in H2O as spray solvents. Total ion count (TIC) profiles, putative annotations, and metabolite abundances were compared in negative ionization mode. The results show similar TIC profiles but differences in TIC profiles of targeted metabolites from glycolysis, TCA cycle and fatty acids. Differences were observed in the total number of putative annotations and metabolite levels, which were higher under 90% ACN conditions. The extent of these differences varies by tissue type, highlighting the need to develop tissue-specific solvent compositions.

The second part of this study involves application of the optimized DESI MSI method to spatial metabolomic analysis of hypoxia-induced kidney damage, in the context of transplantation tissue quality. Damaged areas were identified histologically on H&E stains. Validation of metabolite identifications and quantification are ongoing by LC/MS metabolite analyses (bulk) of differentially laser capture microdissected tissue areas.

We conclude that selection of tissue type-dependent solvent composition is a critical optimization step in DESI MSI to obtain reliable high quality biologically relevant spatially resolved metabolomics data.

8.4 2:25 p.m. – 2:40 p.m.	277 Optimized On-Tissue Chemical Derivatization (OTCD) strategy to enhance detection of carboxylic acids and carbonyls on tissue using MALDI-MSI

PRESENTING AUTHOR: ★ ***Antonia Fecke, Leibniz Institut Für Analytische Wissenschaften, Germany***

CO-AUTHORS: ***Antonia Fecke, Siva Swapna Kasarla, Luiza Martins Nascentes Melo, Alpaslan Tasdogan, Sven Heiles, Albert Sickmann, Karl Smith***

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is a powerful technique capable of mapping spatial distributions of analytes in tissue sections at high lateral resolution. MALDI MSI on high-performance mass spectrometers enables the characterization of molecular changes in healthy and diseased tissues. Despite its growing popularity, MALDI-MSI-based analysis of small molecular weight metabolites still faces challenges, primarily due to low ionization efficiency leading to localization with only modest lateral resolution. On-tissue-chemical derivatization (OTCD) has emerged as a promising strategy to tackle this challenge by enhancing ionization efficiency, sensitivity, and selectivity through chemical modifications.

In this work, we optimized an OTCD workflow for spatial analysis of carbonyl- and carboxyl-containing metabolites in mammalian tissue samples using 4-APEBA (4-(2-((4-bromophenethyl)dimethylammonio)ethoxy)benzenaminium dibromide) in combination with carbodiimide crosslinker EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide). Derivatization conditions such as reagent concentration, solvent composition, and incubation time were optimized to achieve maximum coverage of target metabolites and improved sensitivity. The optimized OTCD MALDI-MSI workflow was applied to map the lateral distribution of key metabolites in kidney and a cancerous tissue model.

Preliminary results indicate a significant improvement in the detection of carbonyl- and carboxyl-containing metabolites on tissue. We observed enhanced sensitivity of low-abundant or low-ionizing metabolites as well as increased metabolic coverage for target analytes in one imaging run. These findings highlight the potential of using OTCD to overcome existing methodological limitations. Our OTCD approach contributes to the field by providing a valuable tool to study metabolic alterations in health and disease with regards to metabolic heterogeneity in tissues, thereby contributing to the understanding of disease mechanisms.

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SESSION 8. MASS SPECTROMETRY IMAGING	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

8.5 2:40 p.m. – 3:00 p.m. **292 Spatial Lipidomics with Cyclic Ion Mobility Mass Spectrometry for Resolving Isotopic, Isomeric, and Isobaric molecules in Hepatocellular Carcinoma Tissue**

PRESENTING AUTHOR: ★ ***Pattipong Wisanpitayakorn, Mahidol University, Thailand***

CO-AUTHORS: ***Pattipong Wisanpitayakorn, Napat Angkathunyakul, Prawat Kositamongkol, Chutwichai Tovikkai, Yongyut Sirivatanauksorn, Sakda Khoomrung***

Desorption electrospray ionization mass spectrometry (DESI-MS) provide spatially resolved molecular information from tissue samples. However, conventional DESI-MS relies solely on the mass-to-charge ratio (m/z) for compound resolution, making it challenging to distinguish isomeric and isobaric lipid species. The absence of chromatographic separation and overlapping spectral signals further complicate lipid annotation, leading to potential misidentifications. To address these challenges, we developed a DESI-MS workflow integrated with cyclic ion mobility-mass spectrometry (cIM-MS) for lipid profiling, enabling precise differentiation of isomeric and isobaric lipids through multi-pass ion mobility separation. This approach effectively resolves isotopic interferences and previously unresolved lipid species while achieving level-2 lipid identification via ion mobility and accurate mass measurements. Furthermore, targeted MS/MS fragmentation within distinct cIM mobility windows enables level-1 lipid annotation against reference standards. We applied this DESI-cIM-MS workflow to the spatial lipidomic analysis of hepatocellular carcinoma tissue sections, revealing distinct lipid distributions between tumor and normal regions. By optimizing separation times, we achieved a balance between sensitivity and ion mobility resolution, enabling high-confidence structural elucidation of complex lipids in clinical samples. Our results highlight the power of multi-pass cIM-MS in spatial lipidomics, providing a robust framework for biomarker discovery and molecular characterization in cancer research.

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SESSION 9. MICROBIAL METABOLOMICS	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

9.1 1:30 p.m. – 1:50 p.m.	190	Bacterial lipidome discovery through combined metabolic incorporation and mass spectrometric approach

PRESENTING AUTHOR: ***Xue Li Guan, NTU, Singapore***

Bacteria exist in diverse environments, from the human body to marine ecosystems, playing essential roles in maintaining ecological balance. These unicellular microbes produce lipids that form protective membranes, which are crucial for cell integrity and defense against harsh conditions. A fascinating yet analytically challenging aspect of bacterial lipids is their remarkable diversity, which varies between species. This diversity is further expanded by the ability of bacteria to utilize environmental substrates and incorporate them directly into lipids. The vast complexity of the bacterial lipidome remains largely uncharacterized due to the limited availability of standards for this diverse and largely unknown spectrum of lipids in nature. To address this challenge, we developed a combinatorial metabolic incorporation and stable isotope labeling approach, coupled with mass spectrometric analysis, for the discovery of novel bacterial lipids. Using this method, we identified two previously unknown phospholipid classes, phosphatidylhomoserine and phosphatidylpropanolamine, in the extensively studied model bacterium Escherichia coli. Further genetic evidence suggests that these lipids are synthesized through the known enzyme phosphatidylserine synthase. Notably, the incorporation of homoserine into phospholipids is influenced by bacterial species and culture conditions. Our findings highlight the substrate promiscuity of bacterial enzymes and demonstrate their ability to incorporate non-canonical substrates, expanding the diversity of lipids in nature. The bacterial lipidome constitutes a vast biochemical reservoir with significant functional potential, warranting further exploration through advanced structural characterization approaches, and investigations into the roles of novel lipids in bacterial physiology and adaptation.

9.2 1:50 p.m. – 2:05 p.m.	176	Integrated metabolomics and metagenomics approaches to understanding fluctuations in gut microbial communities and metabolic activity in jersey cows

PRESENTING AUTHOR: ★ ***Susana Moñino Ramos, SRUC-University of Edinburgh, United Kingdom***

CO-AUTHORS: ***Susana Moñino Ramos, Tessa Moses, Mick Watson, Jolanda van Munster***

The microbiota of the rumen, the first of the four stomach chambers present in ruminal mammals, plays a crucial role in climate change and food security, as microbial activity during plant digestion produces high level of methane emissions. Despite an exemplary ability to digest lignocellulosic material, the specific mechanisms governing microbial digestion in the rumen remain poorly understood mainly due to its high complexity and the substantial variability across diets and individuals. Consequently, longitudinal studies have yet to establish strong links between microbiome composition, microbial activity, and the metabolite profile of the rumen.

To address this gap, we conducted a longitudinal multi-omics study to investigate how individual differences and dietary fluctuations influence microbial communities and metabolite profiles in the rumen. Rumen fluid was collected from rumen-fistulated Jersey cows (n=5) over the course of a year and eight sampling points. The metabolic profile of each animal was determined through untargeted metabolomics, employing zwitterionic-phase hydrophilic interaction chromatography (HILIC-Z) coupled to drift tube ion mobility quadrupole time-of-flight (DTIMqTOF) mass spectrometry. As part of a proof of concept, we have observed differences in the metabolome composition of individuals and metabolic routes via enriched pathway analysis. These findings will be coupled with microbial profiling using bacterial 16S and fungal ITS/28S amplicon sequencing alongside volatile fatty acid (VFAs) profiles and rumen fluid pH measurements at collection time.

By integrating multiple omics approaches, this study will contribute to the characterisation of the bovine rumen metabolome while enhancing our understanding of how microbial communities influence individual metabolic activities in cattle. Ultimately, this study aims to facilitate the systematic integration of omics data in the bovine gut system, enabling a more comprehensive analysis of complex biological samples. We ultimately aim to contribute to the understanding of bovine gut physiology, particularly in carbohydrate degradation, towards more sustainable agriculture practices.

SESSION 9. MICROBIAL METABOLOMICS	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

9.3 2:05 p.m. – 2:25 p.m. **279 Metabolic Interplay Between the Honeybee Gut Microbiota and an Agrochemical Library**

PRESENTING AUTHOR: ***Andrew Quinn, University of Lausanne, Switzerland***

CO-AUTHORS: ***Andrew Quinn, Richard Jacoby, Lenard Kreis, Jamille Viray, Michael Zimmermann, Philipp Engel***

Introduction: Commensal bacteria in the animal gut play an indispensable role digesting food and synthesizing beneficial metabolites. However, their extensive metabolic repertoire also extends to the biotransformation of synthetic compounds including drugs, pollutants, and agrochemicals. Pollinator species, such as bees, are particularly exposed to agrochemicals through foraging activity in treated fields. Therefore, investigating the mechanistic interplay between agrochemicals, bees and their microbiota is crucial to understand the full impact of chronic, sub-lethal exposure that is now common across diverse ecosystems. Unfortunately, the vast array of agrochemicals makes comprehensive experiments documenting interactions in bees nearly impossible.

Methods: We utilized a combinatorial pooling strategy to screen 656 agrochemicals for degradation and biotransformation by 20 bacterial isolate species from the Pseudomonadota, Actinomycetota and Bascillota phyla that encompass the species level diversity within the gut of the western honeybee (Apis mellifera). These chemicals were selected from a larger library of 1054 agrochemicals based upon their lack of toxicity to the microbiota, as well as their measurability via high resolution LC-MS using a single extraction and analysis method.

Findings: We identified microbial degradation and resulting biotransformation features in 15% of the agrochemicals that we screened. Biotransformation was particularly prevalent amongst the sulfonylurea and cyclohexanedione oxime herbicides as well as amongst the organothiophosphate insecticides. Well known biotransformation reactions, such as nitroreductase activity, were frequent across our dataset, however we also found numerous instances of unreported degradation and conjugation reactions.

Contribution to the Discipline: Our results indicate that microbial metabolism is a key factor modulating the fate of ingested agrochemicals in bees. They pave the way for future experiments investigating the impact of biotransformation on bee health and physiology. Furthermore, as a consequence of using sequenced isolate strains, our dataset will serve as a valuable tool for annotating xenobiotic metabolism across varied animal gut associated microbial taxa.

9.4 2:25 p.m. – 2:40 p.m. **508 Comparative metabolomics reveals distinct metabolic adaptations in Crabtree-positive and Crabtree-negative yeast species**

PRESENTING AUTHOR: ***Harini Sridharan, Georgia Institute Of Technology, United States***

CO-AUTHORS: ***Harini Sridharan, April Miguez, Mark Styczynski***

The Crabtree effect is an evolutionary paradox: some yeast species, like Saccharomyces cerevisiae, favor fermentation over respiration despite its lower energy yield, highlighting metabolic adaptation to high-glucose environments. Yeast, with its conserved metabolic pathways and ease of genetic manipulation, serves as an ideal model for studying metabolic adaptation. The Crabtree effect serves as a model for metabolic flexibility, demonstrating how organisms rewire energy production under environmental pressures. Understanding this shift provides insights into yeast biology with applications in microbial fermentation, industrial strain improvement, and metabolic disorder research. While transcriptomics and proteomics have provided valuable insights into gene expression and protein regulation, they do not fully capture the dynamic metabolic shifts that define cellular function. Despite its significance in metabolic adaptation, it remains poorly explored through this lens, underscoring the need for metabolomics-driven investigations to uncover its regulatory mechanisms. We use gas chromatography–mass spectrometry to assess how central carbon metabolism gene deletions impact metabolism in Crabtree-positive (S. cerevisiae) and Crabtree-negative (Kluyveromyces lactis) yeasts. We analyzed metabolic shifts during exponential and stationary phases to capture transitions from glucose consumption to resource limitation. Our analysis revealed species-specific differences, including novel regulatory shifts in membrane stability and oxidative stress defenses, linked to sterol and amino acid metabolism, respectively. The deletion of Pyruvate Decarboxylase, a key enzyme that drives fermentation, significantly impaired membrane integrity in K. lactis but uniquely altered oxidative-stress response metabolites such as mannitol and proline in S. cerevisiae. Disruptions at the pyruvate branchpoint expose species-specific vulnerabilities, guiding strain optimization and targeted metabolic interventions. Sterol metabolism influences membrane fluidity and ethanol tolerance, while amino acid metabolism enhances oxidative stress defense, strengthening yeast resilience in bioprocessing. This study highlights the role of metabolomics in characterizing the metabolic rewiring associated with the Crabtree effect in model yeast species.

SESSION 9. MICROBIAL METABOLOMICS	Tuesday, June 24	1:30 p.m. – 3:00 p.m.

9.5 2:40 p.m. – 3:00 p.m. **255 CROSS-FEEDING INTERACTION WITHIN MICROCYSTIS PHYCOSPHERE : New perspectives from the combination of metagenomics, metametabolomics and genome-scale metabolic modeling approaches**

PRESENTING AUTHOR: ***Binta Diémé, Université Clermont Auvergne, INRAE, UNH, PFEM, France***

CO-AUTHORS: ***Juliette Audemard, Mohamed Mouffok, Charlotte Duval, Melissa Eon, Jeanne GOT, Sebastien Halary, Julie Leloup, Marie Lefebvre, Benjamin Marie, Gabriel Markov, Anne Siegel, Clémence Frioux, Nicolas Creusot, Binta Diémé***

In the face of global changes, cyanobacterial blooms are of increasing concern due to their impact on the environment, animal and human health, with potential significant economic consequences. Despite evidence regarding environmental factors that promote such harmful events, there remains a limited understanding of the physiological and ecological mechanisms behind them. However, cross-feeding interactions between cyanobacteria and their microbiome may be involved, facilitating an optimal and symbiotic use of nutrients within the phycosphere.

To elucidate these mechanisms, this study aims to characterize the cross-feeding interactions within the phycosphere of 12 Microcystis strains and their associated heterotrophic bacteria, sampled during a bloom in a lake in France. Metagenomics formed the basis for reconstructing genomes and their associated genome-scale metabolic networks (GSMNs). These are particularly relevant due to their ability to predict metabolic networks and microbial interactions. Complementary, metabolomics was used as a tool to validate these predictions.

Taking advantage of this multi-omics approach, we compared the metabolic capacities (e.g., production of microcystins) of the 12 microbiomes. Although the bacterial taxa associated with the 12 Microcystis strains differ, the metabolic functions carried out by the communities are similar. We highlighted differential metabolite synthesis, both at the modeling and metabolomics levels, between individual strains and communities, suggesting complementary functions between Microcystis and its associated bacteria. Metabolomics revealed discrepancies in the chemical composition of the 12 Microcystis communities. Notably, it showed that some metabolites were detectable only in one or a few strains (e.g., Aeruginosins). While several classes of bioactive peptides were identified (e.g., microcystins, microginins), ongoing investigations aim to expand the annotation to support the validation of metabolism predictions from GSMNs.

Overall, our study demonstrates the relevance of multi-omics approaches and computational models to unravel functional and ecological mechanisms related to the relationship between cyanobacteria and their microbiome that potentially contribute to blooms.

SESSION 10: VENDOR SESSION **DETAILS AVAILABLE ON PAGES 8-12** Click to jump to the start of the section



SESSION 11. PREGNANCY, NEONATAL, AND EARLY LIFE METABOLISM	Tuesday, June 24	3:30 p.m. – 5:10 p.m.

11.1 3:30 p.m. – 3:50 p.m.	381 Longitudinal targeted plasma and urine metabolomics reveals novel inositol isomers patterns from preconception into pregnancy

PRESENTING AUTHOR: ***Jean-Philippe Godin, Nestle Research, Switzerland***

CO-AUTHORS: ***Jean-Philippe Godin, Shiao-Yng Chan, Luca Lavalle, Jose Manuel Ramos-Nieves, Fang Huang, Chiara Nembrini, Irina Monnard, Hsin Fang Chang, Ling Wei Chen, Han Zhang, Jui-Tsung Wong, Heidi Nield, Keith Godfrey, Wayne Cutfield, Irma Silva-Zolezzi, NiPPeR study group***

Myo-inositol plays a crucial role as a bioactive compound during pregnancy and in placental-fetal development. There is limited data regarding longitudinal changes in concentrations of plasma and urinary inositol isomers from preconception and into pregnancy. Also, there are a lack of clinical studies reporting the concentration of myo-inositol and other inositol isomers in plasma and urine after long-term myo-inositol supplementation. Thus, our ability to fully exploit the therapeutic potential of myo-inositol is hampered.

Samples from 585 healthy women recruited preconception from Singapore, New Zealand, and United Kingdom were analyzed; 290 took a standard micronutrient supplement without myo-inositol (control) and 295 received the intervention (NiPPeR mix containing myo-inositol and other micronutrients). Plasma and urine samples were collected at preconception baseline, and with supplementation at preconception, early pregnancy (~7-weeks' gestation) and late pregnancy (~28-weeks). Plasma and urinary inositol isomers (myo-inositol, scyllo-inositol, chiro-inositol, neo-inositol) were quantified by liquid-chromatography-tandem-mass-spectrometry.

This lecture will unveil, for the first time, the distinct longitudinal changes in the concentrations of inositol isomers within a diverse multi-ethnic cohort, spanning from preconception through late pregnancy. We explore sociodemographic and clinical factors linked to isomer concentrations and examine the impact of myo-inositol supplementation on plasma and urinary levels. Our groundbreaking findings indicate isomer-specific regulation of inositol concentrations in plasma and urine during the preconception phase and throughout pregnancy. Additionally, this extensive study brings to light an often-overlooked inositol isomer, scyllo-inositol, emerging as the second most abundant isomer in plasma, which warrants further investigation to understand its biological significance.

11.2 3:50 p.m. – 4:10 p.m.	470 Prenatal organophosphate pesticide exposure and the maternal pregnancy metabolome in the NYU CHES cohort

PRESENTING AUTHOR: ***Haleigh Cavalier, New York University School of Medicine, United States***

CO-AUTHORS: ***Haleigh Cavalier, Akhgar Ghassabian, Sara Long, Susan Sumner, Susan McRitchie, Yu Chen, Kannan Kurunthachalam, Mengling Liu, Leonardo Trasande***

Prior research links prenatal exposure to organophosphate (OP) pesticides to adverse health outcomes via molecular mechanisms such as oxidative stress, neurotransmitter disruption, and mitochondrial dysfunction. This study investigates such mechanisms by estimating relationships between prenatal OP pesticide exposure and the urinary maternal metabolome using data from the New York University Children's Health and Environment Study (NYU CHES) cohort (n=890). Urine samples were collected at three timepoints during pregnancy (T1, T2, T3) and analyzed for six dialkyl phosphate (DAP) metabolites and a set of 188 metabolites related to key biological functions. Associations between DAP concentrations and individual metabolites were estimated using linear and logistic regression models, adjusting for potential confounders. Statistical analysis revealed significant associations between OP exposure and the maternal metabolome, with the most associations observed during late pregnancy (T3). The most robust associations across all models and timepoints were observed for acylcarnitine profiles, which were consistently elevated in association with OP exposure. This study identifies specific metabolic signatures associated with OP pesticide exposure during pregnancy, providing insights into potential mechanisms of toxicity and implications for maternal and child health. Further research is needed to validate these findings and further understand the functional significance of the identified metabolic disturbances.

SESSION 11. PREGNANCY, NEONATAL, AND EARLY LIFE METABOLISM	Tuesday, June 24	3:30 p.m. – 5:10 p.m.

11.3 4:10 p.m. – 4:30 p.m.	339 Metabolomic alterations in cord blood improve the prediction of childhood-onset neurodevelopmental disorders

PRESENTING AUTHOR: *Polina Girchenko, University Of Oulu, Finland*

Background

Neurodevelopmental disorders (NDDs) complicate the lives of 15-20% of children. The fetal cord blood metabolome may provide a valuable opportunity to identify biomarkers that indicate the risk of NDDs already at birth. This study aimed to (1) identify cord blood metabolic measures associated with NDDs and evaluate their ability to enhance risk prediction beyond known early-life risk factors and (2) investigate whether and how these metabolic measures are associated with established early-life risk factors for NDDs, including maternal age, education, substance use during pregnancy, maternal obesity, diabetes, hypertensive disorders, antenatal depression, sex of the child, gestational age, and birth weight.

Methods

Participants were drawn from a pregnancy cohort of 923 children followed from birth until 14–16 years of age. The Nightingale Health Quantification Library NMR metabolomics platform quantified 110 metabolic measures encompassing pathways related to lipids, amino acids (AAs), fatty acids (FAs), glycolysis, ketone bodies, fluid balance, and inflammation. NDD diagnoses were extracted from a national health register.

Results

Elastic net regression identified 12 cord blood metabolic measures collectively associated with NDDs. These measures included metabolites related to HDL synthesis, concentration, and composition, as well as FAs and AAs. These 12 metabolic measures improved the prediction of NDDs beyond early-life risk factors. Additionally, prenatal environmental adversities known to increase the risk of NDDs were found to influence cord blood metabolite levels in a manner suggesting an elevated risk of NDDs.

Conclusions

Cord blood metabolites appear to enhance the prediction of NDD risk in children followed from birth until 14–16 years of age. These metabolic measures have the potential to serve as early biomarkers for NDDs, facilitating timely diagnosis, treatment, and prevention.

11.4 4:30 p.m. – 4:50 p.m.	312 Early life gut microbial and metabolic shifts reflected in stool consistency

PRESENTING AUTHOR: *Santosh Lamichhane, University of Turku, Finland*

CO-AUTHORS: *Anna Katariina Aatsink, Heidi Isokääntä, Matilda Kråkström, Leo Lahti, Minna Lukkarinen, Abhijit Paul, Thomaz Bastiaanssen, Eveliina Munukka, Pieter C. Dorrestein, Hasse Karlsson, Linnea Karlsson, Matej Oresic, Alex M Dickens, Santosh Lamichhane*

Stool consistency serves as a proxy for gut transit time, which is crucial for gut health. However, the relationship between the gut microbiota, metabolome, and stool consistency in infants and young children remains poorly understood. In this study, we analyzed stool microbiota and metabolome data from 618 stool samples of children aged 2.5 months (n = 360), 6 months (n = 229), 14 months (n = 274), and 30 months (n = 169) from the FinnBrain Birth Cohort Study, correlating these with stool water content and parent-reported stool consistency. Our findings revealed that microbiota community composition and the relative abundance of specific genera, such as Clostridium, Hungatella, and Lactobacillus, were associated with stool consistency. Among the metabolites, concentrations of conjugated bile acids, including newly discovered microbial bile acid amidates (conjugated to 22 different amino acids), were linked to constipation and stool water content. Stool water content also reflected the functional potential of microbial communities. Specifically, we found that bile salt hydrolase, primarily involved in the deconjugation of primary bile acids, was negatively associated with stool water content. Furthermore, short-chain fatty acids, particularly acetic acid, were positively associated with stool water content, whereas branched short-chain fatty acids showed a negative association. These findings suggest a shift from bacterial saccharolytic to proteolytic metabolism in the gut with constipation. Overall, our results highlight the importance of objective classification of stool consistency in fecal metabolomics and microbiome research.

SESSION 11. PREGNANCY, NEONATAL, AND EARLY LIFE METABOLISM	Tuesday, June 24	3:30 p.m. – 5:10 p.m.

11.5 4:50 p.m. – 5:10 p.m. **234 Exploring circulating lipoprotein and metabolite profiles associated with gestational diabetes and offspring outcomes in post-bariatric surgery pregnancies**

PRESENTING AUTHOR: *Jia Li, Imperial College London, United Kingdom*

CO-AUTHORS: *Asma Mohamed, Nusrat Kamal, Jihang Wu, Tanya Maric, Chidimma Kanu, Deesha Patel, Kaoutar Abaakil, Xuan Yang, Makrina Savvidou, Jia Li*

Background: Bariatric surgery (BS) is the most effective treatment for morbid obesity and approximately 70% of women receiving BS are of childbearing age, resulting in increased post-BS pregnancies. While BS reduces gestational diabetes mellitus (GDM) risk, it increases the risk of small-for-gestational age infants. Therefore, we aimed to explore the association between maternal circulating metabolic profiles and GDM and birth weight percentiles (BWP) in both post-BS and no-BS pregnancies.

Methods: Serum samples were collected from pregnant women with pre-pregnancy sleeve gastrectomy (SG, n=27) or Roux-en-Y gastric bypass (RYGB, n=25), and from pregnant women without BS (n=71), whose early-pregnancy body-mass-index (BMI) matched with BS groups. 1H Nuclear Magnetic Resonance (NMR) spectroscopy was applied to serum collected at 11-14 weeks of gestation. Lipoprotein and small metabolites were quantified using NMR spectra through in vitro diagnostics research (IVDr) methods.

Results and Discussion: BWP was significantly lower in BS compared to control (control 68.00 vs. SG 17.00, p=0.001; control 60.00 vs. RYGB 26.00, p=0.003). BS showed lower circulating concentrations of amino acids (AAs) and higher concentrations of dimethylsulfone compared to non-BS group. These observations could be due to BS-induced anatomical and physiological changes. Histidine was presented in significantly higher concentrations in women who developed GDM compared to those who did not in the BS group, but this trend was opposite in the control group. Furthermore, circulating AAs, e.g., tyrosine and valine (Pearson's correlation, r=0.45, p<0.005), and very-low-density lipoprotein (VLDL) subfractions (r=0.40, p<0.01) were associated with BWP in the BS group, suggesting that post-BS maternal circulating AAs could play a role in birth weight. In the non-BS group, several high-density lipoproteins (HDL) (r=-0.34, p<0.01) were inversely associated with BWP.

Conclusion: We showed the association between BS-induced circulating metabolic changes and BWP. Further analysis will seek longitudinal changes during pregnancy that are associated with offspring outcomes.

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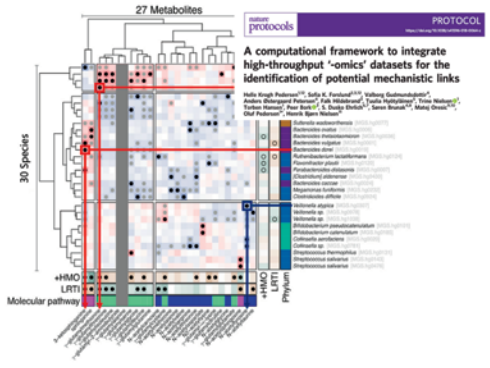
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SESSION 12. AGRICULTURAL AND ANIMAL PRODUCTS	Tuesday, June 24	3:30 p.m. – 5:10 p.m.

12.1 3:30 p.m. – 3:50 p.m.	133 Metabolic Profiling of Bovine Colostrum: Unravelling the Influences of Diet and Seasonality on Functional Dairy Components

PRESENTING AUTHOR: ***Raghunath Pariyani, University College Cork, Ireland, Ireland***

CO-AUTHORS: ***Raghunath Pariyani, Gabriele Rochetti, Michael Dineen, Lorraine Bateman, Luigi Lucini, Tom F. O'Callaghan***

Bovine colostrum, the first milk produced postpartum, is a bioactive-rich secretion essential for neonatal immunity, growth, and development. Beyond its role in calf nutrition, bovine colostrum has gained interest in functional food and performance nutrition due to its high concentrations of immune-modulating proteins, growth factors, and bioactive lipids, beneficial for gut health, muscle recovery, and athletic performance. Given that diet, environmental factors, and metabolic adaptation of cows influence dairy composition, this study aimed to investigate the metabolic shifts in bovine colostrum on account of the differences in pre-partum diet and environmental variables associated to seasonality. We hypothesise that environmental and pre-partum dietary factors drive distinct metabolic differences in colostrum, potentially affecting its nutritional and functional properties.

Ninety colostrum samples, collected from Holstein Friesian cows calved in autumn that were fed pasture-based diets, and cows calved in spring fed on grass silage, were extracted using acetonitrile and formic acid solvent system and analysed via UHPLC-Orbitrap MS-based untargeted metabolomics approach. The multivariate statistical analyses revealed distinct metabolic signatures, with autumn colostrum showing two-fold higher levels of 24 metabolites belonging to lipids and lipid-like molecules, particularly phospholipids, bioactive peptides, organic acids, and nucleotides. Key metabolites, glycyl aspartate and D-erythro-imidazole-glycerol-phosphate, were identified as metabolic biomarkers capable of differentiating the two classes of colostrum. A correlation analysis revealed that the colostrum metabolome was significantly influenced by extrinsic factors such as diet (pasture vs. silage) and environmental temperature, while intrinsic factors such as parity and genetic merit (Economic Breeding Index) showed minimal association.

These findings provide novel insights into the adaptability of the colostrum metabolome with respect to diet and seasonality, emphasising its implications for calf nutrition, dairy management, and colostrum-based functional food formulations. Understanding these metabolic shifts may support targeted feeding strategies to optimise colostrum bioactivity and enhance its applications in nutraceutical and dairy industries.

12.2 3:50 p.m. – 4:10 p.m.	338 Insights from Dual-Platform Metabolomics on Durian Flowers: An Alternative Source of Procyanidins from Agricultural Waste with Bioactivities

PRESENTING AUTHOR: ★ ***Supakorn Potijun, Chulalongkorn University, Thailand***

CO-AUTHORS: ***Supakorn Potijun, Hubert Schaller, Supaart Sirikantaramas***

Procyanidins, polyphenolic compounds found in grape seeds, apples, and berries, are well known for their anti-inflammatory and antioxidant properties. In this study, we investigated durian flowers—an underutilized agricultural waste—as a novel and sustainable source of procyanidins. Although durian trees bloom profusely, not all flowers develop into fruit, leaving behind significant biomass. Dual-platform metabolome analysis using ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF-MS/MS) and gas chromatography–mass spectrometry (GC-MS) identified key polyphenols, including (–)-epicatechin, procyanidins B1, B2, and C1. Extraction optimization with 80% (v/v) ethanol yielded a crude extract containing 7.68 mg/g total procyanidins. Bioactivity assays demonstrated that the procyanidin-rich extract significantly reduced oxidative stress and inflammation. In vitro studies using HaCaT cells exposed to UVA-induced oxidative stress revealed that durian flower crude extracts reduced total ROS and superoxide levels by 28%, and decreased pro-inflammatory markers TNF-α and IL-6 by 48.9% and 41.6%, respectively. These findings position durian flowers as a promising alternative source of procyanidins, particularly relevant to Southeast Asia, where durian cultivation is widespread. This is the first study to propose durian flowers as a sustainable, cost-effective procyanidin source with potential applications in the nutraceutical and cosmeceutical industries, contributing to agricultural waste valorization through green technology.

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SESSION 12. AGRICULTURAL AND ANIMAL PRODUCTS	Tuesday, June 24	3:30 p.m. – 5:10 p.m.

12.3 4:10 p.m. – 4:30 p.m.	149 Metabolomic and Microbiota Alterations in Goat Milk During Experimental Subclinical Mastitis by Staphylococcus warneri

PRESENTING AUTHOR: ★ *Ana Júlia Silva Moreira, Federal University of Viçosa, Brazil*

CO-AUTHORS: *Ana Júlia Silva Moreira, Richard Costa Polveiro, Luiz Felipe Marques Rodrigues, Bianca Uliana Picolo, Arlene Bispo Dos Santos Nossol, Hebréia Oliveira Almeida-Souza, Mário Machado Martins, Luiz Ricardo Goulart Filho, Maria Aparecida Scatamburlo Moreira*

Goat milk is a valuable nutritional resource, but caprine mastitis, often bacterial, compromises production, quality, and safety. This study investigated microbiota and metabolomic alterations in goat milk during experimental subclinical infection caused by Staphylococcus warneri, an emerging mastitis pathogen. Six primiparous Pardo Alpina goats (Capra aegagrus hircus) underwent intramammary infection with S. warneri (1.2×10^8 CFU/mL), with one half of the udder infected and the other serving as an internal control, as each functions as an independent mammary gland. Milk microbiota was analyzed via 16S rRNA sequencing, while metabolomic profiling (GC-MS) data was processed in MS-DIAL (v5.5.24) and analyzed in MetaboAnalyst (v6.0). Alpha diversity showed a transient increase in microbial richness in infected halves (Chao1, $p<0.05$), but Shannon diversity returned to baseline after eight days ($p>0.05$), suggesting self-limitation. Metabolomic analysis identified 884 metabolites after manual curation of 17,663 analytes, displaying significant temporal variations (sPLS-DA, $p<0.05$). Infected halves clustered separately from pre-infection samples, while control samples remained stable (k-means, $p<0.05$), indicating treatment effects. Among the top 25 metabolites, Eplerenone – an anti-inflammatory compound – was initially downregulated (days 0 to 4) in both halves, suggesting an initial systemic response. It recovered (upregulated) in controls between days 4 and 8, but remained downregulated in infected halves during the same period (t-test, $p<0.05$), indicating prolonged inflammation only in infected glands. Dodecylamine, potentially involved in host immune response with possible antimicrobial activity, shifted from down- to upregulation in infected halves (t-test, $p<0.05$), suggesting a delayed metabolic response. These findings indicate that S. warneri disrupts milk microbiota and metabolome. Despite microbial diversity stabilization and apparent disease self-limitation, dysbiosis and metabolic alterations persisted, indicating extended inflammation. This study is the first to integrate microbiota and metabolomic analyses in caprine mastitis. Further metabolite annotation may help identify potential subclinical mastitis biomarker for disease monitoring.

Acknowledgements: CAPES, CNPq, FAPEMIG

12.4 4:30 p.m. – 4:50 p.m.	202 Natural variation in the wheat root exudate metabolome and its plant-microbe interactions

PRESENTING AUTHOR: *Cristina Lopez-Hidalgo, University of Vienna, Austria*

CO-AUTHORS: *Cristina Lopez-Hidalgo, Arindam Ghatak, Alexandros E. Kanellopoulos, Andrea Malits, Yuhang Meng, Florian Schindler, Shuang Zhang, Steffen Waldherr, Hugo Ribeiro, Melina Kerou, Logan Hodgkiss, Maximilian Dreer, Reyazul Rouf Mir, Sandeep Sharma, Gert Bachmann, Dimitrios G. Karpouzas, Christa Schleper, Evangelia S. Papadopoulou, Palak Chaturvedi, Wolfram Weckwerth*

The rhizosphere, a thin soil layer surrounded and influenced by plant roots, represents one of Earth's most dynamic interfaces. In this environment, plant roots exude compounds significantly mediating plant-microbe interactions, influencing microbial community composition and plant nutrient uptake efficiency.

Wheat is the primary crop consumed worldwide. However, yield efficiency and productive variety selection have been carried out under intensive fertilizer use. Consequently, modern commercial cultivars depend on these fertilizers and reduce their nutrient assimilation capacity or ability to interact with microorganisms for nutrient availability. Compared with modern cultivars, many species, including wild relatives, landraces, and old cultivars, are rich in genetic diversity and are likely to contribute superior traits for varietal improvement.

This study aimed to investigate natural variation in root exudate wheat varieties and identify key metabolites responsible for inhibitory effects on multiple ammonia-oxidizing bacteria (Nitrosospira multiformis and Nitrosomonas ureae) and archaea (Nitrososphaera viennensis and Nitrosocosmicus franklandianus). Root exudates from 44 diverse wheat genotypes (Triticum aestivum) from India and Austria were analyzed using combined gas and liquid chromatography-mass spectrometry (GC-MS and LC-MS) approaches. The metabolomic analysis covered primary and secondary bioactive molecules, including amino acids, organic acids, vitamins, phenols, flavonoids, and sugars. The results demonstrated significant genotype-dependent variation in composition and growth inhibition, clearly differentiating between Austrian and Indian varieties. Through deep learning analysis and multivariate statistics, we identified several key metabolites, including oxalic acid, oleamide, various amino acids, and secondary metabolites, such as isoshaftoside and shaftoside, that contribute to growth inhibition.

Our results provide the first comprehensive characterization of wheat root exudate metabolomes and their inhibition potential, establishing a critical link between plant diversity and inhibition of rhizosphere microorganisms. These findings advance our understanding of plant-soil interactions and offer new opportunities for breeding wheat varieties with enhanced nutrient assimilation through natural interactions.

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SESSION 12. AGRICULTURAL AND ANIMAL PRODUCTS	Tuesday, June 24	3:30 p.m. – 5:10 p.m.

12.5 4:50 p.m. – 5:10 p.m.	491	Predictive metabolomics to unravel phenotypic traits in pearl millet grains from Brazilian germplasm panel

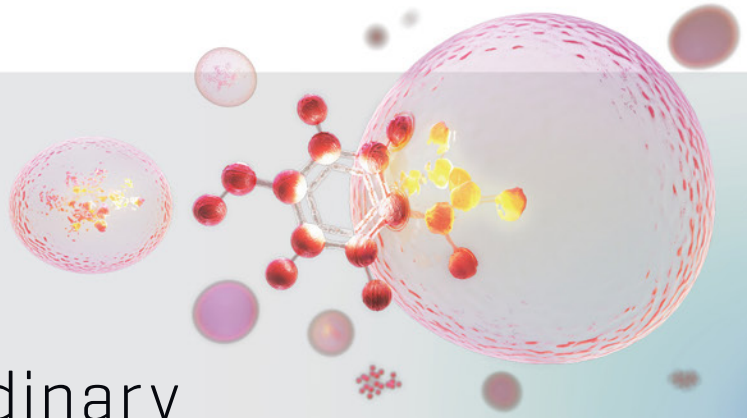
PRESENTING AUTHOR: ★ *Mariana Pinheiro Costa Pimentel, Food and Nutrition Graduate Program (PPGAN-UNIRIO), France*

CO-AUTHORS: *Mariana Pinheiro Costa Pimentel, Alexandre Martins Abdão dos Passos, Sylvain Prigent, Cédric Cassan, Flavio Dessaune Tardin, Mariana Simões Larraz Ferreira, Pierre Pétriacq, Millena C. Barros Santos*

Pearl millet emerges as an alternative to major cereal crops due to its notable nutritional value and gluten-free nature. Beyond it, it exhibits strong resilience to adverse agro-climatic conditions reinforcing its role in addressing agricultural challenges and enhancing food security. Despite this, little is known about how pearl millet's genetic diversity influences physiological functions, particularly metabolic responses. To address this gap, predictive metabolomics—combining metabolomics with machine learning—enables a comprehensive top-down modelling approach, linking phenotype to the underlying mechanisms of phenotypic traits. Thus, this study aimed to associate Brazilian germplasm core collection metabolome of 203 pearl millet genotypes with phenotypic traits and find metabolic predictors. Grains from these accessions were analysed using UHPLC-LTQ-Orbitrap mass spectrometry. Raw data were processed in MS-DIAL, resulting in 7,790 RT/m-z features. After data cleaning and normalisation, 2,767 features were used to predict 21 agronomic traits via supervised machine learning: generalised linear models (GLM) and random forest. Models were developed using the Caret package with three types of penalisation: LASSO, Elastic-Net, and Ridge. Training used 50% of the individuals, while the remaining 50% tested prediction accuracy. The GLM model successfully predicted eight qualitative traits with accuracies ranging from 74% to 87% and seven quantitative traits with R^2 values up to 0.5. Qualitative accuracies increased to over 80% and quantitative R^2s above 0.6 when the model was rerun using the top predictors (>80% occurrence). The top 896 metabolic predictors (595 level 1-3 and 301 level 4, from Metabolomics Standards Initiative) were annotated. Notably, most metabolic predictors belonged to the carbohydrate, amino acid, flavonoid, and terpene subclasses. This is the first study leveraging a pearl millet Brazilian germplasm bank for metabolome characterisation and predictive modelling of key phenotypic traits, paving the way for improved breeding strategies with benefits for agronomy, nutrition, and health.



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SESSION 13. MULTI-OMICS AND INTEGRATIVE METHODS | Wednesday, June 25 | 10:15 a.m. – 12:00 p.m.

13.1 KEYNOTE 10:15 a.m. – 10:45 a.m. | **353 Multi-modal exploration for plasma biomarkers of post-MI major adverse cardiovascular outcomes in New Zealand and Singapore – computational tools, lessons and the vision forward.**

PRESENTING AUTHOR: ***Hyungwon Choi, National University Of Singapore, Singapore***

Mass spectrometry-based quantitative lipidomics has gained traction in clinical translation lately as a novel diagnostic tool for cancer or a system for risk stratification in cardiovascular diseases. For scalable application of the technology, it is imperative to establish a seamlessly automated workflow for sample preparation, LC-MS analysis, and data analysis. Moreover, clinical omics studies are often designed to employ multiple phenotyping modalities other than MS, hence it is becoming a routine task to interpret the role of lipidomic data in the context of a single multi-omics study. In this talk, I will first present our recent work in MRMhub, an almost fully automated and scalable LC-MSMS (MRM) data processing and quality control. I will next describe our network-based workflow for multi-omic data integration in the context of outcome prediction using a series of open-source tools developed in house, such as graphical model estimator ACCORD and sparse predictive network discovery method (iOmicsPASS+).

I will demonstrate the power of these tools using a biomarker study for secondary major adverse cardiovascular outcomes (MACE). In this multi-modal investigation, we acquired plasma proteomics and lipidomics data along with conventional biochemistry tests and performed echocardiographic imaging in two patient cohorts with acute myocardial infarction (AMI) from New Zealand and Singapore. Sampling points were standardized at 30 days after hospital discharge following successful reperfusion treatment, and the patients were followed up to 9 years for incident MACE. During the lipidomic data processing step, our MRMhub workflow significantly reduced data processing time in deriving concentration values and filtering high quality analytes in the quantitative data, all achieved in less than an hour in terms of the actual computation time. Our network-based predictive signature produced highly interpretable signatures focusing on a few plasma proteins along with well-established markers, largely enabled by the predictive values reported by iOmicsPASS+.

13.2 10:45 a.m. – 11:05 a.m. | **461 Integration of Omics and Spectroscopic Data for Enhanced Prediction of HCC**

PRESENTING AUTHOR: ***Antonio Malinconico, University of Naples Federico II, Italy***

HepatoCellular Carcinoma (HCC) is among the most common malignancies worldwide, with late diagnosis due to the initial absence of symptoms, significantly impacting survival rates [1].

Plasma samples from 101 individuals infected with the hepatitis C virus (HCV), including 69 patients diagnosed with HCC, were analyzed using mass spectrometry and infrared spectroscopy to identify differences between the two groups. Metabolomics, Lipidomics, and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopic data were pre-processed and subjected to both univariate and multivariate analyses.

Two classification models were developed using data fusion approaches based on Sequential and Orthogonalized Covariance Selection (SO-CovSel) combined with Linear Discriminant Analysis (LDA) [2,3]: one combining metabolomic and spectroscopic data, and the other integrating lipidomic and spectroscopic data. The performance evaluation on the test set revealed that both models attained an accuracy >90.00%.

The variables selected by these models highlight common metabolic alterations associated with tumor progression, including decreased levels of lysophosphatidylcholines, acylcarnitines, and ceramides, along with an overstimulation of the kynurenine pathway, which promotes immunosuppression. Spectroscopic data contributed to both models by providing insights into protein content variations. In conclusion, this preliminary study lays the foundation for the identification of potential biomarkers for the early diagnosis of hepatocellular carcinoma, with the ultimate goal of improving patient survival rates.

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SESSION 13. MULTI-OMICS AND INTEGRATIVE METHODS	Wednesday, June 25	10:15 a.m. – 12:00 p.m.

13.3 11:05 a.m. – 11:25 a.m. **252 Multi-omics integration reveals restored and resistant liver metabolic pathways following a lifestyle intervention in mice**

PRESENTING AUTHOR: ★ *Jordi Rofes, Universitat Rovira i Virgili, Spain*

CO-AUTHORS: *Jordi Rofes, Joan Miro, Christian Heyer, Pau Gama, Pablo Miguel Garcia-Rovés, Matthias Schlesner, Oscar Yanes*

Metabolic plasticity enables organisms to adapt their metabolism in response to external stimuli, but obesity disrupts this flexibility, leading to long-term metabolic alterations. Given the strong link between gut microbiota composition and obesity, and the partial metabolic improvements observed with microbiota restoration, we aimed to explore how diet and lifestyle interventions reshape microbiota-host interactions, particularly the microbiota-liver metabolism-epigenetics axis, and their role in restoring metabolic plasticity.

To investigate this, we applied a multi-omics approach to a C57BL/6J mouse model subjected to a lifestyle intervention. Mice were divided into three groups: a standard diet group, an ad libitum high-fat diet (HFD) group, and a group that received HFD followed by a lifestyle intervention (INT) with a healthier diet and exercise (n = 10 per group). We profiled liver RNA sequencing, whole-genome bisulfite sequencing, histone proteomics, targeted and untargeted metabolomics, as well as gut microbiome 16S rRNA sequencing, shotgun metagenomics, functional profiling, cecum and colon metabolites, metals, and serum metabolites.

Our results show that diet and exercise modulate gene expression through targeted epigenetic changes in transcription factors, which coordinate with histone modifications. To integrate these datasets, we performed multi-omics factor analysis (MOFA2), identifying two key factors. The first represented pathways restored by the lifestyle intervention, enriched in ribosomal proteins, extracellular matrix genes, apoptosis, and immune system pathways. The second captured metabolic pathways that remained altered despite the intervention, including short-chain fatty acid metabolism, lipid metabolism, amino acid degradation, and disruptions in the citrate (TCA) cycle. Multi-omic correlation analysis further linked liver metabolic changes from both groups to microbiota composition, functional pathways, and specific microbial taxa, suggesting a role for the gut microbiome in metabolic plasticity. These findings provide new insights into microbiota-host interactions and highlight the extent to which lifestyle interventions can restore metabolic balance.

13.4 11:25 a.m. – 11:45 a.m. **241 Using optimal transport to integrate spatial multiomics data**

PRESENTING AUTHOR: *Nami Sakamoto, Tokyo University of Agriculture and Technology, Japan*

CO-AUTHORS: *Nami Sakamoto, Haruki Uchino, Koki Tsuyuzaki, Yuki Matsuzawa, Eiryo Kawakami, Makoto Arita, Hiroshi Tsugawa*

Integration of spatial multiomics data sets such as spatial lipidomics and transcriptomics is an emerging need to illuminate the molecular machinery at a region of interest in biological samples. Optimal transport (OT) is a widely used mathematical model to integrate single cell RNA-seq and spatial transcriptomics data. However, OT has not been used in spatial metabolomics yet, and the applicability of OT is currently unknown. In this study, we employed OT techniques to integrate MALDI-MS derived spatial lipidomics and Visium based spatial transcriptomics data of mouse brains. To create features for integrating two different omics layers, the cell-type deconvolution method was developed by a machine learning using LC-MS/MS based untargeted lipidomics data for fractionated brain cells including neuron, oligodendrocyte, astrocyte, and microglia. We evaluated multiple OT techniques and found that fused Gromov-Wasserstein (FGW) considering structural and feature similarities showed the best performance to align lipidomics and transcriptomics data. Furthermore, we mapped the integrated omics data on the Allen Brain Atlas-defined anatomical regions. Using the integrated dataset, we conducted correlation analyses between the expression levels of all genes and lipid metabolites. The results revealed that the lipid expression levels of sulfatide and hexosyl ceramide were highly correlated with the profiles of Fa2h, Ugt8a, and Gal3st1, which are involved in their biosynthetic pathways. Furthermore, we observed significant differences in the balance of phospholipids containing polyunsaturated fatty acids and long-chain saturated fatty acids between neurons and glial cells, indicating that our approach has a potential to reveal lipid signatures and their molecular machinery at the cellular level, which have not been elucidated in bulk analyses.

SESSION 13. MULTI-OMICS AND INTEGRATIVE METHODS	Wednesday, June 25	10:15 a.m. – 12:00 p.m.

13.5 11:45 a.m. – 12:00 p.m.	499	Metabolomic and Integrative Analysis of COVID-19 and Long-COVID Based on ISARIC and PHOSP-COVID Studies

PRESENTING AUTHOR: ***Desai Chen, Imperial College London, United Kingdom***

The PHOSP-COVID and ISARIC-4C studies, two of the largest UK-based COVID-19 research initiatives, provide a unique opportunity to investigate the metabolic signatures of COVID-19 severity, survival, and progression to Long-COVID. The ISARIC-4C (Coronavirus Clinical Characterisation Consortium) is a prospective cohort study that enrolled over 135,000 hospitalized COVID-19 patients across the UK, collecting extensive clinical, immune, and biomarker data to uncover risk factors and molecular drivers of disease severity and mortality. Meanwhile, PHOSP-COVID focuses on post-hospitalization COVID-19 recovery, following over 10,000 patients longitudinally to understand Long-COVID pathophysiology.

Using metabolomics (^{1}H NMR) and integrative analysis, this study aims to identify key metabolic disruptions associated with both acute and post-acute COVID-19 outcomes.

Our findings in the ISARIC-4C cohort reveal significant alterations in one-carbon metabolism, particularly in the S-adenosylmethionine/S-adenosylhomocysteine ratio, a key regulator of methylation capacity and immune response. A lower SAM/SAH ratio is strongly associated with increased mortality, suggesting impaired methylation potential as a driver of disease severity. In addition, metabolites such as N6-Methyladenosine, HPHPA, and p-Cresol glucuronide were identified as top predictors of survival in plasma, while disruptions in phenylalanine, lipid, and branched-chain amino acid metabolism were linked to both acute and long-term complications.

For Long-COVID, our study leverages PHOSP-COVID longitudinal plasma and urine samples, collected at 3 and 12 months post-hospitalization, allowing for a time-series analysis of metabolic recovery. Early results indicate persistent serotonin pathway dysregulation, altered lipid metabolism, and continued mitochondrial dysfunction, which may contribute to fatigue, cognitive impairment, and systemic inflammation in Long-COVID patients.

By integrating metabolomics with immune profiling (OLINK data), this study seeks to develop predictive models for COVID-19 severity and Long-COVID risk, advancing our understanding of host metabolic responses and potential therapeutic targets. Our work highlights the importance of metabolic monitoring in post-COVID recovery and paves the way for precision medicine approaches to improve patient outcomes.



SESSION 14. MICROBIOME AND HOST-METABOLISM INTERACTIONS	Wednesday, June 25	10:15 a.m. – 12:00 p.m.

14.1 KEYNOTE 10:15 a.m. – 10:45 a.m.	571 Poo, piss and plasma: the dirtier side of exploring the role of metabolites in inflammatory disease

PRESENTING AUTHOR: ***Jennifer Kirwan, Berlin Inst. Of Health At Charite, Germany***

Inflammatory processes are characterized by acute or chronic activation of the immune system. Their effects are seen as a contributory or causal factor in multiple infectious and non-communicable diseases. Metabolomics holds significant promise for advancing precision medicine by offering deeper insight into individual disease mechanisms and how inflammatory processes can be influenced. It also shows promise in explaining why the same process can cause different diseases in different indviduals. Both „top down“ untargeted or „bottom up“ targeted approaches are being applied to investigate how metabolomics can improve diagnosis and prognosis in disease and accelerate drug discovery pipelines.

This talk will highlight our recent research, focussing on inflammatory diseases including infectious and non communicable conditions. It will explore the microbiome-metabolite-immune regulation axis, concentrating on the key roles of tryptophan metabolites and short chain fatty acids in this chain. These metabolites may act both as prognostic markers, and also represent key opportunities for therapeutic monitoring or intervention.

Given the complexity and variability inherent in metabolomic data, it will also highlight the critical importance of pre analytical and analytical rigor. It will touch upon the quality management frameworks we employ to ensure data robustness, with particular emphasis on MSLineaR, our in-house tool designed to enhance existing methods of data filtering to reduce risk of false discoveries.

14.2 10:45 a.m. – 11:05 a.m.	161 Mapping microbial lipid production: the impact of linoleic acid on gut microbiota composition and function

PRESENTING AUTHOR: ***Josep Rubert, Wageningen University And Research, Netherlands***

CO-AUTHORS: ***Josep Rubert, Zongyao Huyan, Nicoletta Pellegrini, Edoardo Capuano***

During digestion, 5-10% of dietary lipids reach the large intestine, interacting with gut microbes. However, the interplay between lipids and the gut microbiota remains largely unexplored. To address this, we investigated the interaction between linoleic acid (LA), a prevalent dietary polyunsaturated fatty acid (PUFA), and the human gut microbiota. We hypothesized that (i) LA selectively enhances the growth of certain bacterial species and is processed through their fatty acid metabolism, and (ii) the resulting fatty acids are integrated into structural and signaling lipids.

To test our hypothesis, we utilized untargeted lipidomics, metagenomics, and isotopically labeled LA (ILLA) to study gut microbiota-LA interactions. Initially, we employed static bioreactors with human feces to identify gut microbes responsible for producing microbial lipids and their metabolic pathways. We then validated our findings using a simulator of the human intestinal microbial ecosystem (SHIME).

Our results indicated that dietary lipids and gut microbiota influence microbial composition and metabolic activity. By integrating isotope-tracking lipidomics and metagenomics, we found that gut microbes actively metabolize LA, incorporating it into complex lipids and generating bioactive PUFAs. The addition of LA shifted gut microbiota composition, increasing the prevalence of species within the Gammaproteobacteria class. This metabolic activation led to diverse microbial lipids, primarily fatty acids, phospholipids, and sphingolipids. The SHIME results further confirmed that bioactive long-chain PUFAs, such as linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, were synthesized shortly after LA exposure, with Gammaproteobacteria playing a crucial role in this lipid production.

Previous studies found that many microbial lipids in stools, tentatively identified through lipidomics, were either unannotated or lacked verification of their microbial origin. Our novel strategy has established a microbial atlas derived from LA, proposing that microbial lipids can serve as indicators of gut health, similar to the role of short-chain fatty acids in microbiome research.

SESSION 14. MICROBIOME AND HOST-METABOLISM INTERACTIONS	Wednesday, June 25	10:15 a.m. – 12:00 p.m.

14.3 11:05 a.m. – 11:25 a.m. **111 Metabolomics-guided discovery of novel antibiotics from the human microbiome**

PRESENTING AUTHOR: ***Karin Meier, Roche, Switzerland***

CO-AUTHORS: ***Karin Meier, Chantal Heer, Alexandre Dentler, Andreas Haldimann, Manuel Tzouros, Alexander Zipperer***

Antibiotic resistance is an urgent threat to global health security and might become the leading cause of death by 2050. Novel resistance-breaking antibiotics are crucial to overcome multidrug resistant infections with high-priority pathogens such as Pseudomonas aeruginosa, Acinetobacter baumannii and members of the Enterobacteriaceae family. Most antibiotics in clinical use today are natural products or derivatives thereof, with actinomycetes being one of the most prolific producers of antibiotics. Unfortunately, high re-discovery rates associated with traditional sources of natural product antibiotics limit discovery of new classes of antimicrobials.

To circumvent this limitation, we leverage the human microbiome's diverse bacteria, which produce a multitude of bioactive molecules, to discover novel antibiotics. We collected human saliva samples, isolated over 4000 bacteria, and phenotypically screened the secreted molecules for antimicrobial activity against Pseudomonas aeruginosa. We combined our phenotypic screen with an LC-MS/MS based chemical characterization of the complex mixtures to pinpoint the molecules linked to antimicrobial activity. This approach led to the discovery of more than a hundred unidentified, highly polar molecules as the common metabolic signature of isolates with antimicrobial activity. By following up on selected isolates with strong growth-inhibiting activity against Pseudomonas aeruginosa, we specified the molecular fingerprint for each and further excluded known natural product molecules with antimicrobial properties through a targeted dereplication process. With this combined workflow, we identified a range of new and uncharacterized candidates supporting drug discovery endeavors, highlighting the human microbiome as a promising source for novel antibiotics.

14.4 11:25 a.m. – 11:45 a.m. **445 A prebiotic diet intervention can restore faecal short chain fatty acids in Parkinson's Disease yet fails to restore the gut metabolome and microbiome homeostasis**

PRESENTING AUTHOR: ***Silke Heinzmann, Helmholtz Munich, Germany***

CO-AUTHORS: ***Silke Heinzmann, Janis Bedarf, Falk Hildebrand***

Introduction and Aim: Parkinson's Disease (PD) remains without disease-modifying therapies, and innovative approaches targeting the gut-brain axis are of growing interest. Intestinal microbiota and their metabolites, specifically short-chain fatty acids (SCFA), have been implicated in PD pathophysiology. This study aimed to investigate the impact of a short-term dietary intervention on SCFA production and microbiome-metabolome alterations in PD patients.

Methods: A controlled pilot study was conducted with 11 couples, each consisting of one PD patient and a healthy control (CO). Participants followed a 4-week diet rich in dietary fiber and supplemented with prebiotic lactulose. Metagenomic analysis, NMR-based metabolomics of faeces and urine samples, and clinical assessments were performed pre- and post-intervention.

Results: The intervention led to a significant increase in SCFA production, correlating with an increase in Bifidobacteria spp. PD-related gastrointestinal symptoms improved in parallel with higher SCFA levels. However, the bacterial dysbiosis commonly seen in PD, such as depletion of Blautia and Dorea persisted. Notably, some pathobionts, like Klebsiella, were reduced. Metabolomic analysis revealed that PD patients exhibited shifts in both faecal and urinary metabolomes, moving closer to the healthy control profile. Besides metabolites we also assessed brain-relevant functional metabolic potential (GBM modules) and found significant improvement in S-Adenosyl methionine, DOPAC, glutathione, tryptophan, and inositol – suggesting enhanced neuroprotective and antioxidant pathways.

Conclusions: This short-term dietary intervention significantly altered the gut microbiome and metabolome in PD patients, with promising effects on gastrointestinal symptoms and functional potential for neuroprotective metabolites. These findings highlight the potential of microbiome-targeted therapies in PD, warranting further investigation into the long-term effects and underlying mechanisms.

This study provides evidence that dietary modifications can influence gut microbial composition and metabolites, with potential implications for PD treatment, contributing to the emerging field of health-related microbial metabolomics.

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SESSION 14. MICROBIOME AND HOST-METABOLISM INTERACTIONS	Wednesday, June 25	10:15 a.m. – 12:00 p.m.

14.5 11:45 a.m. – 12:00 p.m.	199 Herpesvirus exposures are associated with disrupted phospholipid metabolite profiles and incidence of chronic inflammatory diseases

PRESENTING AUTHOR: ★ *Nicole Prince, Brigham & Women's Hospital, United States*

CO-AUTHORS: *Nicole Prince, Sanghun Lee, Kevin M Mendez, Qingwen Chen, Christoph Lange, Rachel S Kelly, H. Benjamin Larman, Jessica A Lasky-Su*

Viral infections are established precursors of many chronic inflammatory diseases, but their underlying pathobiological mechanisms remain largely unknown. Recent technological innovations – such as Molecular Indexing of Proteins by Self-Assembly (MIPSA) – have provided a novel means to comprehensively assess viral exposures on an omic-wide scale. MIPSA coupled to metabolomic profiling represents a powerful new approach to capture relevant biochemistry, and we aimed to utilize these datasets to investigate connections between viral exposures and subsequent development of diseases. In this study, we performed MIPSA viral antibody profiling (Infinity Bio) and global metabolomic profiling (Metabolon, Inc.) in 1600 plasma samples from the Mass General Brigham Biobank cohort (MGBB), spanning up to 30 years of follow-up. Linear regression analyses estimated associations between viral antibodies and metabolite levels. Exposure to 9 herpesvirus species across 5 viral genera was associated with altered metabolomic profiles, including increased levels of phospholipid metabolites; specifically, we observed significant elevations in levels of phosphatidylcholine (Betas=0.25-0.48; P=4.8x10-3-0.03) and lysophospholipid (Betas=0.18-0.24; P=8.9x10-3-4.8x10-3) metabolites. Directions of effect were consistent across herpesvirus species. Next, we investigated associations between herpesvirus exposures and incident diseases using logistic regression. Herpesvirus exposures were associated with increased incidence of chronic inflammatory diseases, such as allergic disease (OR=1.2, P=2.1x10-3), rheumatoid arthritis (OR=1.3, P=0.019), and type 2 diabetes (OR=1.2, P=0.02), among others, suggesting a range of pathobiological impacts. Our study represents the largest comprehensive assessment to date of viral antibody repertoires and chronic inflammatory diseases, and metabolomic profiling suggested a role for increased phospholipid metabolite levels in the underlying pathobiology of multiple inflammatory diseases. Given the role of phospholipids as inflammatory signaling molecules, these results identified a potential mechanistic connection between herpesvirus exposures and disease development. Phospholipid metabolites – particularly those within the phosphatidylcholine and lysophospholipid classes – may represent future therapeutic targets for the prevention of chronic inflammatory diseases.



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SESSION 15. NATURAL PRODUCTS AND PLANT DEFENSE	Wednesday, June 25	10:15 a.m. – 12:00 p.m.

15.1 KEYNOTE 10:15 a.m. – 10:45 a.m.	303 Advances in Chemical Ecology and Mass Spectrometry: Unraveling Natural Products in Biological Communication

PRESENTING AUTHOR: *Norberto Peporine Lopes, University Of São Paulo, Brazil*

Over the past decade, chemical ecology has seen remarkable growth, largely driven by advances in omics-based approaches. Since antiquity, living organisms have served as sources for various traditional medicines used by numerous ethnic groups. One of the greatest challenges in the field of natural products today is to understand how these compounds function, aiming for rational applications in the future. In this context, mass spectrometry methodologies stand out as essential tools for unraveling the complex chemistry of natural substances. Meanwhile, progress in data analysis computing has expanded our ability to catalog a wide range of biological systems. In our laboratory, for instance, we have investigated neuroactive and antimicrobial compounds present in the skin secretions of anuran amphibians. We have observed that some of these components may be involved in chemical communication, both within the same species and between different species. In this presentation, we will discuss the relevance of plants and bacteria as potential sources of chemical signals in animals, illustrating one of the many aspects that make up Brazil's vast biodiversity.

15.2 10:45 a.m. – 11:05 a.m.	375 Plant Defense in Monocots: A Multi-Bladed Swiss Knife with Conserved Metabolic Traits?

PRESENTING AUTHOR: *Gerd U. Balcke, IPB-Halle, Germany*

CO-AUTHORS: *Gerd U. Balcke, Dario Esposto, Guillaume Patoine, Fiona Hui, Henriette Uthe, Alain Tissier*

Wheat, rye and barley are among the most widely cultivated cereals in the world and are used as staple foods, for biofuels and as animal feed. Despite fungicide use, farmers still lose 10-23% of crops to fungal diseases, highlighting the need to uncover gene-function relationships in plant defense for breeding resistant varieties. Since plant defense involves small molecules with anti-fungal properties, their discovery through untargeted metabolomics is crucial to this process.

Here we introduce a novel workflow by metabolite profiling with UPLC-QToF-MS/MS and downstream data processing by MS-Dial, SIRIUS and MetFamily, which assists the identification of individual compounds and the annotation of entire molecular families. Specifically, the coupling of hierarchical MS/MS clustering and classification by chemo-ontologies supports the identification of conserved metabolic motifs across the three monocots.

Wheat, barley and rye are closely related phylogenetically and belong to the tribe Triticeae within the order Poales but exhibit notable differences in genome size and ploidy. Regardless of these differences, root infection with pathogenic fungi or chemical elicition not only triggers the production of the same classes of specialized metabolites but also results in comparable distribution patterns across shoots, roots, and root exudates across the three cereals.

We observe high root accumulation of anti-fungal smiglasides, a class of feruloylated acetylsucroses, while various indole alkaloids, diterpenes, and oxylipins are increasingly exuded upon infection or elicitation. However, only a few defense metabolites have fully conserved structures across all three monocots, indicating the involvement of shared biosynthetic genes. Most metabolic responses reflect variations in conserved biosynthetic motifs across the three monocots.

15.3 11:05 a.m. – 11:25 a.m.	501 Exploring the Mode of Action of 20 Mycotoxins using a Powerful Targeted Metabolomics Approach

PRESENTING AUTHOR: ★ *Andrea Gerdemann, University of Muenster, Germany*

CO-AUTHORS: *Andrea Gerdemann, Matthias Behrens, Melanie Esselen, Hans-Ulrich Humpf*

Filamentous fungi predominantly grow in regions characterized by high humidity and elevated temperatures, where they can infest both crops on the field as well as food and feed post-harvest. These fungi produce highly toxic secondary metabolites known as mycotoxins, which pose a significant food safety concern through contamination of food and feed. While several of these compounds are known to be carcinogenic or immunosuppressive, and exhibit high toxicity, the precise cellular mechanisms remain poorly understood. Furthermore, the discovery of new compounds is still ongoing requiring powerful screening methods to enable fast elucidation of their mode of action.

Therefore, a comprehensive, targeted analytical method utilizing hydrophilic interaction chromatography coupled with tandem mass spectrometry (HILIC-MS/MS) was developed. This method enables the simultaneous analysis of more than 100 polar compounds of the main biochemical pathways allowing a detailed evaluation of cellular responses to xenobiotics in a single analysis.

The human hepatocellular carcinoma cell line HepG2 was used to investigate the metabolic effects of 20 mycotoxins derived from various fungal species and compound classes. Many of the compounds induced a multitude of metabolic effects. For example, the Fusarium toxin moniliformin caused a notable accumulation of oxoglutarate alongside a depletion of succinic acid, suggesting an inhibition of the oxoglutarate dehydrogenase. These findings are in line with previous cell free studies reporting interactions with thiamine pyrophosphate-dependent enzymes, such as pyruvate and oxoglutarate dehydrogenase. Additionally, the Aspergillus toxin penitrem A exerted significant effects on amino acid metabolism and the urea cycle, revealing novel insights into its mode of action, which has not been fully elucidated in prior research.

In conclusion, these results contribute to a deeper understanding of the cellular mechanisms of mycotoxins and highlight the potential of targeted metabolomics screening approaches for the elucidation of cellular effects of newly discovered compounds.

SESSION 15. NATURAL PRODUCTS AND PLANT DEFENSE	Wednesday, June 25	10:15 a.m. – 12:00 p.m.

15.4 11:25 a.m. – 11:45 a.m.	138 A combination of Untargeted Metabolomics and Mass Spectral Imaging: A case study on the interaction between Eucalyptus species and the weevil Gonipterus platensis.

PRESENTING AUTHOR: ***Andy Pérez, Universidad De Concepcion, Chile***

CO-AUTHORS: ***Andy Pérez, Jasna Campos, Ignacio Salinas, Catalina Bravo, Javiera Nahuelcura***

Metabolomics allows for the complete and systematic analysis of all measurable metabolites in a biological sample using high-throughput analytical means. Data-driven studies (Untargeted Metabolomics) focus on profiling a sample's total downstream cellular small molecules. However, metabolomics analysis usually includes the bulk biological samples, which must be homogenized for metabolome extraction with solvents. Although this workflow provides valuable information on the identity and concentration of molecules of interest in a bulk sample, it does not provide insight into their location and spatiotemporal within the sample tissues. Consequently, functional attribution is always ambiguous. We use Mass Spectrometry Imaging (MSI), an analytical tool that directly maps various chemical classes from an intact cryo-preserved and microtome-sectioned biological sample to complement Metabolomics. This technique provides information concerning analyte identity, abundance, and two-dimensional spatial distribution. Our study uncovered the crucial triggered defensive traits in Eucalyptus nitens × globulus genotypes grown in a field trial to deter weevil herbivory through an untargeted metabolomics approach combined with MALDI-Imaging for targeted molecular mapping on tissues. Findings revealed that such hybrids have the key enzymes to synthesize stilbenes constitutively, a trait that provides resistance. These metabolites were localized between the epidermis and mesophyll toward the adaxial face of leaves and distributed across the leaf cross-section, including edges, which should have a direct defensive role over larvae and adults feeding. At the same time, the downregulation of Roseoside, a monoterpene glycoside, was directly related to the resistance of genotypes to herbivory and was mapped exclusively in the secretory channels distributed throughout the leaf cross-section—presumably, this metabolite cleavages upon herbivory to release the volatile Vomifoliol as an active molecule. The findings mark a critical initial milestone in developing resistance for this hybrid species and highlight the potential of combining untargeted metabolomics and MALDI-Imaging for comprehensive study of biological systems.

15.5 11:45 a.m. – 12:00 p.m.	331 Exploring Grapevine Metabolomics: Pathogen Interactions and Defense Pathways

PRESENTING AUTHOR: ***Marisa Maia, FCiências.ID – Associação para a Investigação e Desenvolvimento de Ciências, Portugal***

CO-AUTHORS: ***Marisa Maia, Leonor Guerra Guimarães, Gonçalo Laureano, Jasmine Hertzog, Ana Cruz-Silva, Francisco Baeta, Jorge Cunha, Vincent Carré, Andreia Figueiredo***

Grapevine is one of the most important crops worldwide and every year it faces several outbreaks caused by various pathogens with different life cycles and modes of infection, such as the oomycete Plasmopara viticola and the fungi Botrytis cinerea. To defend itself and survive from these aggressors, grapevine rely on the regulation of several metabolic classes.

The metabolic profile of grapevine leaves, obtained by mass spectrometry, revealed that grapevine genotypes possess innate metabolic differences, which are responsible for their tolerance or susceptibility to pathogens. This study allowed us to propose for the first-time metabolic biomarkers of constitutive susceptibility (catechin/epicatechin).

Upon pathogen infection, our results showed that grapevine's metabolic dynamics has a pivotal role on the activation/shutdown of specific defence pathways. Flavonoids, carbohydrates, and lipids are the metabolic classes with higher modulation exhibiting distinct temporal regulation patterns. Further analysis using mass spectrometry imaging, revealed that stilbene phytoalexins and carbohydrates, are highly accumulated in areas close to infection sites and their accumulation depend not only on the time after infection but also on their molecular complexity.

These results led us to hypothesize that specific metabolites might be translocated to the infection site, at specific time-points, to restrict pathogen progression. The integration of intracellular and extracellular metabolomics data, offers a comprehensive perspective on grapevine's metabolic response to pathogen invasion, revealing crucial metabolites involved in grapevine's defense. To better understand these metabolic dynamics, a metabolic characterization of grapevine's apoplast is ongoing, as this compartment is crucial for pathogen recognition, activation of signaling cascades and pathogen counterattack.

This knowledge is fundamental for elucidating the role of metabolites in grapevine defense mechanisms, contributing for the definition of new and more sustainable approaches to control grapevine diseases.

SESSION 16. AGE-RELATED DISEASES AND CANCER	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

16.1 1:30 p.m. – 1:50 p.m.	324 Decoding Alzheimer's Disease Through Brain Metabolomics: Metabolic Dysregulation and Pathway Insights

PRESENTING AUTHOR: ***Donghai Liang, Emory University, United States***

CO-AUTHORS: ***Donghai Liang, Youran Tan, Emma Casey, Zhenjiang Li, Marla Gearing, Allan Levey, James Lah, Aliza Wingo, Thomas Wingo, Dean Jones, Douglas Walker, Anke Huels***

Background: Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by progressive cognitive decline and neuropathological hallmarks. Despite extensive research, the molecular mechanisms linking metabolic dysregulation to AD pathology remain poorly understood. We conducted a comprehensive high-resolution brain metabolomics study, profiling metabolic perturbations associated with AD neuropathology.

Method: Using untargeted high-resolution metabolomics, we analyzed 162 frontal cortex samples from the Emory Goizueta Alzheimer's Disease Research Center brain bank with comprehensive neuropathological evaluations, including Braak stage, CERAD, and ABC scores. We conducted a metabolome-wide association study of AD neuropathology adjusting for confounders and multiple testing (FDR 5%). Pathway enrichment analysis was performed to uncover biological processes implicated in AD, and chemical annotation confirmed key metabolites with Level 1 evidence. We also examined potential effects of APOE ε4 genotype in modifying these associations.

Results: Our analysis identified 155 metabolic features, and 36 metabolic pathways significantly associated with the AD neuropathology markers, spanning ten metabolic classes such as energy metabolism, nucleotide metabolism, and amino acid metabolism. ABC score was linked to the largest number of pathways, while Braak stage and CERAD exhibited distinct metabolic associations, including nucleotide metabolism with tau pathology and fatty acid-related pathways with neuritic plaque deposition. Further, 18 unique metabolites were confirmed with level 1 evidence, implicating their involvement in amino acid metabolism, lipid metabolism, carbohydrate metabolism, nucleotide metabolism, and metabolism of cofactors and vitamins in AD neuropathology. Genetic variability influenced these associations, with non-carriers of the APOE ε4 allele showing stronger perturbations in metabolites including glucose, mannose, myo-inositol, and adenosine 5'-diphosphoribose.

Conclusion: This study highlights the potential of high-resolution metabolomics to reveal metabolic pathways and molecular mechanisms underlying AD pathology. Findings provide insights into metabolic dysregulation in AD and its interplay with genetic factors, warranting future longitudinal studies to confirm these associations and explore functional significance.

16.2 1:50 p.m. – 2:05 p.m.	213 Metabolomics and Aging: Identifying Biomarkers of Obesity, Diabetes, and Hypertension

PRESENTING AUTHOR: ***Anas Abdel Rahman, King Faisal Specialist Hospital & Research Centre, Saudi Arabia***

CO-AUTHORS: ***Maha Almogren, Reem Almalki, Randah Alahmari, Moudi Alwehaibi, Abdullah Albogami, Shoaib Khan, Mohammad Waqar Ahmad, Abdulaziz Alsaif, Anas Abdel Rahman***

Background: Aging is a multifaceted biological process shaped by genetic, environmental, and metabolic factors. Understanding the metabolic underpinnings of aging is crucial for identifying biomarkers that distinguish healthy longevity from age-related diseases.

Methodology: In this study, we applied a comprehensive untargeted metabolomics approach to profile circulating metabolites in a well-characterized human cohort (n=423). This cohort covers disease-free and age-related diseases in the 18-87 age range.

Results: We analyzed the overlap among obesity (n=123), diabetes (n=96), and hypertension (n=95) in a patient cohort. Twenty-one individuals presented with all three conditions, while others had one or two; 66 patients had obesity alone, and 26 had only diabetes. These patterns highlight the complexity of comorbidities, underscoring the need for targeted interventions and personalized treatments. After processing the data, including filtering out missing values and annotating peaks from 12,560 detected features, we identified 312 metabolites, of which 156 were annotated, and 68 were classified as endogenous metabolites linked to age. Among the strongest metabolic predictors of age-related disease were 5a-Dihydrotestosterone sulfate (AUC = 0.787, 95% CI: 0.713–0.854, p<0.001, downregulated with age), L-carnitine (AUC = 0.882, 95% CI: 0.822–0.936, p<0.001, upregulated), and PG(6 keto-PGF1alpha/i-12:0) (AUC = 0.821, 95% CI: 0.732–0.891, p<0.001, upregulated). Some metabolites were also linked to age-related conditions such as diabetes, hypertension, and cardiovascular disease, indicating their potential role in disease susceptibility. Pathway analysis revealed disruptions in lipid metabolism, amino acid turnover, and redox homeostasis—hallmarks of aging and disease progression. These findings illuminate metabolic shifts in aging and suggest targets for early interventions.

Conclusion: This study highlights the interplay between obesity, diabetes, and hypertension, emphasizing the need for a metabolomics-based approach to understand these conditions. The metabolomic panel could facilitate early diagnosis, risk assessment, and personalized treatment. Validation in larger, independent cohorts is essential for medical translational goals.

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SESSION 16. AGE-RELATED DISEASES AND CANCER	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

16.3 2:05 p.m. – 2:25 p.m.	452 Dissecting Metabolic Alterations in Pancreatic Cancer

PRESENTING AUTHOR: ★ *Carolin Schneider, M3 Research Center, Germany*

CO-AUTHORS: *Carolin Schneider, Gyuntae Bae, Markus Löffler, Günter Schneider, Christoph Trautwein*

Introduction

Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, remains among the most lethal malignancies due to late-stage diagnosis and limited treatment options.

Metabolic reprogramming is crucial for driving disease progression and resistance. Simultaneously, it creates cancer cell-specific dependencies that may be therapeutically exploited, making it a promising area of research [1].

Aim

By applying cutting-edge metabolomic tools and complementary -omics approaches, we strive for a deeper understanding of the metabolic landscape of cancer cells and aim to characterize the spatial metabolic footprints within the tumor microenvironment of PDAC.

Methods

We integrated (i) quantitative biofluid ^{1}H nuclear magnetic resonance-based metabolomics and lipidomics, using an in vitro diagnostics regulation-compliant assay on sera from 34 PDAC patients with (ii) bulk transcriptomic data from PDAC samples to identify cancer cell-specific metabolic signatures. Resulting signatures were validated using targeted MALDI imaging, generating spatial profiles at single-cell resolution in patient-derived tumor samples.

Results

We identified distinct metabolic alterations in serum of PDAC patients, when compared to healthy controls [2], which overlapped with results of transcriptomic analyses, particularly in lipid and amino acid metabolism as well as the citric acid cycle. Moreover, MALDI imaging revealed cancer cell-specific metabolic circuits that aligned with respective metabolic pathways.

Conclusion

Metabolomics is a powerful tool for advancing our understanding of PDAC biology. Integrating multi-omics technologies provides a comprehensive view of disease mechanisms, offering substantial potential for identifying biofluid-based biomarkers for non-invasive diagnosis and uncovering metabolic vulnerabilities of cancers that could be targeted by future therapeutic strategies.

References

[1] Schneider C et al. A Novel AMPK Inhibitor Sensitizes Pancreatic Cancer Cells to Ferroptosis Induction. Adv Sci (Weinh). 2024;11(31):e2307695.

[2] Bae G et al. Quantitative Metabolomics and Lipoprotein Analysis of PDAC Patients Suggests Serum Marker Categories for Pancreatic Function, Pancreatectomy, Cancer Metabolism, and Systemic Disturbances. J Proteome Res. 2024;23(4):1249-1262.

16.4 2:25 p.m. – 2:40 p.m.	466 Baseline Serum Metabolomic Profiling Identifies 3-Hydroxybutyrate and Acetone as Prognostic Biomarkers in Relapsed/Refractory DLBCL Patients Treated with R2-GDP

PRESENTING AUTHOR: *Joan Badia Cabré, IISPV, Spain*

CO-AUTHORS: *Joan Badia Cabré, Raquel Cumeras, Sara Fernández-Castillejo, Luis de la Cruz Merino, Alejandro Martín Garcia-Sáncho, Fernando Carnicero-González, Natalia Palazón-Carrión, Eduardo Ríos-Herranz, Fátima de la Cruz-Vicente, Antonio Rueda-Domínguez, Natividad Martínez-Banaclocha, José Gómez-Codina, Jorge Labrador, Francisca Martínez-Madueño, Núria Amigó, Antonio Salazar-Silvestre, Delvys Rodríguez-Abreu, Laura Gálvez-Carvajal, Margarita Sánchez-Beato, Mariano Provencio-Pulla, Maria Guirado-Risueño, Esteban Nogales, Víctor Sánchez-Margalet, Carlos Jiménez-Cortegana, Guillermo Rodríguez-García, Josep Gumà*

Patients with relapsed or refractory (R/R) diffuse large B-cell lymphoma (DLBCL) who are ineligible for high-dose chemotherapy have limited treatment options and poor prognoses. The phase II R2-GDP-GOTEL trial (EudraCT 2014-001620-299) evaluated the R2-GDP regimen—a combination of lenalidomide, rituximab, gemcitabine, dexamethasone, and cisplatin—demonstrating its feasibility and effectiveness. Serum samples were collected at baseline from 69 enrolled patients, with a mean follow-up of 41 months. Baseline serum metabolomic profiles were assessed using NMR (Fernández-Castillejo et al., Cancers, 2025;17(3):532), identifying two independent metabolites, 3-hydroxybutyrate (3OHB) and acetone, significantly associated with overall survival (OS) and progression-free survival (PFS). Higher baseline serum levels of 3OHB (>141µM) and acetone (>40µM) were linked to poorer survival outcomes. Specifically, 3OHB was significantly associated with PFS (hazard ratio[HR] 7.7, 95% confidence interval[CI] 2.5–24.1) and OS (3OHB: HR 9.32, 95% CI 2.75–31.6), while acetone was also significantly associated with PFS (HR 9.32, 95% CI 2.75–31.6) and OS (HR 1.92, 95% CI 1.36–2.69). Elevated 3OHB levels were specific to the Activated B-cell-like (ABC) DLBCL subtype, whereas acetone levels were elevated in both types but more pronounced in ABC cases. In a multivariate survival analysis including the International Prognostic Index (IPI) score and refractoriness status(R/R), 3OHB and acetone remained significant. To facilitate risk stratification in R/R-DLBCL patients receiving the R2-GDP regimen, we developed PFS and OS nomograms incorporating 3OHB or acetone levels, IPI score, and refractoriness status. The nomogram with 3OHB and refractoriness status achieved a time-dependent AUC of 0.86 for 6-month PFS and 0.84 for 12-month OS, providing a robust tool for individualized prognosis and treatment optimization. These findings highlight 3OHB and acetone as independent prognostic biomarkers of poor outcomes in R/R-DLBCL, irrespective of IPI score, chemorefractoriness, sex, age, or BMI. Their incorporation into clinical decision-making may improve patient stratification and guide therapeutic strategies.

SESSION 16. AGE-RELATED DISEASES AND CANCER	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

16.5 2:40 p.m. – 3:00 p.m. **95 Metabolomic Profiles for Ovarian Cancer Early Detection**

PRESENTING AUTHOR: *Oana Alina Zeleznik, Harvard Medical School and Brigham & Women's Hospital, United States*

CO-AUTHORS: *Naoko Sasamoto, Julian Avila-Pacheco, Shelley S. Tworoger, Britton Trabert, Daniel W. Cramer, Kathryn L. Terry, Oana Alina Zeleznik*

Background: Ovarian cancer has poor survival outcomes, largely due to most cases being diagnosed at an advanced stage. CA125, the most widely used ovarian cancer biomarker, has limited sensitivity and specificity, especially for ovarian cancer early detection. Here, we propose to develop a metabolite-based early detection score for ovarian cancer.

Methods: Our study included 251 plasma metabolites measured by LC-MS/MS (Broad Institute, Cambridge, MA) in samples collected up to three years before ovarian cancer diagnosis from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO; n=100, training dataset) and the Nurses' Health Studies (NHS; n=98, replication dataset). In PLCO, LASSO was used to select a group of metabolites that is most predictive of ovarian cancer. We assessed model performance by estimating the area under the receiver operating characteristic curve (AUC) and 95% confidence interval (CI) in PLCO and NHS.

Results: LASSO selected nine metabolites into the score: C16:0 cholesteryl ester (CE), C22:6 CE, C30:0 phosphatidylcholine (PC), C32:1 PC, C40:10 PC, C38:2 phosphatidylethanolamine, C45:0 triglyceride, acetaminophen, and 4-hydroxyhippurate. Adding the metabolite score to a model based on CA125 alone significantly improved the AUC in PLCO, from 0.66 (95%CI=0.59–0.72) to 0.87 (95%CI=0.80–0.94; p<0.01), as well as in NHS, from 0.63 (95%CI=0.56–0.71) to 0.72 (95%CI=0.61–0.83; p<0.05).

Conclusion: A metabolite-based score significantly improved ovarian cancer early detection compared to CA125 alone in samples collected up to 3 years before diagnosis. These findings, replicated in an independent dataset, suggest plasma metabolites as promising early detection biomarkers for ovarian cancer.

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SESSION 17. BIOMONITORING AND ENVIRONMENTAL METABOLOMICS	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

17.1 1:30 p.m. – 1:50 p.m.	219	Don't Smile at a Polluted Crocodile: PFAS Contamination and Its Impact on wild-caught Australian Saltwater Crocodiles (Crocodylus porosus)

PRESENTING AUTHOR: *David Beale, CSIRO, Australia*

CO-AUTHORS: *David Beale, Sandra Nilsson, Jian-Wei Liu, Daniel Guymer, Simon Booth, Suzanne Vardy*

Suspected PFAS contamination from Cairns Airport and nearby defence sites is a significant environmental concern in Far North Queensland (Australia), impacting the Trinity Inlet (TI) and Baron River (BR) ecosystems. This study investigates PFAS bioaccumulation in wild-caught saltwater crocodiles (Crocodylus porosus) to assess health impacts as indicators of broader ecosystem effects. Serum from crocodiles in PFAS hotspots and a reference site (Russel River; RR) were analysed using LCMS. Historical surface water PFAS levels at the hotspots showed maximum concentrations of 2.29 µg/L (PFOS), 3.05 µg/L (PFOS + PFHxS), and 0.09 µg/L (PFOA) at TI, and 0.03 µg/L (PFOS + PFHxS) and <0.01 µg/L (PFOA) at BR. According to the PFAS National Environmental Management Plan, these levels pose risks to animals through the food chain. Serum PFAS concentrations (∑45 PFAS) from sampled crocodiles ranged from 6.8 to 71 ng/mL (BR, n=6; 19.47 ± 25.25), 2.3 to 62 ng/mL (TI, n=6; 14.27 ± 23.29), and 1.1-1.8 ng/mL (RR, n=4; 1.43 ± 0.39). Omics-based measurements revealed that PFAS-impacted crocodiles had increased levels of cystatin-like proteins, periostin, von Willebrand factor, beta-defensin 10, and secreted phosphoprotein 1, indicating physiological stress responses like inflammation, tissue damage, immune activation, and vascular issues. PFAS exposure also raised several metabolites and lipids, pointing to oxidative stress, energy imbalance, and potential impairments in muscle, liver, or kidney functions. Compared to reference crocodiles, PFAS-exposed animals had lower levels of pantetheinase-like protein, affecting energy metabolism and inflammatory responses, and complement component C6-like protein, weakening the immune system. Reduced levels of ATP, AMP, DHAP, citric acid, and DL-isocitric acid suggested disruptions in energy metabolism, while decreased lipids and O-phosphorylethanolamine and oxidized glutathione pointed to lipid metabolism issues. These findings highlight the extent of PFAS bioaccumulation and its health effects on these long-lived reptiles, with broader implications for ecosystem health that warrant further research.

17.2 1:50 p.m. – 2:05 p.m.	259	Using 18O-assisted phosphometabolomics to elucidate key enzymatic pathways regulating cellular phosphorus metabolism under stress

PRESENTING AUTHOR: ★ *Cheng Shi, Eawag, Switzerland*

CO-AUTHORS: *Cheng Shi, Elena Evertz, Federica Tamburini, Thomas Hofstetter*

Climate change, biodiversity loss, chemical pollution, and other environmental changes driven by anthropogenic perturbations are deemed to impact ecosystem services negatively and, thus, threaten food and clean water supply. However, there is limited understanding on how microorganisms adapt their metabolism to these perturbations. We hypothesize that the holistic metabolic activity is shifted under stress, and thus limiting the microorganisms' ability to provide ecosystem services.

In this study, we aim to assess the metabolic activity through quantifying changes in phosphorus metabolism using 18O-assisted phosphometabolomics. We chose phosphorus metabolism to estimate the holistic metabolic activity due to the essential role of phosphoryl-transfer reactions in cellular metabolism, such as intracellular energy transfer and storage, signal transduction, and post-translational modification. We posit that the enzymatic dynamics of phosphoryl-transfer reactions can be estimated by the isotope ratio of 18O/16O in cytosolic phosphate (cytosolic δ18O(PO43-)) as a proxy.

To test this hypothesis, we developed a method to track microbial phosphorus fluxes through different phosphoryl-transfer reactions under stress conditions, such as heat shock and nutrient limitation. Specifically, we used cell media with 18O labeled water to trace 18O incorporation into phosphometabolites in model organisms, such as Escherichia coli and Bacillus subtilis. By quantifying the enrichment of 18O in both targeted and untargeted phosphometabolites with LC-HRMS, we elucidated the changes in the phosphoryl-transfer network under stress (Figure 1). Combining the measurements of cytosolic δ18O(PO43-), we expect to evaluate how oxygen isotope fractionation expressed in cytosolic phosphate reflects the phosphometabolites turnover, and their dynamics induced by stress. Preliminary results suggested shifts in the turnover of nucleotide triphosphates (e.g., ATP, GTP) under stress, which are also reflected in cytosolic δ18O(PO43-). Phosphometabolomics, moreover, support further development of 18O metabolic modeling. The outcomes of this study will contribute to a framework aiming to predict holistic metabolic activities influenced by environmental perturbations.

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SESSION 17. BIOMONITORING AND ENVIRONMENTAL METABOLOMICS	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

17.3 2:05 p.m. – 2:25 p.m.	503 1H NMR metabolomics and embryotoxicity tests: Insights into the effects of polystyrene microplastics and bisphenol A in the black sea urchin Arbacia lixula

PRESENTING AUTHOR: *Tiziana Cappello, University of Messina, Italy*

CO-AUTHORS: *Maria Concetta Eliso, Barbara Billè, Giuseppe De Marco, Mariachiara Galati, Mery Terranova, Maria Maisano, Tiziana Cappello*

In the contemporary era, named plasticene, the extensive presence of microplastics (MPs, < 5 mm) in all the environmental matrices constitutes a global challenge, whose impact on living organisms is still largely unknown. Furthermore, there are increasing concerns on the capacity of MPs to act as environmental vectors or sinks for hydrophobic organic pollutants, and there is still a gap in understanding the mechanisms of embryotoxicity of pollutant mixtures in marine organisms. The Italian project PRIN 2020 (20204YRYS5_003) focuses in assessing the impact of 1 and 5 µm polystyrene MPs (PS MPs; 10 µg/mL) and bisphenol A (BPA; 5 and 25 µM), alone and combined, on the embryogenesis of the black sea urchin Arbacia lixula. After evaluating the adsorption behaviour of BPA on PS MPs, embryotoxicity tests were conducted for 48 h. The uptake of MPs was then evaluated in the pluteus larvae, which showed a significantly altered larval development after exposure to BPA alone, with skeletogenic defects at 5 µM BPA and development block at 25 µM BPA. Interestingly, the normal embryogenesis of sea urchins was not reduced by both sizes of PS MPs, while co-exposure of PS MPs (both sizes) with the two BPA doses induced morphological abnormalities comparable with those observed following exposure to BPA alone. The protonic Nuclear Magnetic Resonance (1H NMR)-based metabolomics revealed disorders in the cholinergic and serotoninergic neurotransmission pathways by altered levels of choline and N-acetyl serotonin, as confirmed by inhibited acetylcholinesterase (AChE) activity. The NMR spectra showed also interferences in the energy metabolism and biomineralization processes by altered levels of lactate and glycine, respectively, suggesting a perturbed skeletogenesis. Overall, insights on the toxicodynamics of PS MPs and BPA during sea urchin embryogenesis were provided. NMR-based metabolomics combined with embryotoxicity tests are an effective tool to accurately evaluating the embryotoxicity of environmental contaminants.

17.4 2:25 p.m. – 2:40 p.m.	217 Global meta-metabolomics reveals marine chemical diversity

PRESENTING AUTHOR: ★ *Maïa Henry, Friedrich Schiller Universität, Germany*

CO-AUTHORS: *Maïa Henry, Stéphane Pesant, Mireille Pujo-Pay, Gilles Reverdin, Hannes Richter, Georg Pohnert*

Most of the ocean's biomass consists of microscopic organisms, collectively referred to as the marine microbiome. While their genetic and functional diversity has been extensively studied, the biosynthetic potential of these organisms and the chemical interactions driving microbiome functioning remain largely unexplored. Indeed, most secondary metabolites produced by marine prokaryotes are still unknown, and extensive datasets capturing the marine meta-metabolome across latitudes, depths, timescales, and microbial size fractions remain scarce.

Here, we present a global study using LC-MS to investigate the marine chemical space and the functional roles of marine metabolites within geochemical cycles and oceanographic processes. As part of two oceanographic expeditions dedicated to surveying the global marine microbiome (Mission-Microbiomes-AtlantECO and TREC-Tara Europa, 2021–2024), we deployed a metabolomic experiment to monitor variations in the marine meta-metabolome. This effort covered over 300 sampling sites, spanning from South America to the Baltic Sea, and resulted in the collection of more than 4,000 samples.

As a proof of principle, we introduce the analysis of three sampling sites in the Exclusive Economic Zone of Brazil and French Guiana, covering different microbial size fractions and depths: the Amazon River Plume, an anticyclonic eddy influenced by the Amazon River Plume waters, and a reference site representing seawater without freshwater input.

Leveraging high-resolution Orbitrap mass spectrometry coupled with UHPLC, along with computational mass spectrometry and metabolomic annotation developments, we reveal an unexpectedly rich dataset, encompassing known and newly characterized molecules, as well as potential new secondary metabolites, including chlorinated polyketides. Finally, we envisage how key metabolomic and functional variations among the Amazon River Plume microbiomes link microbial community structuring and oceanographic processes to marine chemical diversity.

The introduced metabolomic dataset demonstrates how meta-metabolomics supports marine environmental studies, chemical ecology, and metabolite discovery.

SESSION 17. BIOMONITORING AND ENVIRONMENTAL METABOLOMICS	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

17.5 2:40 p.m. – 3:00 p.m. **117 Metabolomics for monitoring seagrass responses along a nutrient gradient**

PRESENTING AUTHOR: ***Steven Melvin, Griffith University, Australia***

CO-AUTHORS: ***Steven Melvin, William Bennett, Nicholas Doriean, Kaitlyn O'Mara, Anthony Carroll, David Welsh***

Seagrass meadows play a crucial role in coastal ecosystems but are increasingly threatened by anthropogenic pressures, particularly nutrient enrichment and eutrophication. While traditional monitoring approaches rely on morphology and stable isotopes as indicators of nutrient enrichment, these approaches provide limited insights into the physiological health of seagrasses. We used Nuclear Magnetic Resonance (NMR) spectroscopy to characterise the metabolite profiles of seagrass (Zostera muelleri) along an anthropogenic nutrient gradient in Wallis Lake, NSW, Australia, and compared the results to traditional indicators. Nitrogen and δ15N content of seagrass shoots reflected the prevailing anthropogenic gradient, whereas sediment nutrients were more variable. Seagrass morphology was also indicative of the anthropogenic gradient, with lower biomass and reduced length: width ratio of shoots moving away from the anthropogenic source. Principal Component Analysis of the seagrass metabolome revealed clear differences between sites moving along the gradient. Metabolites associated with nitrogen metabolism (e.g., nitrogen-containing amino acids) closely mirrored sediment nutrients, suggesting these molecules offer a robust representation of current nutrient availability. Conversely, metabolites associated with carbon metabolism (e.g., carbohydrates and TCA cycle intermediates) exhibited a more linear pattern of decrease, moving from the most nutrient rich site to locations less impacted by anthropogenic activities. Additionally, the production of phenolic compounds increased moving away from the source of anthropogenic nutrients, supporting prior evidence that these compounds may be synthesized in response to nutrient limitation. Taken together, these findings highlight the value of molecular diagnostics for monitoring the health of seagrass meadows and contribute to the development of metabolomic-based indicators for assessing coastal ecosystems and informing management strategies.



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SESSION 18. METABOLOMICS BEYOND LC-MS: NMR AND MORE	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

18.1 1:30 p.m. – 1:50 p.m.	171 NMR-Based Metabolomics to Investigate the Coupling of Arginine Methylation and Metabolism

PRESENTING AUTHOR: ***Tobias Madl, Medical University of Graz, Austria***

Background

Protein arginine methylation (ArgMet) is a key post-translational modification involved in transcription, RNA processing, and signal transduction. Dysregulation of ArgMet is linked to neurodegenerative diseases and cancer, yet its metabolic impact remains unclear due to the lack of quantitative analytical methods. Nuclear Magnetic Resonance (NMR) spectroscopy offers a powerful, label-free, and quantitative approach to study ArgMet and its metabolic consequences. Here, I will present our method development in NMR-based ArgMet quantification and highlight our metabolomics studies in neurodegeneration and cancer.

Methods

We developed a robust NMR-based platform for the quantification of global ArgMet levels in proteins, cells, organoids, and tissues. This method enables high-sensitivity detection of ArgMet dynamics, its modulation by small-molecule inhibitors, and the interplay between ArgMet and cellular metabolism. Our metabolomics analyses utilized high-field NMR spectroscopy combined with multivariate statistical approaches to assess metabolic alterations.

Results

I will present our NMR-based platform for ArgMet quantification. Our metabolomics study on post-mortem brain tissue from FTD and AD patients revealed region-specific metabolic signatures distinguishing FTD subtypes from AD. Distinct differences emerged in the frontal and occipital cortices, identifying potential biomarkers for differential diagnosis.

In sarcoma, we established and characterized clear cell sarcoma cell lines from patient-derived primary and metastatic lesions. NMR-based metabolomics revealed that ArgMet is altered in tumor cells compared to matched normal fibroblasts. Notably, inhibition of PRMT1, a key enzyme in ArgMet, resulted in metabolic reprogramming and selective apoptosis of tumor cells, while normal fibroblasts remained unaffected. This highlights PRMT1 inhibition as a potential therapeutic strategy targeting ArgMet-associated metabolic vulnerabilities in cancer.

Conclusion

Our NMR-based approach provides a quantitative, and high-resolution method to study ArgMet and its metabolic consequences. The findings demonstrate that ArgMet dysregulation is linked to metabolic changes in both neurodegeneration and cancer, with potential applications in biomarker discovery and therapeutic development.

18.2 1:50 p.m. – 2:05 p.m.	29 Metabolomic Insights into Amphetamine-Type Stimulant Misuse: Unraveling Biochemical Pathways and Biomarkers

PRESENTING AUTHOR: ***Ruzmayuddin Mamat, University Malaya Centre For Addiction Science, Malaysia***

CO-AUTHORS: ***Ruzmayuddin Mamat, Baharudin Ibrahim, Sim Maw Shin Gareth, Rusdi Abd Rashid, Nuratikah M Nordin, Azmir Ahmad***

Amphetamine-type stimulants (ATS), such as methamphetamine, amphetamine, and MDMA, are highly risky substances linked to neurochemical disruptions, metabolic disturbances, and systemic toxicity. Despite substantial research on their neurotoxic effects, the metabolic pathways involved in ATS dependence remain poorly understood. This study aimed to characterize the metabolic signatures associated with ATS dependence using NMR-based metabolomics to identify systemic metabolic disruptions related to chronic ATS use. A cross-sectional study was conducted involving 583 participants, comprising ATS-dependent individuals from Malaysian drug detention centers and healthy controls. Plasma samples were analyzed using 1H-NMR, CPMG, and HSQC spectroscopy to obtain comprehensive metabolomic profiles. Multivariate analyses, including PCA-X, OPLS-DA, and logistic regression, were employed to identify metabolites that differentiated ATS patients from controls. Metabolites were cross-referenced with BMRB and HMDB databases for validation. ATS-dependent individuals showed significant alterations in metabolic pathways, with reductions in cholic acid, L-valine, L-alanine, lactic acid, creatinine, histidine, taurine, and homovanillic acid (all $p < 0.005$), indicating disruptions in energy metabolism, neurotransmitter biosynthesis, and oxidative stress defenses. Elevated L-arginine levels ($p < 0.001$) suggested nitrogen metabolism dysregulation. OPLS-DA analysis demonstrated robust group separation ($R^2Y = 0.762$, $Q^2Y = 0.756$, AUROC = 0.987), with sensitivity, specificity, and classification accuracy of 86.9%, 97.4%, and 91.5%, respectively. This study presents the first NMR-based metabolomic profile of ATS dependence in Malaysia, identifying critical metabolic disruptions linked to chronic ATS use. Key biomarkers, including cholic acid, L-valine, and homovanillic acid, highlight potential targets for biomarker development and precision medicine strategies to improve the diagnosis, treatment, and understanding of ATS use disorder.

SESSION 18. METABOLOMICS BEYOND LC-MS: NMR AND MORE	Wednesday, June 25	1:30 p.m. – 3:00 p.m.

18.3 2:05 p.m. – 2:25 p.m. **446 Measurement of isotopologue ratios for metabolic flux analysis via parallel, quasi-simultaneous EI and CI-TOFMS detection with a novel, flexible CI source**

PRESENTING AUTHOR: *Pegah Mousazadehfazeli, BOKU University, Austria*

CO-AUTHORS: *Pegah Mousazadehfazeli, Christina Troyer, Teresa Steininger-Mairinger, Eliška Ceznerová, Sonja Klee, Arnd Ingendoh, Matthew R. Lewis, Stephan Hann*

Accurate analysis of ^{13}C labeled metabolites is essential for stable isotope tracer experiments and metabolic flux calculations, which are key techniques for pathway analysis in metabolic engineering. Mass spectrometry (MS)-based methods offer high accuracy in isotopologue distribution analysis. A novel ionization source was recently developed for a GC-TOF system that significantly facilitates the generation of data of both the intact non-fragmented carbon backbone of metabolites as well as fragments carrying positional information of ^{13}C labeled atoms. The system is based on parallel electron ionization (EI) and chemical ionization (CI) of the analytes after post-column splitting, followed by quasi-simultaneous detection via time-of-flight (TOF) MS. Thus, both CI and EI data are generated within a single GC-run cutting instrument time and cost in half.

This GC-TOF was used to measure isotopologue abundances of central carbon metabolites such as amino acids, organic acids, sugars, and sugar phosphates in standard mixtures and cell extracts. A validated in-house method for automated two-step derivatization and an optimized GC separation were applied to test chemical ionization reagent gases employing the benefits of automated reagent gases switching.

The measured isotopologue intensities were corrected for the contribution of natural abundant isotopes including those added by derivatization. As reference materials are still lacking, trueness of the isotopologue ratios was assessed using an in vivo labeled material (ethanolic cell extract) following Pascal's triangle. The EI&CI-TOFMS results were compared with state-of-the-art GC-TOFMS data (EI or classical CI with methane) in terms of trueness, bias, and precision. Monte Carlo simulation was used to assess the impact of possible sources of error in isotopologue ratio analysis.

18.4 2:25 p.m. – 2:40 p.m. **361 In vivo real-time metabolic isotopic tracing via breath analysis upon deuterated water ingestion**

PRESENTING AUTHOR: *Zhihong Yin, University of Basel, Switzerland*

Stable isotope deuterium (D) has been widely used as a tracer in various research such as the synthesis of biomolecules, lipids, carbohydrate, protein, and the measurement of total body water. However, the availability of in vivo, non-invasive dynamic monitoring tools is still scarce. Here we introduce an online breath analysis platform based on secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) that allows such measurement.

We enrolled a total of 11 healthy volunteer from two centres for online breath analysis using SESI-HRMS. Each participant contributed 20 sets of breath samples during this study, including 4 breath samples before intake of deuterated water, 8 breath samples after intake of deuterated water, and 8 breath samples after intake of food. A total of 213 samples was obtained in this study.

Here we show that SESI-HRMS is able to resolve natural 13C isotopic peaks from the peaks corresponding to the incorporation of deuterium. And a total of 2691 isotopic D/H pairs were identified in breath samples. Butanoic acid, along with other metabolites in Cluster 4, exhibited a sharp D/H ratio increase after D2O intake, followed by a decline, and then a secondary rise after food consumption. This pattern suggests these metabolites, including butanoic, propanoic, and pentanoic acids, reflect metabolic activities influenced by both D2O intake and refeeding. In contrast, Clusters 7 and 10 showed a significant rise only after feeding, indicating metabolic responses specifically linked to refeeding.

Secondary electrospray ionization-high resolution mass spectrometry allows monitoring in vivo D-incorporation of metabolites in a non-invasive and real-time setup and opens new opportunities to use deuterium isotope tracing to apply current metabolic studies.

18.5 2:40 p.m. – 3:00 p.m. **342 Multum in parvo: how to derive three one-dimensional NMR spectra from a single acquired spectrum**

PRESENTING AUTHOR: *Leonardo Tenori, University Of Florence, Italy*

CO-AUTHORS: *Leonardo Tenori, Alessia Vignoli, Stefano Cacciatore*

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for analyzing complex mixtures due to its ability to manage matrix complexity, provide detailed molecular insights, and preserve sample integrity. In metabolomics, NMR enables the identification, quantification, and characterization of metabolites with minimal sample preparation, a broad dynamic detection range, and high reproducibility. Blood-derived samples (namely serum and plasma), in which both metabolites and macromolecules are present in solution, offer a practical example of the flexibility of the technique: different types of NMR experiments can provide complementary insights into the sample molecular composition. The one-dimensional (1D) Nuclear Overhauser Effect SpectroscopY (NOESY) experiment produces a spectrum in which the signals of all the 1H NMR-detectable chemical species present in solution (e.g., metabolites, proteins, lipoproteins) are visible, independent of their molecular weights. The 1D Carr-Purcell-Meiboom-Gill (CPMG) experiment suppresses signals from macromolecules and other broad resonances by manipulating spin-echo decay, yielding the selective detection of low molecular weight molecules. The diffusion-edited experiment yields the selective suppression of small metabolite signals by distinguishing between molecules based on size and diffusion behavior. The 1H-1H J-resolved spectroscopy (JRES) experiment helps resolve overlapping signals by separating chemical shifts and J-coupling information. However, each additional NMR experiment demands extra spectrometer time, and post-processing effort. Thus, acquiring multiple NMR spectra for biofluids such as serum or plasma can be resource-intensive and time-consuming, especially for high-throughput studies. Here we propose a simple and efficient approach, based on Partial Least Squares (PLS) regression, to computationally derive from a single acquired NOESY spectrum other types of NMR spectra, i.e. CPMG, diffusion-edited and JRES spectra, using serum samples as case study. The machine learning approach here presented constitutes a template for any future similar applications, even beyond metabolomics.

★ AWARD WINNERS

SESSION 19. METABOLITE ANNOTATION	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

19.1 3:30 p.m. – 3:50 p.m. **406 Reverse metabolomics enables public data mining and identifies a drug–bile acid conjugate in inflammatory diseases**

PRESENTING AUTHOR: *Vincent Charron-lamoureux, University Of California San Diego, United States*

CO-AUTHORS: *Vincent Charron-lamoureux, Marta Sala-Climent, Shipei Xing, Ipsita Mohanty, Helena Mannochio-Russo, Yasin El Albiead, Nina Zhao, Victoria Deleray, Prajit Rajkumar, Abubaker Patan, Harsha Gouda, Zhewen Hu, Dionicio Siegel, Manuela Raffatellu, Karsten Zengler, Monica Guma, Pieter C. Dorrestein, ADDITIONAL AUTHORS*

Untargeted metabolomics repositories are rapidly expanding, currently hosting over 2 billion MS/MS spectra and thousands of publicly available datasets. There is growing interest within the metabolomics community to mine publicly available data and harness this underutilized resource. In this work, we use reverse metabolomics, a discovery-based data science strategy that links any phenotype-relevant information−such as health phenotype−to each candidate bile acid found in the public domain. By integrating molecular networking to reverse metabolomics, a tool designed to find analogs based on spectral similarity, we initially discovered that in two patients with rheumatoid arthritis reporting using the prodrug sulfasalazine, one of the active components, 5-aminosalicylic acid (5-ASA) is conjugated to both bile acids and 3-Phenylpropionic acid, a well-known microbial metabolite. Although two patients may seem like a low number of biosample, the power of reverse metabolomics lies in its ability to simultaneously mine thousands of datasets in which these new molecules might be found. Leveraging this approach, we expanded our analysis to independent cohorts, and remarkably, we found the bile acid – drug conjugates in three independent IBD cohorts in which the drug 5-ASA is routinely prescribed. Next, we cultured gut microbes to show that commensal and pathobiont species can conjugate 5-ASA to multiple bile acids potentially modulating the drug activity. To add another layer of biological context and relevance to reverse metabolomics, we developed microbiomeMASST, a tool that bridges microbial metabolites and host biology to accelerate discoveries and facilitate data interpretation. Taken together, reverse metabolomics and microbiomeMASST serve as a framework for exploring complex biological data, identifying associations, and reinforcing discoveries by uncovering hidden metabolic interactions at an unprecedented scale.

19.2 3:50 p.m. – 4:10 p.m. **86 Twin neural network-based spectrum transformer for small molecule analog discovery from untargeted mass spectrometry**

PRESENTING AUTHOR: ★ *Juan Sebastian Piedrahita Giraldo, University of Antwerp, Belgium*

CO-AUTHORS: *Juan Sebastian Piedrahita Giraldo, Manuela Da Silva, Mohammed Reza, Mingxun Wang, Thomas De Vijlder, Kris Laukens, Wout Bittremieux*

Tandem mass spectrometry (MS/MS) is a key tool for analyzing the small molecule composition of biological samples. Untargeted metabolomics data analysis entails matching the experimental MS/MS spectra to spectral libraries based on spectrum similarity, typically using the cosine similarity. However, such heuristic techniques often fail to capture the structural similarity between molecules. With the purpose of discovering novel analogs, which are non-identical yet structurally related molecules, we developed a transformer deep neural network model that has learned the relationship between MS/MS data and chemical structures.

Our approach, called SIMBA, consists of a twin transformer encoder that receives pairs of MS/MS spectra and predicts the structural similarity of their molecules. The model was trained in a multi-task setting to predict both the “substructure edit distance,” a novel domain-inspired metric that reflects the number of modifications between molecules, and the maximum common edge subgraph (MCES), thus learning both the number of structural differences and their atomic cardinality. Harnessing the powerful learning capabilities of transformers, the model was trained on 200 million spectrum pairs from the NIST20 and MassSpecGym spectral libraries.

SIMBA significantly surpasses the state of the art for analog discovery, predicting the MCES with a higher Spearman correlation (r=0.93) compared to modified cosine (r=0.46). Likewise, SIMBA identifies analogs with high performance on the CASMI2022 dataset: SIMBA is able to retrieve analogs with a lower normalized MCES distance of 0.16 compared to traditional modified cosine (0.23) as well as deep learning approaches such as Spec2Vec (0.19) and MS2DeepScore (0.20).

Given that structural similarity is not always reflected in classical spectral similarity, our deep learning algorithms promise to be a useful tool for detecting molecules with similar chemical characteristics.

★ AWARD WINNERS

SESSION 19. METABOLITE ANNOTATION	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

19.3 4:10 p.m. – 4:30 p.m. | **196 Interpreting 200 million metabolite mass spectra using DreaMS-Net – a neural network-based molecular networking**

PRESENTING AUTHOR: ***Roman Bushuiev, IOCB Prague, Czechia***

CO-AUTHORS: ***Roman Bushuiev, Anton Bushuiev, Josef Sivic, Tomáš Pluskal***

Public metabolomics repositories, such as MassIVE GNPS, contain millions of uncharacterized LC-MS/MS spectra from diverse biological and environmental samples. Identifying the metabolites behind these spectra could advance molecular biology, drug discovery, and environmental research. Here, we introduce DreaMS-Net, a neural network-based molecular networking approach that organizes 200 million tandem mass spectra from MassIVE GNPS into DreaMS Atlas, a large-scale molecular network facilitating their interpretation.

We validated DreaMS-Net by benchmarking its spectral similarity performance (i.e., network edges) across key tasks, including chemical similarity approximation, spectral library matching, and analog search. It consistently outperformed traditional methods such as modified cosine similarity and deep learning-based approaches such as MS2DeepScore. To ensure accessibility, we integrated DreaMS-Net into the mzmine software and developed a stand-alone Python package.

DreaMS Atlas organizes 200 million metabolite mass spectra into an easily navigable molecular network, allowing annotation propagation across distinct LC-MS/MS datasets. Using this framework, we putatively identified a fungal pesticide in psoriasis samples, suggesting a potential link to disease etiology. Other examples include a plant-specific metabolite present across multiple species, a family of lipids potentially serving as cancer biomarkers, and a bile acid found in various human samples and other organisms. On a broader scale, DreaMS enables comparative analysis of complete metabolic profiles, which we demonstrated by characterizing “foodomes” of diverse food samples.

19.4 4:30 p.m. – 4:50 p.m. | **471 Novel transformation product annotation with SIRIUS – Combining spectral libraries and structure databases**

PRESENTING AUTHOR: ***Markus Fleischauer, Friedrich Schiller University Jena, Germany***

CO-AUTHORS: ***Markus Fleischauer, Kai Dührkop, Martin Andre Hoffmann, Sebastian Böcker***

Identification of small molecules from LC-MS/MS data remains a major bottleneck in metabolomics. Traditional spectral libraries are incomplete, and analogue searches alone cannot provide exact structural annotations. Machine learning-based methods such as CSI:FingerID efficiently search large structure databases but act as black boxes, making annotations difficult to interpret. Here, we introduce a novel workflow seamlessly integrated into SIRIUS, combining machine-learning-driven structure identification, analogue searches, and combinatorial fragmentation to enhance transformation product annotation from LC-MS/MS spectra.

Our workflow integrates four key components: (1) generation of potential transformation products using BioTransformer; (2) molecular structure annotation via SIRIUS+CSI:FingerID; (3) a rapid spectral library and analogue search; and (4) combinatorial fragmentation for annotating MS/MS peaks with potential substructures. We optimized spectral library searches through a novel dynamic programming algorithm that calculates modified cosine similarity in linear time, achieving 14 million comparisons per second for cosine similarity and 2 million per second for modified cosine similarity.

Spectral libraries are automatically annotated with fragmentation trees and molecular formulas, facilitating direct comparison of molecular formulas for matching peaks. We integrate molecular formula annotations from analogue searches via a Gibbs sampling approach. Additionally, by employing a combinatorial fragmenter, we annotate MS/MS peaks with potential fragments from both the database hit and the analogue compound, enhancing confidence in structure database hits through shared fragment explanations.

We reanalyzed Luxembourgish surface water samples for common drugs and their transformation products using data from Singh et al. (2021). From 772 unique drug structures (577 with reference spectra in MassBank+MoNA), our workflow annotated 80 drugs and generated a custom database of 483,203 potential transformation products. Subsequent analysis resulted in 85 (confidence score >0.64) putatively annotated transformation products, including 56 previously unrecorded in MassBank+MoNA, highlighting the efficacy of our integrated approach.

19.5 4:50 p.m. – 5:10 p.m. | **187 MVP-JESTR: MultiView Projection-Joint Embedding Space Technique for Ranking Candidate Molecules for the Annotation of Untargeted Metabolomics Data**

PRESENTING AUTHOR: ★ ***Yan Zhou Chen, Tufts University, United States***

CO-AUTHORS: ***Yan Zhou Chen, Soha Hassoun***

Metabolomics, driven by mass spectrometry, offers great potential for advancing biology and addressing challenges in medicine and the environment. However, structure elucidation from mass spectra is a bottleneck, with usually only 10% of experimental spectra assigned a structure. Here, we introduce a new model, MultiView Projection (MVP), for spectra annotation. Conceptually, MVP builds on JESTR (Joint Embedding Space Technique for Ranking candidate molecules), our prior tool that learns a joint embedding space on molecules and spectra. MVP considers molecular fingerprints and consensus spectra as additional views of the same data point. MVP is trained to maximize the mutual information among the four views of each data point while minimizing the similarity of non-matching views. Given an experimental spectrum, we rank a set of molecule candidates by measuring the embedding similarity between the candidate and the spectra or the candidate and the consensus spectra if the molecule was measured at different collision energies or detected with different adducts. Evaluation on the MassSpecGym benchmarking dataset shows improvement over the current best method by 145.82% and 25.24% in terms of rank@1 and 39.34% and 18.71% in terms of MCES@1 when retrieving candidates by mass and formula, respectively. Importantly, we demonstrate the value of using consensus spectra as input to a model over aggregating the ranking result of multiple individual spectra.

★ AWARD WINNERS

SESSION 20. CARDIOVASCULAR & ENDOCRINE DISEASE	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

20.1 3:30 p.m. – 3:50 p.m.	77 Metabolome-wide association of carotid intima media thickness identifies ferredoxin (FDX1) as a determinant of cholesterol metabolism and cardiovascular risk in Asian populations.

PRESENTING AUTHOR: ★ *Nilanjana Sadhu, Nanyang Technological University, Singapore*

CO-AUTHORS: *Nilanjana Sadhu, Rinkoo Dalan, Pritesh Jain, Mick Lee, Leroy Pakkiri, Kay Yi Tay, Theresia Mina, Dorrain Low, Yilin Min, Matthew Ackers-Johnson, Thi Tun Thi, Vishnu Kota, Yu Shi, Yan Liu, Hanry Yu, Vicky Lai, Yang Yang, Darwin Tay, Hong Kiat Ng, Xiaoyan Wang, Kari Wong, Max Lam, Xueli Guan, Nicolas Bertin, Eleanor Wong, James Best, Rangaprasad Sangarajan, Paul Elliott, Elio Riboli, Jimmy Lee, Eng Sing Lee, Joanne Ngeow, Patrick Tan, Christine Cheung, Chester Drum, Roger Foo, Gregory Michelotti, Haojie Yu, Patricia Sheridan, Marie Loh, John Chambers*

The burden of cardiovascular disease (CVD), and CVD-related mortality is rising in the Asia-Pacific region, in contrast to its falling mortality rates in Europe and North America. With the aim of exploring the metabolic signature of atherosclerosis and discovering new insights into the molecular pathways underlying CVD risk in Asian populations, we performed a metabolome-wide study of carotid intima media thickness (cIMT) in 8,124 participants from the Health for Life in Singapore (HELIOS) study (53±12 years, female=59%, Chinese=67%, Malay=14%, Indian=19%).

cIMT was assessed in the carotid bulb and distal common carotid artery using 2D/3D carotid ultrasonography. 883 metabolites, quantified by untargeted mass spectrometry on Metabolon's Global Discovery Panel, were assessed for their relationship with cIMT using linear regressions, while controlling for age, sex, ethnicity, BMI, blood pressure, total cholesterol, smoking and diabetes status.

We found that plasma concentrations of 3beta-hydroxy-5-cholestenoate (3BH5C) associated inversely with cIMT (Beta[SE]=-0.013[0.002]). Genetic instruments supported a causal relationship between 3BH5C and cardiovascular risk, wherein increased plasma concentrations of 3BH5C associated with decreased risk of coronary artery disease. We noted a 5-6 fold higher effect size in Asians (OR-GSMR[95% CI]=0.89[0.87-0.92], OR-IVW[95% CI]=0.86[0.80-0.92]) compared to Europeans (OR-IVW[95% CI] = 0.98[0.96-0.99]). Colocalization analyses using whole blood cis-expression quantitative trait loci (QTL) data from HELIOS (N=1,228) and GTEx (N=670), and protein QTL data from the UK Biobank (N=49,748), revealed the presence of a shared causal variant between 3BH5C plasma levels and ferredoxin (FDX1) expression, a protein essential for sterol and bile acid synthesis. We functionally validated FDX1 as a key regulator of 3BH5C synthesis in hepatocytes and macrophages. We also demonstrated that FDX1 modifies cholesterol efflux in macrophages and aortic smooth muscle cells.

This study advances our understanding of the metabolic basis for atherosclerosis, highlighting population specific differences, and identifies FDX1 as potential therapeutic target for the prevention of CVD.

20.2 3:50 p.m. – 4:10 p.m.	432 Lipidomic profiles and their relationship to incident diabetic retinopathy in type 2 diabetes: The Fremantle Diabetes Study Phase II

PRESENTING AUTHOR: *Nicola Gray, Murdoch University, Australia*

CO-AUTHORS: *Timothy Davis, Jocelyn Drinkwater, Alanah Grant-St James, Wendy Davis, Nicola Gray*

Background

Diabetic retinopathy (DR) is a major complication of diabetes mellitus, and a leading cause of blindness globally. Early detection and intervention are key for DR treatment and remains a challenge. The underlying biological mechanisms are also poorly understood. This study examined comprehensive plasma lipid profiles using a targeted approach to detect potential biomarkers of DR.

Methods

Ultra-performance liquid chromatography-tandem mass spectrometry was used for plasma lipid profiling of 762 participants from the Fremantle Diabetes Study Phase II. We investigated the association of lipid measures at baseline with DR status at both baseline and follow-up at Year 4 or 6. Of the participant samples analysed, 252 had DR at baseline and 38 of these had moderate NPDR or worse. For the 510 with no DR at baseline, 35 developed DR between baseline and Year 4/6. Comprehensive statistical analysis including multiple logistic regression models on lipid measurements and conventional risk factors (diabetes duration, HbA1c, blood pressure, intensity of diabetes treatment, and urinary albumin:creatinine ratio) were conducted to detect candidate markers of any new or worsening DR.

Results

We detected five lipids independently associated with subsequent development of DR and/or its progression, including cholesterol ester (20:4), fatty acid (20:3), lysophosphatidylglycerol (18:0), lysophosphatidylglycerol (18:1) and phosphatidylcholine (18:0_20:3). The inclusion of lipid parameters to conventional risk factors modestly improved predictive models for any new or worsening DR. For incident DR, the AUROCs were 0.64 (0.54, 0.74) (conventional model) versus 0.76 (0.68, 0.84) (conventional model plus lipid biomarkers), P=0.086.

Conclusions

This study demonstrates disturbances in lipid metabolism are associated with the presence and incidence of DR. Our findings revealed novel lipid-based biomarkers that have potential to improve prediction of DR risk in combination with conventional clinical factors. Our data also provide new insights into the mechanistic pathways underlying the development and progression of DR.

SESSION 20. CARDIOVASCULAR & ENDOCRINE DISEASE	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

20.3 4:10 p.m. – 4:30 p.m.	238 Untargeted tracer-based multi-omics identifies a novel role for pyruvate dehydrogenase kinase 1 (PDK1) in cardiomyocyte metabolism.

PRESENTING AUTHOR: *Michael Atser, University Of British Columbia, Canada*

CO-AUTHORS: *Michael Atser, Chelsea Wenyonu, Justin Hong, Haoning Howard Cen, Sareh Panahi, Armando Alcazar Magana, James D. Johnson*

Pyruvate dehydrogenase kinases (PDKs) regulate the intersection between glucose and lipid metabolism. Under fasted conditions, PDKs phosphorylate pyruvate dehydrogenase activity (PDH) to inhibit the entry of glucose-derived carbon units into the Krebs cycle, leading to the activation of fatty acid-derived carbon entry into the Krebs cycle. This regulation is critical to the proper functioning of healthy cardiomyocytes. PDK1, one of four isoforms, is abundant in the heart but its distinct role in cardiomyocyte metabolism remained elusive. In this study, we characterized the role of PDK1 on glucose and lipid metabolism in cardiomyocytes. We performed lentiviral knockdown of Pdk1 in H9c2 cells, a surrogate cardiomyocyte cell line and, surprisingly, observed no change in PDH activity and glycolysis rate. We then labelled cells with 13C6-glucose and performed untargeted metabolomics using UHPLC-HRMS/MS. Mass isotopologue distribution analyses at isotopic steady state revealed no differences in fractional enrichment of M+2 Krebs cycle intermediates, indicating similar rates of glucose-derived carbon entry between control and knockdown cells. Together, these findings demonstrate a non-essential role for PDK1 in glucose oxidation. In contrast, Pdk1 knockdown significantly affected lipid metabolism, causing a decrease in mitochondrial fatty acid utilization following nutrient deprivation. Untargeted lipidomics profiling of control and knockdown cells using UHPLC-HRMS/MS revealed no changes in the relative abundance of lipid classes. However, we observed a lowered rate of triacylglycerol turnover in nutrient-deprived knockdown cells. Labelling with 13C16 palmitate revealed minimal acyl chain shuffling within triacylglycerol, confirming that triacylglycerol hydrolysis, specifically, was dysfunctional in PDK1 reduced cells. Further, algorithmic prediction from our lipidomics dataset identified ATGL as a candidate enzyme with decreased activity. Collectively, our studies define a non-canonical role for PDK1 in the control of triacylglycerol turnover to increase fatty acid oxidation, independent of PDH activity.

20.4 4:30 p.m. – 4:50 p.m.	302 NAD+ Depletion Drives Metabolic Dysfunction in Diabetic Kidney Disease: Integrating Clinical Biomarkers with Mechanistic Insights

PRESENTING AUTHOR: *Jianhong Ching, Duke-NUS Medical School, Singapore*

CO-AUTHORS: *Jianhong Ching, Kee Voon Chua, Jian-Jun Liu, Lye Siang Lee, Jean-Paul Kovalik*

Introduction: Diabetic kidney disease (DKD) remains a leading cause of end-stage kidney disease (ESKD) worldwide, yet the metabolic mechanisms underlying disease progression remain incompletely understood.

Aim: To identify novel metabolic pathways and potential therapeutic targets in DKD using an integrated metabolomics approach.

Methods: We conducted targeted metabolomic profiling on mass spectrometry in three independent clinical cohorts of patients with type 2 diabetes. Findings were further investigated in DKD mouse models and HEK293 cells using stable isotope tracing with 13C-labeled glucose and palmitate under diabetic conditions. NAD+ supplementation experiments evaluated potential therapeutic mechanisms.

Results: In clinical cohorts, urinary TCA cycle intermediates (fumarate and citrate) independently predicted progression to ESKD after adjustment for clinical risk factors. Plasma tryptophan was inversely associated with ESKD risk, while the kynurenine-to-tryptophan ratio was positively associated. Kynurenic acid and xanthurenic acid showed protective effects. Disruption to the TCA cycle and tryptophan metabolism suggested NAD+ depletion as a central theme in DKD pathogenesis. Stable isotope tracing revealed that kidney cells primarily utilize palmitate for energy production even under normal conditions. Under diabetic stress, NAD+ depletion led to incomplete fatty acid oxidation, evidenced by accumulation of hydroxylated acylcarnitines derived directly from palmitate. In DKD mouse models, we observed increased tissue levels of lactate and specific acylcarnitines, confirming dysregulated energy metabolism. NAD+ supplementation reduced inflammation and fibrotic gene expression in kidney cells exposed to diabetic conditions.

Conclusions: Our findings reveal a novel metabolic vulnerability in DKD where NAD+ depletion disrupts the kidney's intrinsic reliance on fatty acid oxidation. The tryptophan-kynurenine pathway emerges as a critical regulator of NAD+ availability, connecting altered urinary TCA cycle metabolites with systemic metabolic dysregulation. The NAD+ metabolic network represents both a source of prognostic biomarkers and a promising therapeutic target in DKD.

★ AWARD WINNERS

SESSION 20. CARDIOVASCULAR & ENDOCRINE DISEASE	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

20.5 4:50 p.m. – 5:10 p.m.	59	Lipid metabolism drives diet-influenced metabolic health: insights from high-throughput profiling of 10,339 individuals in the Australian Obesity, Diabetes, and Lifestyle Study

PRESENTING AUTHOR: ★ *Habtamu Beyene, Baker Heart And Diabetes Institute, Australia*

CO-AUTHORS: *Habtamu Beyene, Kevin Huynh, Tingting Wang, Michelle Cinel, Natalie Mellett, Gavriel Olshansky, Jonathan Shaw, Dianna Magliano, Corey Giles, Peter Meikle*

Introduction

Diet influences the onset and progression of many diseases, yet the underlying metabolic pathways involved remain unclear. We aim to identify associations between dietary intake and lipid metabolism and evaluate how lipidome-defined diet quality impacts metabolic health and all-cause mortality.

Methods

Using data from the Australian Obesity, Diabetes, and Lifestyle study (AusDiab; n = 10,339), we examined the association between diet (based on a validated 74-item food frequency questionnaire) and plasma lipidome. Lipidomic profiling was carried out using a targeted, liquid chromatography tandem mass spectrometry assay. Diet–lipidome associations were examined using linear models and a novel lipid set enrichment analysis. Cox regression models were used to assess associations between lipidome-derived diet quality scores and all-cause mortality accounting for possible confounding factors. Hazard ratios (HR) are per SD increase in each diet quality score.

Results

Characteristic lipidomic profiles associated with dairy [increase in sphingomyelins, hexosylceramides, and lipids esterified with 14:0, 15:0, 17:0 or 17:1 fatty acid and reduction in triacylglycerol and diacylglycerol species with polyunsaturated fatty acids], red meat and poultry [higher alkyl- and alkenyl-phosphatidylcholine and phosphatidylethanolamine notably, with arachidonic acid and decreased linoleic (18:2) and very long-chain fatty acids (e.g.,24:0)], and fish [higher 22:6, and lower 22:4 fatty acids] reported. Intake of nuts (coefficient = -0.07, $p = 7.92 \times 10^{-18}$) and fish (coefficient = -0.05, $p = 3.21 \times 10^{-8}$) were associated with lower lipidome derived CVD risk score. Lipidome-derived diet quality for the Global Diet Quality Score and the Australian Dietary Guideline Index were associated with a 13% (HR = 0.87, 95% CI = 0.83–0.92) and an 11% (HR = 0.89, 95% CI = 0.84–0.93) lower risk of all-cause mortality, respectively.

Conclusions

Our findings revealed links between diet, the plasma lipidome and mortality risk highlighting the role of diet in shaping lipid metabolism and overall metabolic health.



SESSION 21. GOING SMALL – LOW VOLUME AND LOW SAMPLE AMOUNT	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

21.1 3:30 p.m. – 3:50 p.m.	334 A multi-omics method for the study of plant natural product biosynthesis in single cells

PRESENTING AUTHOR: *Lorenzo Caputi, Max-Planck Institute For Chemical Ecology, Germany*

CO-AUTHORS: *Hai Anh Vu, Moonyoung Kang, Chenxin Li, Lorenzo Caputi, C. Robin Buell, Sarah E. O'Connor*

Plant secondary metabolites are synthesized by the sequential action of dedicated enzymes that can be located within different cell compartments or in different cell types. Thus, the correct assembly of the final products requires a highly regulated and coordinated action of enzymes, transporters and accessory proteins across different cell types within the tissue. To fully understand these processes, it is necessary acquire gene expression and metabolomics data at cellular level.

Here, we present a new metabolomics method for targeted and untargeted analysis of isolated plant single cells (protoplasts). The workflow is designed so that RNA-seq data can be obtained from the same cells, providing a very powerful tool to study complex metabolic pathways. As a test case, we selected the medicinal plant Catharanthus roseus, a medicinal plant well known for producing the anticancer drug vinblastine.

After enzymatic tissue dissociation, the cells are trapped on a nanowell membrane. A high precision cell picking robot allows imaging and collection of the cells and their transfer to 96-well plates. Cells are lysed and analyzed by high resolution UPLC/MS. Approximately 200 cells/day can be analyzed. In our untargeted metabolomics experiments, we have been able to robustly identify up to 300 chemicals in a single cell and to assign them to specific chemical classes with good confidence. Targeted analysis of compounds of interest in single cells is revealing the presence of metabolic pools in different cell types in the examined tissues and the accumulation of certain metabolites to high millimolar levels.

We have also integrated RNA-seq analysis on the same cells and our preliminary data showed that an average of 5000 genes can be detected in each cell, in addition to the metabolites. We believe that this approach could greatly benefit the wider community of researchers in plant natural product biosynthesis.

21.2 3:50 p.m. – 4:10 p.m.	289 Subcellular lipidome and proteome profiling utilizing one-pot lipidomic/proteomic organelle immuno-precipitation

PRESENTING AUTHOR: *Brian DeFelice, CZ Biohub – San Francisco, United States*

CO-AUTHORS: *Brian DeFelice, Ira Gray, Max Frank, Frank McCarthy, Eileen Wang, Duo Peng, Manual Leonetti, Josh Elias*

We recently published a comprehensive map of subcellular protein organization, harnessing the power of parallel native immunoprecipitation (IP) (Hein et al., Cell 2024). Now we apply this approach to better define the subcellular lipidome. To this end we developed high sensitivity lipidomics approaches to simultaneously profile lipids and proteins in a one-pot experiment quantifying proteins and lipids from the same immunoprecipitated cell line samples. A549 and HEK293T cell lines were CRISPR-Cas9 engineered to express proteins containing 3xHA tags on cytosolic-facing terminus of subcellular compartments. IP was performed in parallel by splitting a single organelle slurry across two tubes, and preparing each for proteomics and lipidomics respectively.

We present the paired lipidome and proteome from epitope tagged cells lines representing the mitochondria, lysosome, golgi, endoplasmic reticulum, peroxisome, and endosome creating an atlas of paired organellar lipids and proteins. Integrating proteome data from each IP provides valuable insight into the efficiency of each IP. In this study we have implemented a nanoflow chromatography lipidomics approach to maximize the number of lipid species annotated from a single well of a 6-well tissue culture plate.

In preliminary data we have processed and curated data from five subcellular compartment protein targets: ATG101 (autophagosome), CLTA (early/late endosomes), LAMP1 (lysosome and late endosome), PEX3 (peroxisomes), TOMM20 (mitochondria) and a non-tagged control HEK293T (wtHEK293T). All files were processed together using MS-Dial v4.60, reported lipids were required to be present in at least 3 out of 4 replicates in at least one cell line to be retained. All annotations were manually reviewed. In total 1624 unique lipids were annotated. When comparing LAMP1 to wtHEK293T the average enrichment of Bis(monoacylglycero)phosphates was >50 fold-change across 17 annotated species. In TOMM20 samples compared to wtHEK293T 50 cardiolipins were annotated and an average enrichment of >15 fold change.

SESSION 21. GOING SMALL – LOW VOLUME AND LOW SAMPLE AMOUNT	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

21.3 4:10 p.m. – 4:30 p.m. **197 Can blood microsampling devices rival plasma for metabolomics?**

PRESENTING AUTHOR: ***Daniel Marques De Sa e Silva, Greece***

CO-AUTHORS: ***Daniel Marques De Sa e Silva, Marlene Thaitumu, Philippine Louail, Johannes Rainer, Glykeria Avgerinou, Anatoli Petridou, Vassilis Mougios, Georgios Theodoridis, Helen Gika***

Blood microsampling (BμS) has emerged as a promising approach in the analysis of endogenous metabolites, especially for its minimally invasive sample collection and simplified analysis logistics. Different BμS devices are currently available; although they share similar concepts for sample collection, matrix variations might impact the analytical outcomes. The present work aims to evaluate the global metabolic profiles obtained from different BμS devices, namely Dried Blood Spots (Whatman), Volumetric Absorptive Microsampling (Mitra), and Capitainer cards, compared to plasma.

For this purpose, venous blood was collected from 10 healthy, overnight-fasted individuals and loaded onto the devices. Plasma was produced from the same venous blood. Two untargeted metabolomic profiling methods were used: a reversed-phase UHPLC system coupled to a TIMS-qTOF MS operating in DDA mode and a GC-MS method with methoxyamine and trimethylsilane derivatization. For LC-MS, ESI-MS conditions were optimized to enhance sensitivity and reduce in-source fragmentation. Extraction protocols, optimized for each technique, involved a mixture of MeOH-H_2O (60:40, v/v) for LC-MS analysis and neat MeOH for GC-MS. Data pre-processing was performed using MSDial, XCMS, and Hystar software. Statistical analysis was performed in SIMCA. Quality control samples – injected periodically throughout the run – and internal standards were used to account for instrumental drift, perform normalization, and verify the data quality.

The different matrices were compared regarding the number, intensity, and annotation of features. Results indicate that BμS devices, especially Mitra and Capitainer, provide metabolic profiles comparable to, or even more informative than, those obtained from plasma.

(The work has been Funded by the European Union under the HORIZON-MSCA-2021 project 101073062 : HUMAN – Harmonising and unifying blood metabolic analysis networks.)

21.4 4:30 p.m. – 4:50 p.m. **362 Microflow liquid chromatography coupled to multinozzle electrospray ionization mass spectrometry for discovery lipidomics**

PRESENTING AUTHOR: ***Isabel Meister, University of Geneva, Switzerland***

CO-AUTHORS: ***Sergey Girel, Mathieu Galmiche, Mathis Fiault, Valentin Mieville, Patrycja Nowak-Sliwinska, Serge Rudaz, Isabel Meister***

Most bioanalytical laboratories rely on LC-MS-based workflows operating at analytical flow rates of 0.2-1mL/min with the use of electrospray to produce charged ions for MS detection. However, this configuration tends to suffer from matrix effects and ion suppression, hindering the detection of low-abundant compounds or weakly ionizable analytes. Nanoflow (10-1000 nL/min) is well recognized as offering major improvements in ionization efficiency and matrix effects but requires long run times and is prone to clogging and damage. This work presents a microflow LC set-up coupled to a novel microfabricated multinozzle electrospray (mnESI) emitter for untargeted lipidomics. This emitter splits the flow into 5 or 8 streams, enabling access to nanoflow ionization when working in microflow conditions. We present here our lipidomics profiling platform operating at 600 nL/min per nozzle for a run time of 19 minutes. Compared to our established analytical flow platform, the mnESI setup produced a 16-fold median increase in intensity across 69 deuterated lipid standards, with markedly less in-source fragmentation and reduced formation of adduct ions. The performance of mnESI was also evaluated in the context of the profiling of 3D clear cell renal cell carcinoma model exposed to an optimized multidrug combination. The annotation rates increased from 752 to 1270 lipids in the mnESI setup. Among those, 762 achieved <10% variation on pooled QC samples for the mnESI method compared to only 361 in the routine platform, demonstrating the reliability of the additional annotations obtained with the mnESI setup. We also observed as much as 79 oxidized triglycerides and 38 cholesterol esters (only 5 and 4 detected in routine, respectively), as well as 12 sitosterol esters, not yet reported in mammalian cell cultures, highlighting the potential of mnESI for discovery workflows.

SESSION 21. GOING SMALL – LOW VOLUME AND LOW SAMPLE AMOUNT	Wednesday, June 25	3:30 p.m. – 5:10 p.m.

21.5 4:50 p.m. – 5:10 p.m.	350 Rapid Microbore Metabolic Profiling (RAMMP) for High-Throughput Lipid Analysis in Blood Microsampling Devices

PRESENTING AUTHOR: *Ana Sanchez Lorenzo, Imperial College London, United Kingdom*

CO-AUTHORS: *Ana Sanchez Lorenzo, Philippine Louail, Johannes Rainer, Tim Ebbels, Elizabeth Want*

Microsampling devices enable the rapid collection of thousands of blood samples, making them particularly advantageous for high-throughput metabolic profiling in large-scale studies. However, their integration with a fast analytical method enhances their compatibility, improving the workflow by minimizing the sample volume, reducing analysis time, accelerating data acquisition, and decreasing the risk of technical bias compared to conventional methods.

Rapid Microbore Metabolic Profiling (RAMMP) methods were developed to provide ultra-fast liquid chromatography analysis cycles of up to five minutes while maintaining high retention time repeatability. Although RAMMP has been successfully applied to urine and plasma, its performance in blood microsampling devices or whole blood has not yet been evaluated. This study assesses the feasibility of the RAMMP method for metabolic analysis of blood microsampling devices against plasma and whole blood and compares its performance with a conventional UPLC-MS approach.

Conventional untargeted reversed-phase lipid profiling was performed using a UPLC ACQUITY™ BEH C8 (2.1 × 100 mm, 1.7 µm) column with a 0.6 mL/min flow rate over 13.25 minutes and 2 µL volume injection. This method was scaled down using a UPLC ACQUITY™ BEH C8 (1.0 × 50 mm, 1.7 µm) column, with a reduced flow rate of 0.3 mL/min over 3.7 minutes and 1 µL injection volume. Blood and plasma samples from 10 healthy volunteers were provided by the HUMAN Doctoral Network. Data acquisition was conducted on a Synapt G2-S system in positive ionisation mode, with data processing performed using Metabonaut and R for mass spectrometry package.

Results demonstrate that RAMMP significantly reduces analysis time while maintaining comparable chromatographic performance to conventional UPLC-MS methods. Although RAMMP exhibits lower peak capacity and detects fewer ions, it achieves similar lipid group discrimination, supporting its potential as a high-throughput alternative for large-scale microsampling-based studies.

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SESSION 22. NUTRITION AND EXPOSOME RESEARCH	Thursday, June 26	10:30 a.m. – 12:15 p.m.

22.1 KEYNOTE 10:30 a.m. – 11:00 a.m.	550	Unveiling Diet–Health Links: The Power of Diverse Study Designs in Precision Nutrition, Recent Advances in the Application of Metabolomics for Nutrition and Health

PRESENTING AUTHOR: ***Cristina Andres-lacueva, University of Barcelona – Ciberfes, Spain***

Understanding and studying diet and nutrition is fundamental not only for identifying and preventing chronic diseases but also for promoting long-term health and healthy aging. Nutrition plays a central role in maintaining physiological balance, supporting immune function, and reducing the risk of conditions such as cardiovascular disease, type 2 diabetes, obesity, and certain cancers. Moreover, as populations age globally, it becomes increasingly important to focus on how dietary patterns influence aging trajectories. Therefore, advancing nutritional research is essential for developing effective strategies aimed at disease prevention, health promotion, and achieving healthy aging at both individual and population levels. Different study designs offer unique perspectives and contribute complementary insights. In recent years, dietary biomarkers have emerged as a promising alternative for improving dietary assessment. These are small molecules, typically under 1000 Da that are derived from specific foods, absorbed and metabolized, including microbiota metabolites and can be detected in biospecimens. Metabolomics has been adopted as one of the key methods in nutrition research. The increasing availability of large biological and nutritional databases has accelerated the identification, validation, and implementation of dietary biomarkers. By leveraging nutrikinetics, randomized controlled trials (RCTs), and observational studies, researchers have gained valuable insights into the behavior and applicability of these biomarkers. Integrating data from these diverse research methodologies is helping to build more robust and comprehensive tools for dietary assessment. These efforts are at the core of Precision Nutrition, as promoted by the Healthy Diet for a Healthy Life Initiative. Although challenges remain—particularly regarding full biomarker validation—the insights gained so far represent a major step forward in enhancing the quality of nutrition research and its application in both public health and personalized interventions.

22.2 11:00 a.m. – 11:20 a.m.	378	Finger sweat analysis enables individual metabolic monitoring of nutritional intervention

PRESENTING AUTHOR: ★ ***Daniel Wasinger, University Of Vienna, Austria***

CO-AUTHORS: ***Daniel Wasinger, Michael Wolf, Gregor Hahn, Samuel M. Meier-Menches, Christopher Gerner***

Eccrine sweat from the finger tips has been shown to contain not only a large part of the endogenous human metabolome, but also orally or intravenously administered xenobiotic compounds within minutes. Pinching filter paper between the thumb and index finger for one minute deposits detectable sweat, analyzable via mass spectrometry. This flexible, non-invasive method may serve as an alternative or complement to blood plasma for biomonitoring and metabolic phenotyping. Using this approach, an intervention study with red wine was carried out using an untargeted LC-MS workflow.

Samples were taken from 17 individuals, with 13 drinking 3 mL/kg body weight red wine and 4 receiving water instead as a negative control. Samples were taken hourly, with 4 timepoints prior to intervention and 4 timepoints afterwards. All participants received the same meals in-between to minimize confounding of other nutritional factors.

Differential time-course analysis identified >100 significantly regulated features (adjusted p value $<10^{-6}$) with highly conserved kinetic profiles. These included signals contributable to well known constituents of wine such as gallic acid and malonic acid. The selfsame features were verified analyzing wine extracts.

Interestingly, some significantly induced features were not found in wine extracts, likely constituting metabolic breakdown products or endogenous responses. Most notably, some individuals lacked signals for specific molecules, which were otherwise consistent across most participants. Moreover, this phenomenon seems to affect only certain classes of molecules but not others. This suggests a phenotypic difference that could be of high interest in the study of pharmacokinetic relationships, where distributions of substances across tissues are of great importance.

Collectively, these findings demonstrate that sweat from the finger tips may be able to not only capture specific responses to nutritional intervention but also allow for personalized metabolic phenotyping.

SESSION 22. NUTRITION AND EXPOSOME RESEARCH	Thursday, June 26	10:30 a.m. – 12:15 p.m.

22.3 11:20 a.m. – 11:40 a.m.	336 Metabolomic and transcriptomic signatures of steatotic liver disease, with focus on sex-specific impacts of PFAS

PRESENTING AUTHOR: *Matej Orešič, Örebro University, Sweden*

CO-AUTHORS: *Shashank Gupta, Oveis Jamialahmadi, Rosellina Mancina, Tuulia Hyötyläinen, Stafano Romeo, Matej Oresic*

Per- and polyfluoroalkyl substances (PFAS) are environmental pollutants linked to metabolic disorders, including metabolic dysfunction-associated steatotic liver disease (MASLD). Here we investigated the associations between PFAS exposure and MASLD progression, focusing on metabolomic and transcriptomic signatures, and sex-specific responses. Metabolomic and lipidomic analyses of liver biopsies from MASLD patients (n=211) were performed by LC-MS(/MS) to examine changes in metabolite composition across steatosis and fibrosis stages. A total of 2,005 lipids and 701 polar metabolites were detected, of which 257 and 78 were identified, respectively. A total of 10 PFAS compounds were included in the analysis. Transcriptomic data were analyzed using weighted gene co-expression network analysis (WGCNA), with KEGG pathway enrichment for functional insights. Lipidomic analysis revealed shifts in lipid composition with steatosis severity, including increased triacylglycerols (TG) containing saturated (SFA) and monounsaturated fatty acid levels in MASLD and decreased phosphatidylinositol and phosphatidylcholine. Transcriptomic analysis identified significant modules in both sexes. These modules were inversely associated with arginine biosynthesis and amino acid metabolism. Metabolomic analysis confirmed these findings, implicating a disruption in amino acid metabolism with disease progression. Positively associated transcriptomic modules in females were linked to cell cycle, steroid biosynthesis, and fatty acid metabolism. Positive associations were observed between PFAS (PFUnDA, PFDoDA) and phosphatidylinositol, while acylcarnitines showed inverse associations. Mediation analysis showed that specific metabolites partially mediated the relationship between PFAS exposure and steatosis. In males, lactosylceramide (LacCer) mediated the effect of PFHpA and PFNA on steatosis, where LacCer had a protective effect for PFNA (negative association) but not for PFHpA (positive association). In females, TG-SFA and phosphatidylethanolamine mediated the effect of PFBS on steatosis. The mediation effect of phosphatidylethanolamine was protective, whereas TG-SFA was not. In conclusion, study provides insights into the mechanisms linking PFAS exposure to MASLD progression, highlighting disrupted amino acid and lipid metabolism and sex-specific responses.

22.4 11:40 a.m. – 12:00 p.m.	183 Early life exposure to endocrine disruptors: Understanding the neuro-endocrine impact from metabolome mapping of rat models

PRESENTING AUTHOR: *Pim Leonards, Vrije Universiteit Amsterdam, Netherlands*

CO-AUTHORS: *Pim Leonards, Sara Evangelista, Margret Schlumpf, Jesus Tresguerres, Beatriz Linillos Pradillo, Marja Lamoree, Walter Lichtensteiger*

Exposure to endocrine-disrupting chemicals (EDCs) in humans during pregnancy and early stages of life can impair normal brain development and reproductive function patterns, leading to severe pathologies later in life. Studies have shown developmental neurotoxicity effects of bisphenols (BPA mostly) and phthalates. However, for many chemicals, such as pyrethroids, per- and polyfluoroalkyl substances (PFAS), organophosphate flame retardants, and plasticizers, hardly any information is available on the relationship between endocrine disruption (ED) and neurodevelopmental (DNT) effects. To better understand the connection between the ED system and DNT outcomes, F0 rat dams were exposed to six EDCs (BPF, BBzP, DINCH, PFOS, permethrin, triphenyl phosphate) from pre-mating through lactation, and their offspring were assessed in adulthood for activity, learning and memory, anxiety, and social behavior. Steroid and thyroid hormones, neurotransmitters and untargeted lipidomics were determined in rat hippocampus (PND6), and the data were used to map the metabolic pathways affected by the EDCs and were correlated to memory function of the littermates. Changes in levels of neurosteroid hormones, neurotransmitters, and hundreds of lipids in the hippocampus were sex- and chemical-specific, and correlated with adverse memory outcomes (Morris water maze) in adult rat littermates. For instance in females pregnenolone-sulphate (PREG-S) and GABA, and in males PREG-S, acetylcholine, histamine and corticosterone, and in both sexes hundreds of lipids were significantly regulated, and correlated to adverse memory function. Previous studies have demonstrated a significant correlation between PREG-S and cognitive function, as well as its role in stimulating acetylcholine release, findings also observed in our study. Altered lipid levels in hippocampus (PND 6) by EDCs correlated with sex-dependent behavioral outcomes. Our findings contributes to understanding the molecular mechanisms of toxicity in the developing neuro-endocrine systems, but also provides new insights in their connections to cognitive function. This project received funding from EU Horizon 2020 (No. 825759, ENDpoiNTs).

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SESSION 22. NUTRITION AND EXPOSOME RESEARCH	Thursday, June 26	10:30 a.m. – 12:15 p.m.

22.5 12:00 p.m. – 12:15 p.m.	479	Comprehensive characterisation of children's exposure to air pollutants using personal samplers and a multiplatform High-Resolution Mass Spectrometry approach

PRESENTING AUTHOR: ★ *Camilla Guerrini, Universitat Rovira i Virgili, Spain*

CO-AUTHORS: *Camilla Guerrini, Mahsa Naghavi Sheikholeslami, Maria Vinaixa, Noelia Ramirez*

Globally, 93% of all children live in environments with air pollution levels above the WHO guidelines. More than one in every four deaths of children under 5 years old is directly or indirectly related to environmental risks. Evidence suggests that exposure to pollutants can cause epigenetic and metabolic alterations, leading to irreversible damage later-in-life, including neurological, metabolic, cardiovascular, and respiratory disorders. However, few studies thoroughly investigate the atmospheric chemical exposome, as traditional targeted approaches focus on specific compound classes,overlooking its intricate impacts on health.

This work aims to accurately determine the personal exposome in children using personal wearable samplers and advanced analytical and data processing strategies. We utilized silicone wristbands (SWBs) as cost-effective, easy-to-use, and child-friendly passive samplers for volatile and semi-volatile organic compounds. Our analytical workflow integrates gas chromatography-high-resolution mass spectrometry (GC-HRMS) and wide-scope suspect screening liquid chromatography-high-resolution tandem mass spectrometry (LC-HRMS/MS),using the R package HERMES to improve the quality of the mass spectra and capture a broad array of pollutants. We applied this methodology to a cohort of 218 children (aged:8-12) living in the Tarragona area, near the Southern Europe's largest petrochemical complex.Through LC-HRMS/MS, we detected 634 atmospheric pollutants, including tobacco smoke toxicants, personal care products, insect repellents, and plastic additives. Among these, 29 were annotated at level 2, and 8 were identified with standards. GC-HRMS analysis annotated 121 compounds with a cosine similarity exceeding 0.8. As a case study, we confirmed the underestimation and ubiquity of tobacco smoke exposure, with nicotine, cotinine, nicoteline, and myosmine detected in 80% of the participants, despite lower reported home exposure rates.This study demonstrated how complementary analytical platforms and passive samplers enhance our understanding of pervasive outdoor and indoor air pollution exposures, highlighting the need for integrating environmental exposome analysis in public health and policies to reduce exposure among vulnerable populations.



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SESSION 23. FOOD, HEALTH, AND SUSTAINABILITY	Thursday, June 26	10:30 a.m. – 12:15 p.m.

23.1 KEYNOTE 10:30 a.m. – 11:00 a.m.	551 Efficient identification of underexplored health-promoting metabolites through integration of food and human metabolome data and nutritional epidemiology

PRESENTING AUTHOR: ***Sastia Prama Putri, Osaka University, Japan***

Common approaches for investigating food bioactive properties often do not consider the vast array of identifiable metabolites in food. Without information metabolite of this food's constituents, less-common metabolites with bioactive properties present in low concentrations may be overlooked. Even in cases where data capturing most of the food metabolome is available, assessing the bioactive properties of these metabolite constituents often poses a significant challenge. Applying conventional biochemical assays to assess the biological activities of the entire food metabolome would be impractical and costly. Recently, cheminformatics approaches have been developed to predict the bioactivity of food metabolites, such as predictions based on metabolites' chemical structures and interactions. While these approaches are more scalable and can be used to rapidly and systematically predict the bioactive properties of food metabolites, these in silico predictions and biochemical assays are not based on human health data, such as from epidemiological studies or clinical trials. The integration of human health data into biological activity prediction is crucial to provide physiological context and improve accuracy, as in silico and in vitro testing alone often demonstrate poor correlation with in vivo biological activities. To address these challenges, a data-driven multidisciplinary framework for investigating bioactive metabolites in foods is proposed. This framework integrates three key components: a food metabolome library, publicly available metabolite pharmacological databases, and a human metabolite library derived from epidemiological studies. The utility of this initial groundbreaking approach was recently demonstrated through a pilot study which led to the identification of an underexplored LDL-cholesterol lowering compound, meglutol, from soybean fermented food tempe. The methods employed here are highly expandable to other food that have been considered to have health promoting benefits but relatively understudied. The approach can facilitate more efficient screening process of health beneficial component for the development of new functional food.

23.2 11:00 a.m. – 11:20 a.m.	462 1H NMR spectral database for Czech wine quality control

PRESENTING AUTHOR: ***Jaroslav Havlik, Ceska Zemedelska Univerzita v Praze, Czech Republic***

CO-AUTHORS: ***Jaroslav Havlik, Anna Mascellani Bergo, Petr Gondas, Lubomir Matak***

Nuclear magnetic resonance (NMR) is a powerful spectroscopic technique for detecting wine fraud. In collaboration with the National Wine Centre of the Czech Republic, a ^{1}H NMR-based spectral database was developed to support wine quality control and local viticulture.

A dataset of 5,000 NMR spectra was compiled, including 15 varietal wines with detailed metadata, including variety, origin, harvest year, and chemical composition (e.g., alcoholic strength, acids, residual sugars, residual extract), determined using standard ISO methods. Spectra were acquired using a Bruker 500 MHz spectrometer at 298 K, pH 4.0, with DSS as internal standard.

Chemometric models for wine quality control were developed based on a subset of 900 wine spectra. These models successfully discriminated between red and white wines, categorized wines based on residual sugar content (dry, semi-dry, and sweet), and identified specific varietal differences. A trained Random Forest (RF) model classified Pinot Noir with 96% accuracy, Blaufränkisch 96%, Riesling 92%, Cabernet Sauvignon 77%, Chardonnay 76%, Gewürztraminer 60%, Hibernal 60%, Grüner Veltliner 52%, Pinot Gris 48%, Sauvignon Blanc 45%, and Pálava 40%. The NMR quantification of glucose and fructose correlated highly with chemical analyses of residual sugars (RMSE 1.8, R^2 = 0.99), though it showed lower accuracy for alcoholic strength determination (R^2 = 0.5). Additionally, Pinot Blanc and Chardonnay were successfully differentiated despite their close ampelographic and sensory characteristics, which often lead to misclassification in vineyards. The wine profiles are regularly used to verify the authenticity of wines in the competition and the market, helping to confirm suspected samples.

The tool has been developed and implemented to support wine control authorities and viticulture professionals in the Czech Republic.

Acknowledgment:

Facilities were provided by METROFOOD-CZ, supported by the Czech Ministry of Education, Youth, and Sports (LM2023064).

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SESSION 23. FOOD, HEALTH, AND SUSTAINABILITY	Thursday, June 26	10:30 a.m. – 12:15 p.m.

23.3 11:20 a.m. – 11:40 a.m.	428 Unlocking the potential of metabolomics for comprehensive insights into edible insects

PRESENTING AUTHOR: *Kateřina Šebelová, University of Chemistry and Technology, Czech Republic*

CO-AUTHORS: *Kateřina Šebelová, Ondřej Pospíšil, Štěpánka Kučková, Kamila Zdeňková, Pavel Vejl, Matej Pospiech, Lenka Kouřimská, Jana Hajšlová*

Edible insects offer a promising solution for the future food supply, particularly in meeting the growing global demand for proteins and other nutrients. In the European Union, four insect species (Acheta domesticus, Tenebrio molitor, Locusta migratoria, and Alphitobius diaperinus) have been authorized and added to the list of novel foods under Regulations (EU) 2015/2283 and (EU) 2017/2470. However, there are still significant gaps in the availability of comprehensive validated laboratory methods that allow for unbiased assessments of their authenticity to avoid potential fraudulent practices and to enable effective quality control of insect-based products. The presented study is focused on metabolomics as a complementary strategy to genomics and proteomics for the authentication of edible insect species. Analyzed samples of insects collected over two years included 10 insect species (authorized and unauthorized). Samples were obtained from different farmers, differing in rearing conditions and ways of killing. Non-targeted screening based on U-HPLC-HRMS/MS followed by multivariate analysis (PCA and PLS-DA) of generated data was employed to characterize the extracts. Safe distinguishing of individual species was achieved, and potential markers of species were selected, tentatively identified based on the exact m/z, isotopic profile, and fragmentation pattern, and added to the in-house database of MS/MS spectra. Their selectivity was tested using real foods from the market that included an insect component. Moreover, it was possible to identify the killing method and rearing sites of given species due to the association between metabolome and particular feed composition. Overall, the great advantage of the metabolomic strategy over genomics and proteomics is the possibility of detecting compounds associated with different killing methods and various feed components, which represents great potential for evaluating the general quality and safety (detection of hazardous compounds) of edible insects and products thereof available on the market.

23.4 11:40 a.m. – 12:00 p.m.	143 Decoding the Geographical Origins of Tea: A Machine Learning Driven Untargeted Metabolomics Approach for Fraud Detection

PRESENTING AUTHOR: ★ *Yicong Li, Queens University Belfast, United Kingdom*

CO-AUTHORS: *Yicong Li, Nicholas Birse, Yunhe Hong, Brian Quinn, Natasha Logan, Christopher T. Elliott, Di Wu*

The global tea industry faces significant challenges related to mislabeling and fraud in geographical origin claims. With complex supply chains, limited transparency, and a wide variety of products such as bulk tea, loose leaves, and premium tea bags on the market, ensuring tea authenticity has become a critical priority for both consumers and industry stakeholders. This study presents an innovative biomarker discovery workflow that integrates high-resolution untargeted metabolomics with machine learning centred automated data analysis to detect global tea fraud. A total of 302 black tea samples from nine major tea-producing regions-Ethiopia, Darjeeling, Assam, China, Kenya, Rwanda, Malawi, and Sri Lanka-were analyzed using UHPLC-QToF-MS/MS under a rigorous quality control protocol. Our research found that different geographical indications (GIs) have distinct metabolite fingerprints. Compared to conventional chemometric approaches, machine learning models demonstrated superior performance in handling imbalanced datasets. To overcome the limitations of traditional VIP-based marker selection workflow, we developed a novel method that integrates multiple parameters, including VIP scores, coefficient of variation, log transformation, p-value, and fold change, improving both predictive accuracy and biological relevance while minimizing false positives. Using this approach, we identified 229 and 145 markers distinguishing African and Asian tea-growing regions, respectively. These markers were validated through internal and external validation, achieving 100% predictive accuracy. After metabolite annotation, flavonoids, terpenoids, catechin gallates, amino acids, and tannins were identified as key discriminators among different tea-producing regions. Region-specific biomarkers were also identified, such as ganoderic acid in Assam, carnitine in Kenya, and isochlorogenic acid in Malawi. To enhance data processing efficiency and reliability, the proposed novel pipeline was automated to streamline data analysis, minimize manual intervention, and improve scalability and reproducibility in biomarker discovery. Our proposed machine learning driven untargeted metabolomics approach can be used as a confirmatory tool to enhance tea traceability, detect and prevent global tea fraud.

SESSION 23. FOOD, HEALTH, AND SUSTAINABILITY	Thursday, June 26	10:30 a.m. – 12:15 p.m.

23.5 12:00 p.m. – 12:15 p.m.	320 Lipidomic Analysis Unveils S. maritima as a Sustainable Source of Food Lipids through Hydroponic Cultivation

PRESENTING AUTHOR: *Marisa Pinho, University Of Aveiro, Portugal*

CO-AUTHORS: *Marisa Pinho, Ana Moreira, Stefano Bonciarelli, Laura Goracci, Tânia Melo, Pedro Domingues, Ana Isabel Lillebø, Javier Cremades, Maria Rosário Domingues*

Halophytes are salt-tolerant plants, increasingly used in cuisine and food preparation. They are valued for their unique flavors, high nutritional content, and adaptability to saline conditions. Salicornia (sea asparagus) is the most well-known halophyte, but other species like Suaeda maritima are being studied for their nutritional and biotechnological potential. These plants have health-promoting effects and a long history of use in traditional medicine. As sustainable crops, they have the unique ability to thrive in saline environments where conventional crops struggle due to poor soil quality, making them ideal for eco-friendly cultivation systems such as hydroponics and aquaponics. This adaptability further enhances their agricultural and economic value. This study aimed to assess the hydroponic cultivation of S. maritima, focusing on biomass production and lipid composition. For the first time, the lipidome of the aerial organs of S. maritima was characterized via C18-RP-LC-MS/MS, and its FA composition was analyzed by GC-MS. The plants grew successfully in hydroponics, with biomass peaking at 8-weeks. A total of 17 FA were identified, including high amounts of essential fatty acids FA18:2 n-6 and FA18:3 n-3, showing higher levels in hydroponic samples. Lipidomic analysis revealed 443 lipid species, including glycerophospholipids, glycolipids, acylglycerols, sphingolipids, sterol lipids, prenol lipids. Hydroponic samples had more glycolipids with high PUFA levels, known for anti-inflammatory properties, while sphingolipids and acylglycerols were more abundant in wild samples. Glycerophospholipids profile showed most variation between 4- and 8-week cultivation times, with species with PUFA showing higher concentrations at 4-weeks. Overall, they have high levels of this class that its a known transporter of n-3 and n-6 PUFA. These findings suggest hydroponic cultivation enhances S. maritima's biochemical stability, reinforcing its potential as a lipid-rich, sustainable crop. Given their resilience and nutritional quality, halophytes should be reconsidered as viable sources for functional food and lipid-based ingredients.



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SESSION 24. DATA PROCESSING AND CHEMINFORMATICS | Thursday, June 26 | 10:30 a.m. – 12:15 p.m.

24.1 10:30 a.m. – 10:50 a.m. | **89** **RforMassSpectrometry: A Community-Driven Ecosystem for Reproducible and Scalable Mass Spectrometry Data Analysis in R – Join Us, We Have Spectra!**

PRESENTING AUTHOR: *Johannes Rainer, Eurac Research, Italy*

CO-AUTHORS: *Johannes Rainer, RforMassSpectrometry Contributors*

A common problem with scientific research software is the lack of support, maintenance and further development. In particular, development by a single researcher can easily result in orphaned and dysfunctional software packages, especially if combined with non-compliance with software development standards.

The RforMassSpectrometry initiative aims to develop an efficient and stable infrastructure for mass spectrometry (MS) data. As part of this initiative, a growing ecosystem of tightly integrated R packages covering various aspects of metabolomics and proteomics data analysis is being developed. Their interoperability enables creation of complete analysis workflows without tedious data conversions. To avoid the aforementioned problems, community contributions are encouraged and emphasis is placed on open development, documentation and long-term support.

At the heart of the package ecosystem are the “Spectra” and “Chromatograms” packages that provide the core infrastructure for processing LC-MS data. Their design allows easy expansion to support additional file types, data representations or storage formats. The “xcms” package for LC-MS data preprocessing was recently updated to leverage this infrastructure enabling preprocessing of very large or remote data sets. Such data sets can also be loaded directly from the MetaboLights repository using the “MsBackendMetaboLights” package allowing seamless reanalysis of public dataset. Annotation of untargeted metabolomics experiments can be performed using the “MetaboAnnotation” package while the required annotation resources are easily accessible through packages such as “MsBackendMassbank”, “MsBackendMsp” or “CompoundDb”, the latter also allowing creation and management of lab-specific compound databases. The “SpectriPy” package enables the direct integration of Python libraries to create even more powerful analysis workflows. Finally, the `MsCoreUtils` and `MetaboCoreUtils` packages provide efficient implementations of commonly used algorithms, designed to be reused in other R packages. Ultimately, and in contrast to a monolithic software design, the RforMassSpectrometry software ecosystem enables implementation of powerful, data set tailored, and reproducible analysis workflows.

24.2 10:50 a.m. – 11:10 a.m. | **163** **xcms at 20 and still in Peak form: Now anchoring a complete ecosystem for metabolomics data preprocessing and analysis.**

PRESENTING AUTHOR: *Philippine Louail, Institute for Biomedicine, Eurac Research, Italy*

CO-AUTHORS: *Philippine Louail, William Kumler, Pablo Vangeenderhuysen, Steffan Neumann, Michael Witting, Carl Brunius, RforMassSpectrometry Contributors, Johannes Rainer*

High-quality data preprocessing is essential for untargeted metabolomics experiments, where increasing dataset scale and complexity demand adaptable, robust, and reproducible software solutions. Modern preprocessing software must evolve to integrate seamlessly with downstream analysis tools, ensuring efficient and streamlined data analysis.

Since its introduction in 2006, the xcms R package has become one of the most widely used preprocessing tools. Its development has followed an open-source, community-driven approach, ensuring long-term stability, continuous innovation, and broad accessibility.

We present recent advancements and extensions of xcms, including methodological improvements, expanded support for diverse mass spectrometry (MS) data formats, and enhanced integration with a growing ecosystem of R packages for MS data analysis. Improved data structures and flexible handling enable efficient processing of large-scale experiments with over 10,000 samples, even on standard computational setups. Lastly, xcms now interfaces with external software such as Sirius, GNPS and various Python libraries, facilitating more comprehensive workflows.

These enhancements effectively establish xcms as a central component of a complete and interconnected software ecosystem, empowering users to create complete, tailored, reproducible data analysis workflow, without format conversions needed. A growing collection of tutorials and teaching materials helps users navigate this ecosystem, making it easier to leverage native R functionalities, Bioconductor packages, and statistical modeling tools to extend and customize their workflows.

Over the last two decades, xcms has embraced a strategy of flexibility, external collaboration, and open-source innovation rather than a monolithic structure. This approach has positioned xcms as a modern, adaptable preprocessing tool that will continue to evolve with the metabolomics community for the next 20 years and beyond.

SESSION 24. DATA PROCESSING AND CHEMINFORMATICS	Thursday, June 26	10:30 a.m. – 12:15 p.m.

24.3 11:10 a.m. – 11:30 a.m. **275 Multiplet Matching in Metabolomic J-Res NMR Spectra using Deep Learning Video Networks**

PRESENTING AUTHOR: *Yan Yan, Imperial College London, United Kingdom*

CO-AUTHORS: *Yan Yan, Michael Judge, Jimenez Beatriz, Timothy Ebbels*

Metabolite annotation remains a major challenge in NMR based metabolomics due to the complexity of the spectra. This is especially true in human biofluid samples, where matrix composition shifts compound signals across spectra. A key task is to detect and match multiplets between different samples. Here, we present a novel approach that treats sequential J-resolved (J-Res) NMR spectra from multiple samples as a "video", exploiting advanced object detection and tracking techniques to facilitate multiplet matching.

Our methodology comprises two main steps. Firstly, we detect the multiplets using a modified YOLO-based video object detection network, enhanced by J-Res NMR-specific augmentations to increase data diversity and a symmetric loss function to accommodate the inherent symmetry of J-Res NMR data. Subsequently, we employ an adapted BOT-SORT tracking algorithm to associate these detected multiplets across sequential spectra. Key modifications include enforcing directionality constraints to reflect pH-dependent shifts and integrating a J-Res NMR-specific similarity measure that enhances multiplet association accuracy. This approach extracts intensity variations for each multiplet throughout the "video", which enables clustering of multiplets with similar intensity profiles across samples, thereby aiding in grouping signals originating from the same metabolite.

Evaluations on both simulated J-Res NMR spectral sequences and real experimental data demonstrate high detection and tracking performance, particularly for doublets, triplets and quadruplets. Singlets, with their non-unique peak shape, are comparatively more challenging to track. Nevertheless, our findings illustrate the potential of this approach to expedite metabolite annotation by reducing reliance on time-consuming and labour-intensive expert analysis.

In conclusion, this YOLO-based detection and BOT-SORT tracking framework provides a powerful and efficient strategy to detect and group NMR multiplets across samples, offering a significant contribution to the field of NMR-based metabolomics. Through continued refinement and validation, this method promises to advance our capacity for precise and comprehensive metabolite analysis in complex mixtures.

24.4 11:30 a.m. – 11:50 a.m. **396 LipidCXSMILES: A dynamic solution to represent lipid structures towards the "Dark Matter" discovery**

PRESENTING AUTHOR: *Zhixu Ni, TU Dresden, Germany*

CO-AUTHORS: *Zhixu Ni, Egon Willighagen*

Current high throughput lipidomics studies commonly report hundreds of lipid species at the discrete level and provide lists of lipid annotations using the shorthand nomenclature. However, the mainstream workflows generally lack precise structural details, such as the information of C=C bond locations and missing other detailed structure information at cis/trans and R/S level. As a result, many lipid shorthand names cannot be directly converted into InChI or SMILES for advanced informatics analysis. Additionally, SMILES based lipidomics methods may limit the search space, miss annotate or over reporting the lipid exact structures and leading to incorrect mapping to the pathways.

Recent advances in instrumentation and analytical workflows have revealed novel lipid species from previously unassigned MS/MS spectra, often referred to as "Dark Matter." While spectral evidence supports the identification of new lipid subclasses, partial substructures remain unresolved, which also prevents the direct generation of corresponding InChI or SMILES.

Thus, the application of many InChI or SMILES based metabolomics techniques require special optimization to be applied in lipidomics. To address these challenges, we developed LipidCXSMILES based on the Chemistry Development Kit (CDK) and extended SMILES (CXSMILES). LipidCXSMILES enables dynamic representation of newly identified lipid structures with uncertain substructures, while maintaining compatibility with lipid shorthand nomenclature. The flexibility of LipidCXSMILES allows to use a single string to represent a collection of possible candidate structure from a lipid shorthand abbreviation at the discrete level (e.g, C=C position isomers of PC 18:1_18:3).

Together with the efforts from advanced analytical methods and new lipid database such as PartialDB from LIPIDMAPS, LipidCXSMILES can enhance the next generation lipid profiling to discover new lipid species from the "Dark Matter" of MS data. Code examples to generate CXSMILES and figures from fatty acids and phospholipids are available on GitHub (https://github.com/egonw/cdk-cxsmiles).

★ AWARD WINNERS

SESSION 24. DATA PROCESSING AND CHEMINFORMATICS	Thursday, June 26	10:30 a.m. – 12:15 p.m.

24.5 11:50 a.m. – 12:10 p.m. **131 Deep Learning-Assisted Single-Cell Data Processing Platform for Metabolite Profiling**

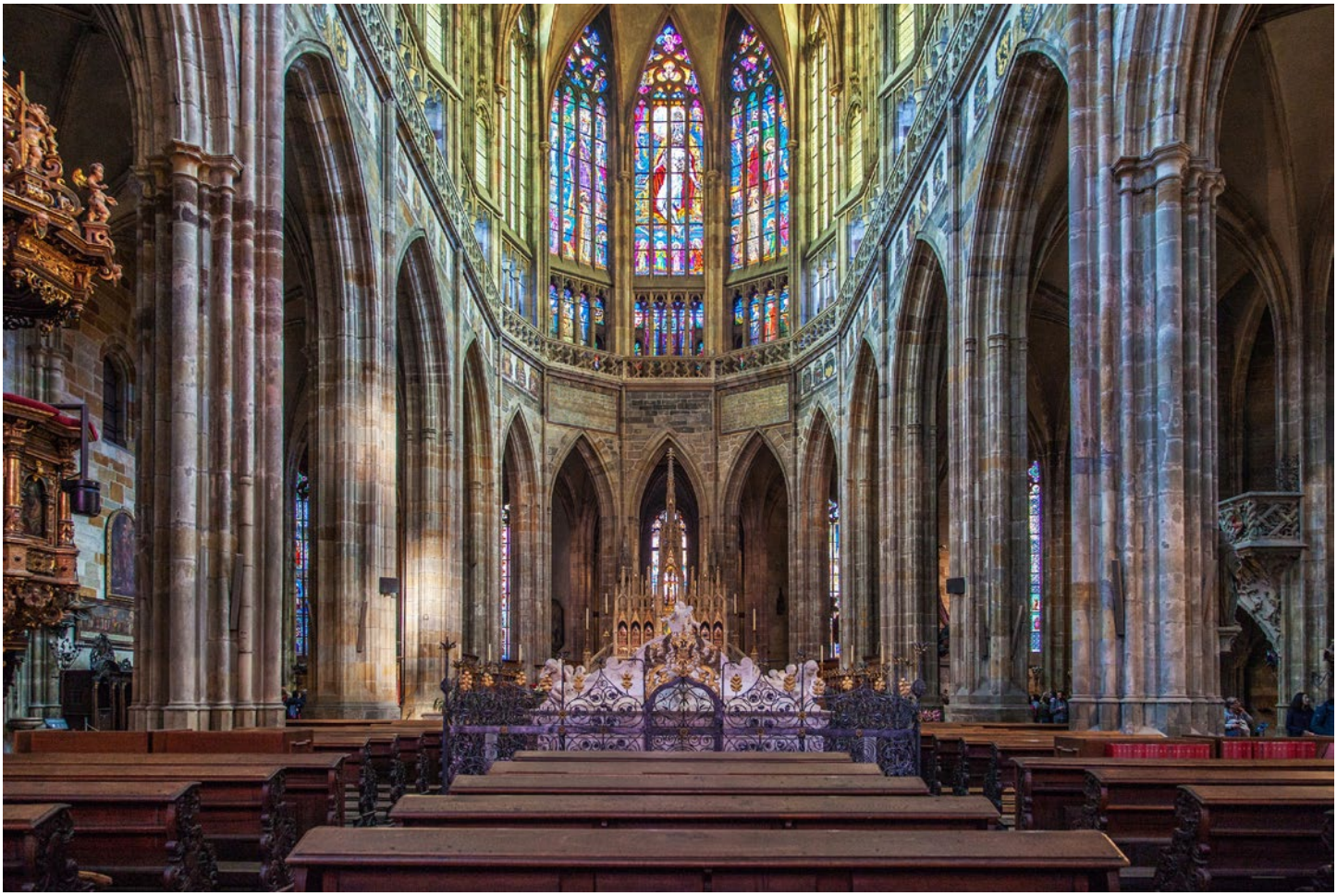
PRESENTING AUTHOR: ★ *Felix Friedrich, Uppsala University, Sweden*

CO-AUTHORS: *Felix Friedrich, Cátia Marques, Ingela Lanekoff*

Mass spectrometry-based single-cell omics reveal the heterogeneity of individual cells. Analyzing hundreds of cells demands selective and sensitive data processing tools. We developed a fast, reproducible data extraction platform for targeted and untargeted methods. Untargeted approaches face challenges in removing background peaks while preserving m/z values from cells. We present and validate a deep-learning image classification algorithm that retains endogenous metabolites from single cells, eliminating background noise.

An Orbitrap™ IQ-X™ Tribrid™ (Thermo Fisher Scientific, USA) was used in positive mode at a mass resolution of 240K. Individual cells were positioned in an array using the CellenONE (cellenion). The content of each cell was desorbed and ionized from a glass slide using a tapered pneumatically assisted nanospray desorption electrospray (PA nano-DESI) probe. The data was acquired in one .RAW file for subsequent data extraction, processing, and feature validation using the developed single-cell data processing pipeline (MATLAB, R2023b). A pre-trained image classification model (EfficientNetb0) was transfer-learned on extracted ion chronograms and validated on unseen data.

Three classes were established based on different signal-to-noise ratios (S/N), encompassing endogenous features with high or low S/N or background species. The validation results demonstrated a high accuracy, with over 97% correct classifications. The sensitivity of the model was evaluated based on the number of retained endogenous analytes, which is essential for achieving high analyte coverage. A high sensitivity of 95% was attained for the true feature class, signifying that very few features are lost due to misclassification. The model's specificity was assessed by removing background features from the combined high and low S/N classes, the endogenous feature class. Only 8% of the endogenous feature class consisted of background, indicating a high-performing model. Thus, a new tool for more streamlined and high-throughput data processing for single-cell metabolomics is now accessible.



POSTER ABSTRACTS



CORPORATE POSTERS

C1	SCIEX

Small molecule omics analysis using the ZenoTOF 8600 system

PRESENTING AUTHOR: *Paul Baker, United States*

CO-AUTHORS: *Jason Causon, Robert Proos, Rebekah Sayers*

Mass spectrometry is the primary means for analyzing lipids and metabolites in studies of human metabolism. Targeted analysis using nominal mass instruments has long been the workflow of choice for quantitative analysis of biomolecules, and high-resolution mass spectrometers have been the preferred option for untargeted analysis. The drive towards the simultaneous acquisition of quantitative and qualitative data has given rise to data-independent analytical workflows such as SWATH. With the launch of the ZenoTOF 8600 system, the gap between quantitative and qualitative analysis has closed with the ability to quantitate biomolecules with the sensitivity similar to that of a high-end triple quadrupole and to structurally characterize molecules with CID- and EAD-based fragmentation. Targeted analysis using the MRMHr scan mode, untargeted analysis using DDA approaches, and a novel workflow, ZT SCAN 2.0 DIA, now enable sensitive, specific, and quantitative analysis of small molecules in biological samples.

Presented here are 4 examples of the power of the ZenoTOF 8600 system for the analysis of lipids and metabolites in human-derived samples. The dramatic improvement in the sensitivity of the ZenoTOF 8600 system compared to the ZenoTOF 7600 system is shown at the TOF MS and TOF MS/MS levels, with a >10-fold increase in sensitivity when the instruments are compared in both targeted and untargeted experiments. The improved sensitivity resulted in increased numbers of lipids and metabolites identified in DDA experiments using the ZenoTOF 8600 system (1.8- and 1.4-fold improvements, respectively). Bile acids were measured using a targeted workflow on both instruments, revealing a >10-fold improvement in the LOQ values for each bile acid analyzed. Furthermore, capitalizing on the EAD-based fragmentation ability of the ZenoTOF 8600 system, unique diagnostic fragments were identified for near-eluting bile acid isomers, to allow for shorter gradients and ultimately increased sample throughput.

C2	Thermo Fisher Scientific

Orbitrap-based Metabolomics Workflows: Discover. Innovate. Exceed

PRESENTING AUTHOR: *Susan Bird, United States*

CO-AUTHORS: *Bashar Amer, Rahul Deshpande, Michal Kaczmarek*

The field of Metabolomics has been advancing at an impressive rate, inspiring key analytical innovations designed to keep pace with the biological needs of a study. Despite these advances, challenges remain around compound annotation and identification. ThermoFisher Scientific is committed to advancing the field of metabolomics through intelligence driven data acquisition, streamlined software processing strategies and key hardware innovations that keep at pace and push beyond the current analytical demands of the field. Herein, we present a novel hybrid orbitrap mass spectrometer with key technological advances that directly address many common metabolomics pain points. Hardware design improvements applied to the ion source and ion transfer regions provide increased sensitivity in typical full scan experiments which is applicable to both qualitative and quantitative aspects of metabolomics research. Additionally, the high-confidence detection and identification of low to medium abundant metabolites in complex matrices is directly addressed. Segmenting the mass range into narrower windows has shown to improve spectral signal-to-noise ratios and increase the detection quality of low-abundance compounds, however, this method is historically manual. We advanced these concepts by implementing intelligent MS1 multiplexing strategies with optimized injection times to directly increase metabolome coverage, specifically of these low abundant species. Utilizing this novel instrument we apply the enhanced dynamic range (eDR) typical to diverse sample matrices such as plasma, liver and plants and highlight how the improved source design and eDR workflow improve upon the number of compounds detected and the sensitivity of detection for key metabolites.

C3	Bruker Life Sciences Mass Spectrometry

Unlocking Metabolomic Insights with Bruker's Cutting-Edge Technology Portfolio

PRESENTING AUTHOR: *Matthew Lewis, United Kingdom*

CO-AUTHORS: *Nikolas Kessler, Sofie Weinkouff, Stefanie Wernisch, Sonja Klee, Claire Canet, Sven Meyer, Aiko Barsch,*

Since the inception of modern metabolomics, Bruker technologies have enabled the field's leading researchers in their quest to discover unique biochemical signatures of health, disease, treatment response and exposures in complex biological samples. Today, our comprehensive suite of mass spectrometry (MS) and nuclear magnetic resonance (NMR) systems continues to revolutionize small molecule research in metabolomics. We empower scientists pushing past the conventional barriers in measurement sensitivity and selectivity, scale and speed of sample analysis, spatial resolution for imaging studies, metabolome coverage, confident metabolite annotation and point of need testing.

The timsTOF series, featuring trapped ion mobility spectrometry (TIMS), offers 4D analysis capability for detailed metabolomic profiling with exceptional selectivity, sensitivity and precision. Our MALDI solutions enable label-free mapping of metabolites, providing detailed molecular insights and revealing regional heterogeneity in complex samples. MRMS (Magnetic Resonance Mass Spectrometry) provides unmatched resolution and mass accuracy, enabling precise identification and quantification of metabolites for challenging applications and sample types. To expand the chemical space of the portfolio, the ecTOF revolutionizes the analysis of (semi)volatile substances by coupling gas chromatography with a dual ionization, high-resolution TOF MS. Using electron impact (EI) and chemical ionization (CI) simultaneously within a single chromatographic run accelerates and improves the process of confident compound identification and quantification.

Finally, our advanced NMR systems enable robust structural elucidation of metabolite interactions and dynamics, and their benchtop counterparts bring these capabilities to the mainstream. Together, these instruments empower researchers to push the boundaries of metabolomics, uncovering new insights into metabolic pathways, disease mechanisms, and therapeutic targets. Bruker's commitment to innovation and excellence ensures that our products not only meet but exceed the demands of modern metabolomics research. Join us at the forefront of metabolomics with Bruker's powerful and expansive instrument portfolio, driving discoveries that shape the future of health and science.

CORPORATE POSTERS

C4 | **Agilent Technologies**

Agilent's End-to-End Solutions Help You Take Your Metabolomics Workflows to the Next Level

PRESENTING AUTHOR: ***Daniel Cuthbertson, United States***

Agilent Technologies offers comprehensive solutions for metabolomics research outlined in this poster. The Bravo Metabolomics Sample Prep Platform enables reliable and standardized sample preparation, enhancing consistency and throughput. You will learn about the targeted Omics Platform using highly curated methods for easy implementation and get insights into Discovery Metabolomics workflows using advanced software for feature extraction, statistical analysis, pathway visualization and flux analysis. Comprehensive Cell analysis solutions are described, which enable deeper, functional insights into Metabolism of cells.



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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1000 A — Datoma: a cloud computing platform for high-throughput metabolomics data analysis

PRESENTING AUTHOR: ***Roger Giné Bertomeu, Universitat Rovira i Virgili (URV), Spain***

CO-AUTHORS: ***Enrique Martínez, Eduard Bel, Faizan Khan, Usama Benabdelkrim, Pedro García, Oscar Yanes***

SUB THEME: ***Cloud computing***

Cloud computing addresses the growing need for fast, scalable and reproducible data analysis workflows. In metabolomics, several web- and cloud-based platforms have emerged in recent years, including Workflow4Metabolomics (Giacomoni et al., 2014), Metaboanalyst (Pang et al., 2022), Phenomenal (Peters et al., 2018), XCMSonline (Tautenhahn et al., 2012), METLIN and GNPS (Aron et al., 2020). However, these platforms face significant limitations in computing performance, scalability for large datasets, and workflow flexibility.

We present Datoma, a cloud-native computing platform that integrates over 20 curated metabolomics tools (e.g., XCMS, rMSI, eRah, MSConvert, MSBuddy, LipidMS, SIRIUS). Users can access Datoma through a web interface or programmatically via a dedicated Python package. Designed for modularity and interoperability, Datoma enables migration of bioinformatics tools (R- or Python-based) using reproducible Docker images and parametric scripts. Custom, user-defined workflows can be built by linking tool output files to subsequent inputs using regular expressions. Additionally, we provide two pre-configured workflows: one for LC-MS quantification and characterization (XCMS, RHermes, qHermes) and another for LC-MS/MS spectral annotation and identification, which consolidates outputs from multiple tools (SIRIUS, ChemEmbed, SingleFrag, MSBuddy) into a single report.

Datoma significantly accelerates large-scale data processing. For instance, we analysed a 164GB imaging-MS dataset (67 images, >1.8M pixels and 3270 features) using rMSI (Ràfols et al., 2017), achieving an 860% speed-up compared to a workstation PC, reducing computation time from 26 hours to just 3 hours.

By offering reproducible, scalable, and high-performance data analysis without dependency management or tool-specific API constraints, Datoma streamlines metabolomics workflows and promotes collaborative research. Datoma is freely available at app.datoma.cloud and via the Datoma Python package at https://pypi.org/project/datoma/.

1001 A — SpectriPy: Bridging R and Python for Cross-Language Mass Spectrometry Data Analysis

PRESENTING AUTHOR: ***Marilyn De Graeve, Eurac Research, Italy***

CO-AUTHORS: ***Wout Bittremieux, Thomas Naake, Matthias Anagho-Mattanovich, Nils Hoffmann, Pierre Marchal, Victor Chrone, Philippine Louail, Carolin Huber, Helge Hecht, Michael Witting, Johannes Rainer***

SUB THEME: ***Collaborative and open data science***

Over the past decade, tremendous efforts have been made to develop powerful algorithms and excellent data analysis software for mass spectrometry (MS) and metabolomics data analysis. These include, among others, R packages from the RforMassSpectrometry initiative such as Spectra, MsCoreUtils, MetaboAnnotation and CompoundDb, as well as Python libraries like matchms, spectrum_utils, Pyteomics and pyOpenMS. Each of these softwares covers different and in part complementary aspects in the analysis of MS data, but their integration into a single workflow remains, in particular across programming languages, challenging.

Here we present SpectriPy*, an R package that efficiently translates MS data structures between R and Python. By leveraging R's "reticulate" system, SpectriPy enables a seamless cross-language integration allowing R and Python MS data analysis algorithms to be combined within unified analysis workflows. A set of example use cases, implemented as Quarto documents, were developed during the EuBIC developers meeting 2025 to demonstrate the advantages and power of this approach.

To summarize, SpectriPy enables and simplifies the integration of R and Python data analysis, empowering data analysts to benefit from the full power of algorithms from both programming languages. Further, software developers can now reuse algorithms across languages rather than re-implementing them, enhancing efficiency and collaboration.

* SpectriPy: https://rformassspectrometry.github.io/SpectriPy/articles/SpectriPy.html; https://github.com/rformassspectrometry/SpectriPy

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1002 A — MetaboSERV – a platform for selecting, exchanging, and visualizing metabolomics data with controlled data access

PRESENTING AUTHOR: *Helena U. Zacharias, Hannover Medical School, Germany*

CO-AUTHORS: *Tim Tucholski, Angela Maennel, Yacoub Abelard Njipouombe Nsangou, Sven Schuchardt, Matthias Gruber, Fabian Kellermeier, Katja Dettmer, Peter J. Oefner, Wolfram Gronwald, Michael Altenbuchinger, Jürgen Dönitz, Helena U. Zacharias*

SUB THEME: *Collaborative and open data science*

The growing number of metabolomics studies, based on high-dimensional data measured by hyphenated mass spectrometry (MS) and/or nuclear magnetic resonance (NMR) spectroscopy, has sparked the creation of several public metabolomics data repositories. Each repository emphasizes different aspects regarding data selection and representation, but most offer only limited options for privacy-preserving data sharing.

We present MetaboSERV, an open-source, browser-based metabolomics platform dedicated to the selection, integration and sharing of quantitative metabolomics data and metadata with controlled data access. MetaboSERV aims to aid researchers in analyzing their results by facilitating means to browse, visualize and compare data across available data sets. It provides different access control functionalities, creating an environment in which data can be shared safely in a privacy-preserving manner to support collaborative and interdisciplinary research. Furthermore, it is designed to be extensible and adaptable to existing data management infrastructures through the creation of self-managed MetaboSERV instances, for which we provide the source code and a set of configurable Docker images. We demonstrate the utility of MetaboSERV in several use cases, spanning targeted and untargeted NMR and MS data.

The public MetaboSERV instance is available at https://metaboserv.ckdn.app, and the source code can be found at https://gitlab.gwdg.de/MedBioinf/metabolomics/metaboserv.

1003 A — Enhancing Lipid Annotation Quality in MZmine Through Automated Expert-Rule Assessment

PRESENTING AUTHOR: *Alberto Gil-de-la-Fuente, CEU-san Pablo University, Spain*

CO-AUTHORS: *Blanca Pueche, Sara Martinez, Miguel Fernández-García, Ana Gradillas, Coral Barbas, Abraham Otero, Alberto Gil-de-la-Fuente*

SUB THEME: *Data processing & software tools*

Lipid annotation remains challenging due to their isomeric/isobaric overlap and the fact that multiple ion species can be generated during LC-ESI-MS ionization. To address this, a curated lipid database for UHPLC-ESI(+-)-QTOF-MS was developed at CEMBIO, containing 592 lipid molecular species annotated with their qualitative and semiquantitave adduct profile by lipid class from NIST SRM 1950 human plasma [1]. This database provides expert knowledge to assess lipid annotation based on experimental adduct/cluster profiles. This adduct profile depends on the mobile phases, additives and the biological matrix and structural constraints inherent to lipid functional groups

A new module was developed in MZmine which uses expert-defined rules to validate lipid annotations based on those adduct/cluster patterns. The rules are implemented using Drools, a rule-based engine that uses “if-then” logic. The module requires prior correlation grouping and lipid annotation, functionality already available in MZmine. It reads the lipid class and associated adduct and it applies rules to determine whether the annotation follows expert-defined patterns. When the annotation follows a pattern, positive evidence supporting the annotation is accrued, and when it does not, negative evidence is accrued. A global evidence score helps the user assess annotation quality. When no annotation is available, the module searches for adducts within a feature group to provide chemical insights. It also considers the mobile phases and sample matrix compatibility.

The previously annotated lipids are now evaluated and validated by using the expert knowledge from the CEMBIO database in a fully automated way. This proves to be an efficient and useful tool that supports or rejects the annotation based on the adduct and cluster profile and the reasoning behind its assignment, supporting high-quality lipidomics workflows. The module can be integrated into other tools since the knowledge base is isolated from MZmine.

References

[1] Martínez S et al. doi: 10.1016/j.jlr.2024.100671.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1004 A — LipidMS Shiny app: Improving Lipid Annotations through Retention Time Modeling in Lipidomics

PRESENTING AUTHOR: *Marta Roca Marugán, FUNDACIÓN PARA LA INVESTIGACIÓN DEL HOSPITAL LA FE CIF: G97067557 La Fe, Spain*
CO-AUTHORS: *Celia Regal Marqués, Maria Isabel Alcoriza Balaguer, Juan Carlos García-Cañaveras, Agustín Lahoz Rodríguez*
SUB THEME: *Data processing & software tools*

Lipidomics is an emerging field focused on the large-scale analysis of lipids in biological systems. Despite its potential, lipid annotation remains challenging, with high rates of incorrect annotations due to issues like misidentification of adducts and in-source fragments. To address these issues, a previous approach developed an algorithm incorporating retention time (RT) to improve annotation accuracy. The method filters lipids by allowing the identification of in-source fragments and removing those that do not follow the expected RT pattern based on their carbon number (CN) and double bonds (DB). Additionally, linear regression models were used to predict RT for candidate lipids based on m/z values, aiming to propose new, more accurate lipid annotations. In this work, we present the implementation of this strategy in the open-source R package LipidMS, incorporating the developed function that models RT behavior across lipid classes based on carbon number (CN) and double bonds (DB), using gamma distribution models. To facilitate the curation of automatically generated annotations and RT-based predictions, we have developed a Shiny application that enables users to interactively visualize lipid class distributions, explore RT trends, and manually filter annotations. This interface significantly enhances the transparency and confidence in lipid annotation workflows, combining automated processing with expert curation. The workflow is currently compatible with lipid annotations generated specifically using LipidMS, ensuring full integration and consistent data formatting. This approach substantially reduces false annotations while proposing new lipid candidates supported by both MS and RT evidence.

1005 A — Confident annotation of GPNAE and HexGPNAE in Caenorhabditis elegans aided by homologous series extension in LC-MS/MS data

PRESENTING AUTHOR: *Michael Witting, Helmholtz Zentrum Muenchen, Germany*
CO-AUTHORS: *Nikolas Kessler, Aiko Barsch, Matthew R. Lewis*
SUB THEME: *Data processing & software tools*

Lipids and lipid-like molecules can be systematically described by their head groups, side chains, double bonds, and modifications. They form grids in LC-MS/MS data based on this systematic nature. We present a workflow that can detect and extend these grids by detecting and extrapolating homologous series for molecular formulas, retention times, and collision cross sections, revealing potential yet unannotated lipid candidates or validating RT and CCS values of annotated lipid candidates relative to their peers.

This new functionality allowed us to semi-automatically derive and visualize homologous series of lipid-like Glycerophospho-N-Acylethanolamides (GPNAE) and Hexosylglycerophospho-N-Acylethanolamides (HexGPNAE) molecules in Caenorhabditis elegans and revealed prospective positions (exact molecular formula and m/z, extrapolated RT and CCS) of additional members.

C. elegans samples have been extracted with H2O/MeOH (v/v, 50/50) and measured using UHPLC-HRMS/MS . Lipid-like molecules were separated on a Waters Acquity BEH C18 column with an H2O/ACN-gradient and detection was carried out in positive and negative ionization mode using data-dependent acquisition. Data analysis was performed in Bruker MetaboScape 2025b.

Known GPNAE, HexGPNAE and LPE species were initially annotated using manually curated Target Lists; other lipids were annotated using rule-based lipid annotation. GPNAEs and LPE represent isomeric structures, which require chromatographic separation. Though they show different fragmentation behaviour, specific members show only low abundance for which no or only low-quality fragmentation spectra can be obtained. In the separation method used in this study, GPNAEs can be differentiated from LPEs, and both form independent homologous series. This is reflected in two almost parallel regression lines in m/z vs. RT plots. Therefore, we utilized trendlines along RT and CCS for enhanced identification.

Employing this novel workflow, we were able to annotate several novel GPNAE and HexGPNAE species, missed during initial annotation.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1006 A — imputomics evolved: expanding imputation methods and redesigning benchmarks for missing data imputation

PRESENTING AUTHOR: *Jarosław Chilimoniuk, Medical University of Białystok, Poland*
CO-AUTHORS: *Krystyna Grzesiak, Michał Burdukiewicz*
SUB THEME: *Data processing & software tools*

Missing values remain a significant bottleneck in metabolomics, impacting data integrity and subsequent biological interpretation. imputomics was originally developed as an open-source benchmarking framework to systematically evaluate imputation methods within the context of metabolomics. As the field evolves, the need for more comprehensive and metabolomics-aware tools for managing missing data also increases.

We are expanding imputomics to encompass a broader range of imputation methods, integrating recent advancements in traditional and machine learning-based approaches. This expansion seeks to capture a wider spectrum of assumptions regarding missingness in metabolomics data, from left-censoring to stochastic dropout, while maintaining a focus on methods commonly used in untargeted metabolomics.

Simultaneously, we are redesigning the benchmarking pipeline to more accurately represent real-world metabolomics use cases. This involves revisiting evaluation metrics to prioritize biological relevance, such as the preservation of sample group separation and pathway-level signals, alongside classical statistical metrics. Additionally, we are curating new datasets across various analytical platforms (e.g., LC-MS, GC-MS, NMR) and reworking our simulation framework to better model missingness patterns observed in experimental data.

This ongoing work seeks to enhance imputomics as a more flexible, rigorous, and metabolomics-centered resource for the evaluation of imputation strategies. By facilitating more informed preprocessing decisions, we aspire to enhance transparency, reproducibility, and the overall quality of metabolomics data analysis.

1007 A — Enhanced spectrum clustering for interpretable molecular networking

PRESENTING AUTHOR: *Janne Heirman, University Of Antwerp, Belgium*
CO-AUTHORS: *Wout Bittremieux*
SUB THEME: *Data processing & software tools*

Introduction & aim

Small molecule tandem mass spectrometry (MS/MS) produces vast datasets, making interpretation challenging. Molecular networking aids analysis by linking spectra based on similarity, identifying groups of similar compounds. Clustering is a crucial preprocessing step to reduce data redundancy and enhance network interpretability. Here we introduce enhanced clustering approaches to improve molecular network interpretability, integrated into Falcon, an efficient clustering tool available via GNPS.

Methods

Low-quality spectra were removed, and noise peaks filtered before clustering spectra using the cosine similarity, followed by molecular networking with the modified cosine similarity. Consensus spectra were generated by a novel consensus algorithm with noise rejection. Clustering methods were evaluated based on cluster completeness, the proportion of clustered spectra, and the incorrect clustering rate. This methodology was tested on 502,993 MS/MS spectra acquired on an Astral mass spectrometer. Ground truth labels were based on feature detection results from MZmine.

Results

We evaluated hierarchical clustering (single, average, and complete linkage) alongside DBSCAN, which was previously used in Falcon. At a maximum threshold of 13% incorrectly clustered spectra, hierarchical clustering with complete linkage significantly outperforms DBSCAN, clustering 89.7% of spectra (11.8% incorrect), while DBSCAN clusters only 39.8% (12.9% incorrect). This improvement results in smaller molecular networks, increasing their interpretability.

A trade-off exists between completeness and clustering accuracy. DBSCAN achieves a higher completeness score (0.856), whereas hierarchical clustering with complete linkage attains a slightly lower score (0.826) but with a better clustering accuracy.

Overall, incorrectly clustered spectra remain high compared to the 1% standard, likely due to strict feature detection. This will be addressed in future work. Still, hierarchical clustering significantly improves clustering accuracy over DBSCAN while maintaining high completeness.

Conclusion

Optimized spectrum clustering reduces redundancy and improves molecular network interpretability, accelerating molecular discoveries in metabolomics.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1008 A — A-SIMA/A-MAP: a comprehensive toolkit for NMR-based metabolomics analysis

PRESENTING AUTHOR: ***Woonghee Lee, University Of Colorado Denver, United States***
CO-AUTHORS: ***Abigail Chiu, Mehdi Rahimi, Woonghee Lee***
SUB THEME: ***Data processing & software tools***

We present two new computer programs, A-SIMA: Advanced-Software for Interactive Metabolite Analysis and A-MAP: A Multivariate Analysis Program. These tools aim to introduce new capabilities for metabolite identification and data analysis, and thereby advancing the computational methodology in NMR-based metabolomics. A-SIMA is designed with an easy-to-use graphical user interface (GUI) which allows users to perform metabolite identification on 1D and 2D NMR data effortlessly with complete control over the identification process. Similarly, A-MAP facilitates multivariate statistical analysis of metabolite data through a straightforward process. It offers analysis options such as Principal Component Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) using regions of interests as inputs. Both A-SIMA and A-MAP are pre-built in the POKY suite with tutorial videos on YouTube for guidance on not only the programs, but also installation. The POKY suite is a popular software program for NMR biomolecular analysis. With the addition of these programs in POKY, researchers and professionals can experience a fully integrated process for every step of their metabolite analysis. Data can also be easily exported from these programs to be applied elsewhere. The introduction of A-SIMA and A-MAP can be promising tools that can lead significant advancements in metabolomics research. These tools offer enhanced capabilities for metabolite analysis and statistical modelling in a user-friendly manner. Their integration into the POKY suite ensures accessibility, usability, and efficiency.

1009 A — Interactive Chemical Space Exploration of MS/MS-based Metabolomics Profiles from Complex Mixtures

PRESENTING AUTHOR: ***Florian Huber, Düsseldorf University Of Applied Sciences, Germany***
CO-AUTHORS: ***Julian Pollmann, Niek de Jonge, Luca Barchet, Kevin Zielke, Justin J.J. van der Hooft, Florian Huber***
SUB THEME: ***Data Visualization***

Complex biological samples often comprise hundreds to thousands of organic compounds detectable by tandem mass spectrometry, yet the majority usually remain unidentified. Recent advances in deep-learning now permit the prediction of chemical similarities between compounds solely based on their MS/MS spectra. In our work, we combine multiple deep-learning predictions across a large reference dataset to generate more reliable analogue predictions accompanied by robust uncertainty metrics. This integrated approach significantly enhances computational efficiency, enabling, for the first time, the analysis of entire samples containing thousands of spectra within seconds.

Beyond improved prediction accuracy and speed as compared to single predictions, we introduce an innovative data visualization method that provides intuitive access to comprehensive sample analysis data. Our interactive chemical map displays detected analogues relative to about 40,000 reference compounds from mass spectral libraries of more than 400,000 spectra. Using library metadata, such as chemical compound class terms, along with predicted chemical similarity and certainty metrics, users can select or highlight query spectra. This holistic visualization facilitates rapid, in-depth exploration of complex mass spectral datasets, enabling the detection of novel patterns, outliers, and systematic changes across large datasets, thereby shifting the analytical focus from individual compounds to a broader sample perspective.

The system is implemented with a performance-optimized Python backend and a responsive JavaScript frontend, ensuring both computational efficiency and a user-friendly interface. This seamless integration of advanced deep-learning techniques with rapid, holistic data visualization represents a significant step forward in the analysis of complex biological mixtures, opening new avenues for discovery in metabolomics and related fields.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1010 A — Multi-View Machine Learning for Integration of Multimodal Mass Spectrometry Imaging Data

PRESENTING AUTHOR: ***Lukas Kopecky, Imperial College London, United Kingdom***
CO-AUTHORS: ***Duncan Roberts, James McKenzie, Elmeri Latvanen, Lauren Ford, Zoltan Takats, Elizabeth Want, Timothy Ebbels***
SUB THEME: ***Machine Learning and AI***

Introduction and Aims:

Mass Spectrometry Imaging (MSI) has emerged as a powerful tool for analysing the spatial distribution of molecules directly on complex tissue samples, enabling the unravelling of disease mechanisms and pathology. However, as for all MS-based techniques, due to the different physicochemical properties of metabolites, multiple analytical assays need to be applied using adjacent sample sections. These assays may be of different ionisation polarities or using different matrix types, resulting in partially overlapping but complementary data blocks.

Conventional approaches analyse each data block separately but fails to capture inter-block relationships, making interpretation of multi-assay molecular interactions difficult. Here we propose a workflow using a multi-block – partial least squares (MB-PLS) model to integrate peak picked data from adjacent slices of multi-modal MSI.

Method:

We employed a MB-PLS model coupled with multi-block variable importance in projection (MB-VIP) and permutation testing to estimate the importance of predictors to the outcome variable. We clustered the selected predictors and compared them to groups defined by their structural properties. To explore this concept, we utilised two adjacent sections (positive and negative ionisation) of colorectal cancer tissue analysed using desorption electrospray ionisation (DESI) MSI. We co-localised these slides using CORGI, a pre-existing in-house tool.

Results:

Our integrative model provided a solid predictive performance (>95%), especially when modelling pixels close to the boundary between tumour and healthy tissues. Additionally, the MB-PLS models shed valuable light on the contribution of each data block. Further, our workflow yielded around 20% additional features compared to modelling each block separately.

Conclusion:

Our workflow provides promising results and offers a streamlined modelling framework for the analysis of multimodal MS images. It has the potential to benefit the metabolomics and MSI communities as it offers interpretable integrative analysis of multimodal MSI data, facilitating elucidation of chemically and spatially diverse predictive signatures.

1011 A — De novo molecular structure generation for unannotated mass spectra

PRESENTING AUTHOR: ***Soha Hassoun, Tufts University, United States***
SUB THEME: ***Machine Learning and AI***

BACKGROUND

The annotation rates of MS/MS spectra collected during untargeted metabolomics remains low due to the enormous molecular diversity in biological samples and the limited scope of reference databases. Advances in generative AI and deep learning presents an exciting opportunity for de novo molecular structure generation, thereby significantly accelerating natural product discovery, and expanding our understanding of previously uncharacterized chemical space.

METHODS

This presentation will introduce two novel models for de novo molecular generation. The first model, MADGEN (Mass-spec Attends to De Novo Molecular GENeration) operates in two stages: scaffold retrieval and spectra-conditioned molecular generation starting with the scaffold. In the first stage, given a query spectrum, we formulate scaffold retrieval as a ranking problem and employ contrastive learning to align mass spectra with candidate molecular scaffolds. In the second stage, starting from the retrieved scaffold, we employ the MS/MS spectrum to guide an attention-based generative model to generate the final molecule. Our approach constrains the molecular generation search space, reducing its complexity and improving generation accuracy. The second model, OMG(Optimized Molecular Generation) is comprised of two steps: molecular generation followed by ranking. In the first step, a model pre-trained on large molecular datasets is finetuned using transfer learning from candidate molecules retrieved from PubChem based on the molecular formula associated with the spectrum. Reinforcement learning is utilized to guide the generation of relevant molecular candidates for a queried spectrum. After sampling the finetuned model, we rank the generated structures.

RESULTS

We evaluate MADGEN and OMG on the CANOPUS and the MassSpecGym datasets. Both MADGEN and OMG show promise in improving state-of-the-art molecular generation techniques available in the public domain. This work presents a significant step in advancing de novo molecular generation for unannotated mass spectrum.

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1012 A — How can I make my results look much nicer

PRESENTING AUTHOR: ***Rasmus Bro, University of Copenhagen, FOOD, Denmark***
SUB THEME: ***Machine Learning and AI***

Metabolomic data often suffer from having many variables but few observations. This increases the risk of presenting overly optimistic models that may not reflect reality well.

In this presentation, we will show how models such as PLS and OPLS can mislead the user and moreover we will show the pitfalls of variable selection in detail. What can be achieved with variable selection and what cannot be achieved. And when can we expect variable selection to work.

1013 A — Generative modelling of small molecule tandem mass spectrometry with next token prediction

PRESENTING AUTHOR: ***Luke Zhang, University Of Toronto, Canada***
CO-AUTHORS: ***Adamo Young, Rory Gao, Bo Wang, Hannes Röst***
SUB THEME: ***Machine Learning and AI***

Tandem mass spectrometry (MS/MS) is a core technology for high-throughput small molecule characterization, but identifying molecular structures from their spectra alone is extremely challenging. Reference libraries of compounds and their measured spectra are commonly used for identification, but their low coverage (~1M spectra representing ~100K molecules) results in poor structure identification rates in untargeted MS/MS experiments. An overwhelming majority of publicly available MS/MS data (conservatively ~100M spectra) thus remains unannotated, a significantly greater number than those found in reference libraries. This disparity represents a critical bottleneck in metabolomics, driving demand for computational tools that can help with identification. However, this wealth of unlabelled spectra also presents an opportunity: through self-supervised learning, we may be able to leverage this data to develop models for spectrum generation and representation.

We introduce MSGPT, a generative language model pre-trained on a large dataset of unannotated MS/MS spectra (the GeMS dataset). MSGPT is pre-trained via next-token prediction (NTP), where each token represents a peak in the spectrum. Given a precursor m/z, it can generate a full spectrum in a peak-wise manner. We evaluate MSGPT's generation quality by analyzing spectrum statistics, and we show that as pre-training progresses, the statistics of the generated spectra converge to those of the ground truth spectra. We also evaluate the quality of MSGPT's full-spectrum embeddings, and show that NTP pre-training enables the model to learn important chemical features that translate to downstream tasks.

MSGPT is a novel language model that learns to generate high-quality spectra through self-supervised pre-training, and our results demonstrate that its representations are rich in chemically informative features and learn accurate distributions of spectral properties. These highlight the utility of our model, a promising step towards accelerated, large-scale MS/MS annotation efforts.

1014 A — Quantifying the unknown – How machine learning enables quantification in non-targeted metabolomics.

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CO-AUTHORS: ***Helen Sepman, Wei-Chieh Wang, Veronika Fitz, Louise Malm, Gunda Koellensperger, Anneli Kruve***
SUB THEME: ***Machine Learning and AI***

Introduction and Aim

Non-targeted metabolomics plays a crucial role in the discovery of novel biomarkers in clinical settings. While significant progress has been made in recent years towards the identification of features of interest, their quantification remains complicated due to the lack of available standards. To circumvent this problem, we introduce a novel machine learning-based strategy that provides quantification estimates for tentatively annotated features and consequently holds great potential for other challenges in the field, such as batch effect correction and normalization.

Methods

The model was trained based on a comprehensive LC-HRMS(/MS) data set, including calibration curves for a metabolite standard panel spiked with fully 13C-labeled yeast as internal standard. Based on differences in retention times, predicted ionization efficiencies (log), and PaDEL-descriptors, the calibration curve slope ratio (log) of two metabolites was predicted by applying a tree-based extreme gradient boosting algorithm. The model's performance was assessed based on fold changes and the root mean squared error (RMSE) of the predicted slope ratios for training and test sets. Further model evaluation will be performed using data from a human clinical study.

Results

Promising prediction performance is indicated by training- and test set RMSEs (log scale) of 0.14 and 0.44, as well as corresponding fold changes of 1.40 and 2.76. Quantification estimates are provided based on slope ratio predictions: 1) either by directly calculating the resulting calibration curve slopes for features of interest, or 2) by selecting a potential surrogate standard based on slope similarities. The resulting quantification estimates for features of interest in a clinical metabolomics study will be benchmarked with existing workflows. Moreover, the versatility of the model will be further exploited regarding batch effect correction based on suggested surrogate standards.

Conclusion

The presented slope ratio prediction model provides concentration estimates for features of interest in non-targeted metabolomics.

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1015 A — Preparing a Large-Scale Dataset for HRMS/MS-Based Foundation Models: A New Era Beyond Annotated Data?

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SUB THEME: *Machine Learning and AI*

Most machine learning-based models for compound annotation and property prediction in metabolomics rely on supervised methods that use labelled mass spectrometry datasets for training. While effective, these models often struggle with the limited availability of annotated training data. For example, recent estimates indicate that even by combining the largest MS/MS repositories—NIST2020, MoNA, MassBank, and GNPS—experimental MS/MS data is available for only around 60,000 molecules, which is an order of magnitude below the typical requirements for training generative deep learning models[1]. This data limitation can be addressed by leveraging the vast amounts of non-annotated high-resolution MS data, where millions of MS/MS spectra are routinely generated. Foundation models offer a promising path forward, redefining how we extract and utilize information from HRMS/MS data. These models, trained on broad and diverse datasets—often using self-supervised learning—can be adapted to various downstream tasks, such as developing new algorithms for spectral similarity. In the past three years, foundation models have begun to emerge in metabolomics and show significant potential[2,3]. However, most of these models are currently trained only on available datasets from public repositories. At Wageningen Food Safety Research, we are compiling, centralizing and curating a large-scale dataset to train a foundation model specifically aimed at advancing food safety. Thanks to the high throughput of our routine analytical platforms, we have accumulated a substantial dataset: between 2022 and 2025, we generated 138,162 raw LC-MS files across four Orbitrap instruments, containing 243,814,022 DDA MS2 and 626,966,090 DIA MS2 spectra (including replicates). This effort represents a large initiative to bring foundation models to food safety applications and the metabolomics field in general. As part of this ongoing project, we would like to invite the scientific community to collaborate and together start to redefine a new horizon of AI in HRMS/MS data analysis.

1016 A — PFAS exposure: computational insights into fetal liver metabolism

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CO-AUTHORS: *Tuulia Hyötyläinen, Paul A Fowler, Frankie Alcock, Matej Orešič*
SUB THEME: *Metabolic modeling*

Perfluorinated alkyl substances (PFAS) are persistent endocrine-disrupting chemicals widely used in many industrial products. PFAS exposure alters metabolite levels, including bile acids, thereby increasing susceptibility to the onset and severity of various non-communicable diseases. Biomonitoring studies reveal that PFAS are detected in almost all humans, including fetuses, thus potentially affecting long-term health. Recently, we observed that PFAS accumulates in the fetal human liver and is notably associated with disturbances in lipid and bile acid metabolism. This highlights a need for more in-depth understanding of specific metabolic pathway perturbations in the human fetuses. The present study aims to investigate the metabolic consequences of PFAS exposure in the human fetal liver using omics data and a constrained-based modelling approach. We integrated the liver proteomics and transcriptomics data from 56 human fetuses (gestational ages between 12 and 19 weeks) with a recent comprehensive genome-scale metabolic model to predict the metabolic flux profiles. Our analysis revealed metabolic reprogramming in the developing human liver and its dysregulation due to PFAS exposure. We observed that replication-related pathways are more active at an early gestational age (12 -14 weeks). PFAS-associated metabolic signatures were also identified, notably affecting fatty acids, amino acids, sugar derivatives, and lipid metabolism. Interestingly, model predictions revealed that PFAS negatively impacts steroid metabolism including cholesterol, estrogen and androgen metabolism. Specifically, PFAS exposure was associated with reduced production of 2-methoxyestradiol-17beta (2-ME2), a key regulator of hypoxia, oxidative stress and inflammation. Negative associations were also observed for fluxes in the mevalonate pathway, which plays an important metabolic route controlling cholesterol production. In conclusion, PFAS exposure is associated with disturbed liver development, including reduced production of multiple important steroids. To validate these findings, we will also measure the steroids in human fetal liver samples and assess their associations with PFAS exposure levels.

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1017 A — Targeting metabolic vulnerabilities to combat resistance to FOLFOX chemotherapy in colorectal cancer

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CO-AUTHORS: *David López-Blanco, Sergi Pujol, Nuria Aleixandre, Pablo Añon, Miriam Tarrado-Castellarnau, Francesc Mas, Pedro de Atauri, Sergio Madurga, Silvia Marin*

SUB THEME: *Metabolic modeling*

Colorectal cancer (CRC), the second leading cause of cancer-related deaths globally, exhibits stark clinical challenges despite advances in targeted therapies and chemotherapy. While platinum-based regimens like FOLFOX remain frontline treatments, their long-term efficacy is often thwarted by chemoresistance and metastatic progression. Emerging evidence underscores tumor metabolism as a pivotal mediator of therapeutic response, yet patient-specific metabolic vulnerabilities remain underexploited in clinical settings.

To bridge this gap, we employed a systems biology approach, integrating multi-omics data from FOLFOX-treated and untreated CRC cells into contextualized Genome-Scale Metabolic Models (GEMs). Unlike conventional methods, our pipeline leverages the CORDA (Cost Optimization Reaction Dependency Assessment) algorithm to reconstruct subtype-specific metabolic networks, revealing dynamic adaptations that drive chemoresistance. By coupling these models with flux balance analysis, we identified subtype-specific metabolic rewiring linked to FOLFOX response in CRC cell lines, and novel synthetic-lethal targets whose inhibition could potentiate FOLFOX efficacy

Our findings not only elucidate how CRC cells rewire central metabolism to evade FOLFOX cytotoxicity but also demonstrate CORDA's unique ability to prioritize patient-specific metabolic vulnerabilities. This framework advances precision oncology by translating multi-omics data into actionable targets, offering a roadmap for designing metabolism-aware combination therapies. By moving beyond static biomarker discovery, we provide a dynamic, model-driven strategy to overcome chemoresistance—a critical step toward improving outcomes in CRC and other solid tumors.

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1018 A — Metabolic networks of engineered Escherichia coli with cofactor boosting system for fatty alcohol production modeled by thermodynamically explicit kinetics simulations

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SUB THEME: *Metabolic modeling*

Xylose reductase/lactose (XR/lactose) system has been employed to enhance fatty alcohol production in a whole-cell bioconversion using Escherichia coli by facilitating cofactor supply, including ATP, NAD, FMN, FAD, and coenzyme A, through the rewiring of sugar metabolisms. This system could enhance up to 4-fold increase of total fatty alcohol by effectively increasing sugar phosphates i.e., 6-phosphogluconate (6-PG), 3-phosphoglycerate (3-PG), and fructose 1,6-bisphosphate (FBP), to balance cofactor demand by cellular metabolisms and fatty alcohol synthesis. Because a large-scale reaction network requires extensive experimental data to construct the model using classical kinetics, an alternative methodology is needed for simulations of metabolic flux. Based on the analysis of untargeted metabolomics data, we reconstructed a metabolic network model of this engineered Escherichia coli with cofactor boosting system for fatty alcohol production using thermodynamically explicit (TDE) kinetics framework. This kinetic model was developed based on thermodynamics of reactions driven by chemical potentials of reactants. TDE parameters can be related to values of activation energy and enzymatic activity of the reaction. We formulated an ordinary differential equation (ODE) model in Julia, high performance computational language, using tools provided by SciML (Scientific Machine Learning). In this work, parameter estimation was applied to simulate a model from the metabolomics measurement, resulting in an insightful data for understanding genetically modified organisms for future optimization of desired strains.

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1019 A — A Polarity-Independent Framework for Metabolomics: Constructing Neutral Molecular Networks from MS/MS Data

PRESENTING AUTHOR: *Nicola Dalla Valle, Nicola Dalla Valle, Italy*
CO-AUTHORS: *Pietro Franceschi, Mar Garcia-Aloy, Peter Robatscher, Michael Oberhuber*
SUB THEME: *Metabolic networks*

Compound identification remains a major bottleneck in untargeted metabolomic mass spectrometry (MS) analysis. Molecular networks (MNs) are commonly used to investigate structural similarity relying on MS/MS spectra. Typically, separate networks are built for positive and negative ionization modes, requiring manual selection of the most informative polarity for each compound, complicating the identification process.

To address these challenges, we propose the creation of a Neutral Molecular Network (NMN), where “neutral” spectra are generated in silico by combining MS/MS data from both ionization modes.

Spectral libraries from the MassBank of North America (MoNA), were used to construct NMNs and compare them with the corresponding polarity-specific MNs. For each compound, neutral spectra were generated by merging [M+H]+ and [M−H]− fragmentation spectra. The three MNs were quantitatively compared in terms of network modularity (which measures the strength of the division of different classes into distinct clusters) and network assortativity (which refers to the tendency of compounds to connect with others of the same class), classifying the individual compounds based on their ClassyFire ontology.

NMNs consistently showed improved modularity and assortativity across most datasets, indicating improved chemical clustering and more reliable network connectivity. These gains were evident at higher thresholds reflecting a more reliable and chemically meaningful MN structure, especially for the edges between compounds with a high similarity score.

The NMN method simplifies untargeted metabolomics analysis by eliminating the need for a polarity-specific network inspection and improving structural organization. Future work will focus on expanding adduct coverage for neutral spectra creation and validating the approach with biological samples.

1020 A — Characterizing body mass index and overweight with metabolic individual-specific networks

PRESENTING AUTHOR: *Anni Malmberg, University Of Helsinki, Finland*
CO-AUTHORS: *Federico Melograna, Kristel Van Steen, Johannes Kettunen, Katri Räikkönen, Johan G. Eriksson, Jari Lahti*
SUB THEME: *Metabolic networks*

Small molecules involved in metabolism do not act independently within an individual but form a complex, interconnected network, with molecular interdependencies that may vary in both direction and strength across individuals. These aspects have largely been overlooked in previous research using metabolomics data to understand the molecular underpinnings of body mass index (BMI) or overweight (BMI ≥ 25). Prior studies focused on direct associations of the metabolic measures or described the molecular interdependencies at a population-level only. Therefore, we obtained individual-specific networks (ISNs) via LIONESS using 137 metabolomic measures assessed in the Helsinki Birth Cohort Study sample (HBCS, N = 1075, 44.7% males, mean age = 66.0 years). Each resulting ISN captured a unique set of possible 9316 interdependencies (edges) between metabolic measures (nodes). We compared traditional univariate metabolome-wide associations (MWAS) and associations of ISN edge weights with cross-sectional BMI and overweight. The number of MWAS hits, ISN edge hits and novel nodes (metabolic measures involved in ISN edge hits but not identified in MWAS), respectively, were 84, 80 and 11 for BMI, and 89, 162 and 12 for overweight (multiple testing corrected). Out of these, 3 (3.6%) MWAS hits, 23 (28.8%) ISN edge hits, and 4 (36.4%) novel nodes were unique to BMI, while 7 (7.9%), 105 (64.8%), and 5 (41.7%) were unique to overweight. For BMI, the top ISN edge hit was ‘total cholesterol in HDL3’ & ‘total phosphoglycerides’ (adjusted p-value = 7.10×10^(-6)) and for overweight ‘total cholesterol in HDL3’ & ‘total cholesterol in HDL2’ (adjusted p-value = 2.96×10^(-8)). In conclusion, although many metabolic measures are associated directly with BMI and overweight, ISNs can provide additional insights by capturing novel trait-relevant biologically plausible nodes that MWAS alone could not detect. However, assessing clinical relevance remains important for future research.

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1021 A — MetaboBank: a public repository for metabolomics data and associated metadata

PRESENTING AUTHOR: ***Takeshi Ara, National Institute of Genetics, Japan***
CO-AUTHORS: ***Toshiaki Tokimatsu, Yuichi Kodama, Asami Fukuda, Takatomo Fujisawa, Masanori Arita***
SUB THEME: ***Metabolite and mass spectral databases***

MetaboBank (https://www.ddbj.nig.ac.jp/metabobank/) is an open repository for metabolomics raw data and associated metadata, operated by the Bioinformation and DDBJ Center (DDBJ; https://www.ddbj.nig.ac.jp) of the National Institute of Genetics (NIG), Japan. As of May 2025, 147 original studies and 1715 MetaboLights (https://www.ebi.ac.uk/metabolights/) studies are searchable with links to the meta- and raw data files. The metadata consists of two MAGE-TABs, called Investigation Description Format (IDF) for study overview and references, and Sample and Data Relationship Format (SDRF) for sample details with measurement conditions and metabolite assignments. Metabolite Assignment File (MAF), describing the annotated results such as metabolite names and identifiers for detected peaks, is optionally accepted. All MetaboBank studies are linked with the BioProject and BioSample databases of the International Nucleotide Sequence Database Collaboration (INSDC) for better linking with genomic data from the same samples.

Raw data from MetaboBank include original mass-spectrometry (MS) vendor files and their mzB-converted files (mzB format was developed by Reifycs Inc.). Through the mzB conversion, users can browse data files from any MS vendor using the free Data Checker program and API functions of the Hive SDK toolkit for C#/Java/Python.

In addition to the existing collaboration with the MetaboLights at European Bioinformatics Institute and the Metabolomics Workbench at University of California San Diego, we also share information with Metabolomics Data Curation Center (MDCC), a part of Korea BioData Station (K-BDS), for standardization of curation and data formats. We plan to make a closer relationship with more Asian countries in the coming years.

1022 A — Improved Drug Metabolite Identification with a Streamlined Software Workflow

PRESENTING AUTHOR: ***Mark Sartain, Agilent Technologies, Inc., United States***
CO-AUTHORS: ***Martin Andre Hoffmann, Lee Bertram, Andrew McEachran, James Pyke***
SUB THEME: ***Metabolite identification/annotation***

Drug metabolite identification is the essential first step to understanding drug behavior in humans, including efficacy, toxicity, and drug-drug interactions. However, drug biotransformation products are most often absent from established spectral libraries, presenting a challenge for their identification. Here we describe enhancements to a previously described strategy that combines open-source and vendor software to accurately predict drug metabolite structures, search these structures against MS/MS data through a molecular fingerprinting approach, and map identifications to molecular features resulting from a differential analysis of drug-treated liver microsomes. Pooled human liver microsomes with buffer were incubated with 250 nM verapamil or without drug, and reactions were quenched at seven time points. Extracts were separated with RP-LC and eluents were analyzed with an LC/Q-TOF. Feature finding and differential analysis were performed within MassHunter Explorer 2.0 software that newly automates extraction of MS/MS spectra, integrates spectral library matching, and transfers MS/MS spectra to SIRIUS/CSI:FingerID for unknown identification. Out of 6,131 compounds, statistics revealed seven significant compounds that were selected for further interrogation. To enable structure elucidation, 1,821 verapamil biotransformation structures representing 146 molecular formulas were predicted with Biotransformer software (https://biotransformer.ca), and these structures were used to create a custom database within SIRIUS/CSI:FingerID. Molecular formulas were derived from the extracted MS/MS spectra, which were then searched against the custom database using a molecular fingerprinting approach. The verapamil drug precursor and seven known metabolites were identified as top search hits, demonstrating the approach differentiated well-documented verapamil drug metabolites over many structurally similar isomers. The workflow can be extended to other classes of xenobiotics and their biotransformations.

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1023 A — ChemEmbed and SingleFrag: Enhancing Metabolite Identification with Deep Learning-Based MS/MS Analysis

PRESENTING AUTHOR: *Oscar Yanes, CIBERDEM & Univ. Rovira i Virgili & IISPV, Spain*

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SUB THEME: *Metabolite identification/annotation*

Machine learning has become essential for annotating the vast number of unidentified MS/MS spectra in metabolomics, overcoming the limitations of conventional spectral libraries. To enable efficient learning, both experimental MS/MS spectra and chemical structures must be transformed into numerical representations that preserve key molecular information. Traditionally, spectral data are encoded into fixed-length vectors, while molecular structures are represented as fingerprints or graph-based embeddings.

Computational tools then generally follow one of two approaches: (i) processing experimental MS/MS spectra and searching for candidate matches in compound databases, or (ii) predicting in silico MS/MS spectra from molecular structures and comparing them to experimental spectra. Here, we introduce ChemEmbed and SingleFrag, two novel deep learning frameworks leveraging these strategies.

ChemEmbed integrates multidimensional vector representations of chemical structures with enhanced MS/MS spectra to improve candidate ranking for metabolite identification. It correctly ranks the target compound first in 42% of cases and within the top five in 76% of cases on test datasets.

SingleFrag introduces a new paradigm for MS/MS spectral prediction. Rather than generating full spectra with a single model, SingleFrag learns individual fragment ion behaviors and predicts peak presence/absence instead of intensity. This approach surpasses rule-based methods (CFM-ID) and deep learning models incorporating 3D molecular structures (3DMolMS), ranking the correct metabolite first in 38% of cases and within the top five in 72% of cases.

When applied to external benchmarks such as CASMI 2016, CASMI 2022, and the Annotated Recurrent Unidentified Spectra (ARUS) dataset—comprising over 25,000 plasma spectra and 68,000 urine spectra—both tools successfully identified tens of previously unannotated compounds. Notably, ChemEmbed outperforms SIRIUS-CSI:FingerID, the current state-of-the-art in computational metabolomics.

1024 A — Are We Running Out of New Metabolites to Discover?

PRESENTING AUTHOR: *Yasin El Abiead, UCSD, United States*

CO-AUTHORS: *Adriano Rutz, Simone Zuffa, Bashar Amer, Shipei Xing, Corinna Brungs, Robin Schmidt, Nicola Zamboni, Pieter C Dorrestein*

SUB THEME: *Metabolite identification/annotation*

The metabolome's "dark matter"—unidentified signals in untargeted metabolomics—regularly sparks debates over its size. Some argue that we may already have most of the information needed to understand life through metabolomics as most data result from analytical artifacts. In contrast, others emphasize the potential for discovery. However, what is the case?

In this work, we investigate to what extent electrospray phenomena (e.g., in-source fragmentation) explain the dark metabolome and explore the remaining discovery potential by utilizing public LC-MS/MS data, including data on ~30,000 analytical standards and mass spectral libraries.

We utilized LC-MSn raw data from ~30,000 analytical standards to show that the median number of in-source fragments observed for highly concentrated analytical standards can range from zero to two, depending on the chemical class and adduct. Median intensities are generally 100 times lower than those of the intact molecular ion.

After analyzing a NIST human fecal reference material and grouping of LC-MS peaks through peak shape correlation and MS/MS signals overlapping with co-eluting features, we obtained 1485 feature groups, 18% of which contained at least one feature that could be annotated with a putative structure through library matching or as putative polymer through retention time - mz patterns. Of the remaining 82% of feature groups, 52% had MS/MS spectra associated with every feature in the group, while 48% had at least one feature without MS/MS or with a chimeric MS/MS spectrum.

To assess whether the discovery of new molecules has slowed, we selected three public human LC-MS/MS datasets for the sample types: fecal, urine, and blood. We observed that the proportion of annotated spectra continuously increased over the last ten years as the GNPS fragmentation library size increased.

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1025 A — Advancing data analysis strategies for spatially resolved metabolomics

PRESENTING AUTHOR: ***Nadia Moltó, Universitat Rovira i Virgili, Spain***
CO-AUTHORS: ***Roger Giné, Toufik Mahamdi, Christoph Bookmeyer, Óscar Yanes, Maria Vinaixa***
SUB THEME: ***Metabolite identification/annotation***

Mass spectrometry imaging (MSI) enables the spatial characterization and quantification of metabolites in tissue sections, providing crucial insights into metabolic heterogeneity. However, accurate metabolite annotation remains a major challenge due to the complexity of spectral data and the vast number of detected peaks. To address this, we have developed a computational workflow that integrates both chemical formula-guided and peak-picking-guided strategies to enhance annotation accuracy in untargeted MSI-based metabolomics experiments.

Our approach leverages rMSI (Ràfols et al., 2017) for the peak-picking-based annotation module, integrating advanced scoring and validation methods that incorporate isotopic patterns, adduct correlations, and mass differences to refine metabolite annotation. Moreover, it incorporates the annotation framework of Hermes (Giné et al., 2021)—originally designed for LC/MS-based metabolomic experiments—as an additional strategy to develop a chemical formula-guided approach tailored for MSI data. A key feature of our workflow is an in-house MS1 and MS2 database, designed to improve adduct identification across different MALDI matrices deposited by low-temperature thermal evaporation (LTE) using an extensive library of analytical standards.

To further streamline spatial metabolomics analysis, we have transitioned our computational workflow to Datoma, a cloud computing platform that enables large-scale data processing and fosters collaborative research.

By addressing critical challenges in MSI-based spatial metabolomics, our analysis pipeline aims to bridge the gap between untargeted MSI data acquisition and actionable biological insights. This will be demonstrated through application examples in cancer research and biomarker discovery, highlighting its potential for improving disease characterization and identifying clinically relevant metabolic signatures.

1026 A — Computational metabolomic profiling of anti-venom and anti-inflammatory South African medicinal plants

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CO-AUTHORS: ***Fidele Tugizimana, Justin van der Hooft, Zamani Cele***
SUB THEME: ***Metabolite identification/annotation***

Medicinal plants are a rich source of bioactive compounds, termed also natural products (NPs), which possess varying therapeutic properties. Medicinal plants have been used since ancient times to treat a vast array of ailments and diseases such as malaria, fever, skin diseases and cancer, amongst many others. Melianthus comosus and Hypoxis hemerocallidea (focus of this work) are South African medicinal plants primarily used for the treatment and management of snakebites, skin wounds and diseases, amongst many other ailments and conditions. However, despite the extensive use of these medicinal plants in South African traditional medicine, there is very little understanding of the (phyto)chemical space as well as the biochemistry that define the medicinal benefits of these plants. Thus, reported herein is a computational metabolomics work aimed at exploring and applying emerging 4IR-driven computational tools and strategies to characterize the metabolome and underlying biochemistry of these South African medicinal plants. The work aims to provide novel comprehensive metabolic landscapes of the plants. Mass spectrometry-based computational tools and strategies such as classical and feature-based molecular networking and MS2Query were used to mine and evaluate the methanolic leaf and root extracts of the medicinal plants under study. The preliminary results revealed the presence of different chemical classes including flavonoids, hydroxycinnamic acid (HCA) compounds, tannins, sugars and lipids at differential distributions across the two plants and their respective tissues. M. comosus was observed to be more dominant in the flavonoid content while H. hemerocallidea was more dominant in the lipid-like molecules. Such differential distribution of metabolic profiles across the leaves and roots of the two medicinal plants reveal clear differences in the chemistries of the plants which in turn points to differences in their metabolism, ultimately contributing to differences in their biological activities and medicinal uses.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1027 A — Comprehensive Proteometabolomic Characterization of Gliomas Reveals Novel Molecular Targets and Metabolic Alterations

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SUB THEME: *Multi-omics/Integrative omics*

Gliomas are a heterogeneous group of glial tumors characterized by infiltrative growth, variable clinical course, and resistance to treatment. Despite advances in molecular classification, their diagnosis still relies on histopathology supported by molecular markers, and a full understanding of functional changes at the proteomic and metabolomic level remains limited. Identifying specific biomarkers for glioma subtypes may significantly improve prognosis and therapy selection. This study represents a significant step forward in glioma research, integrating, for the first time, LC-MS-based proteomics and metabolomics with GC-MS-based metabolomics to provide a comprehensive characterization of the molecular landscape of glioma tissues.

The study included samples from patients who underwent neurosurgery between 2016 and 2021. Tumor grades were determined by histopathology according to the 4th edition of the WHO CNS Tumor Classification. Based on that, patients were grouped into WHO Grades (where n= proteomic samples/ metabolomic samples): G1+2 (n=17/10), G3 (n=10/6), and G4 (n=63/42). The study groups were matched in terms of age, body mass, BMI, and other anthropometric parameters.

Integrated proteomics and metabolomics profiling revealed changes related to mitochondrial function, metabolic stress response, and cytoskeletal remodelling, as well as abnormalities in amino acid metabolism, glycolysis, and lipid processing. In addition to confirming known metabolic adaptations in gliomas, our results also revealed novel, previously undescribed proteins and metabolites, including changes in plasmalogens (LPC P-18:0, LPC O-18:0) and key metabolic enzymes such as TKT and ME1. These findings expand our understanding of the mechanisms of glioma progression and highlight potential biomarkers and therapeutic targets identified through this comprehensive multi-omics approach.

Funding: Ministry of Education and Science within the project “Excellence Initiative - Research University”, Medical University of Bialystok (B.SUB.24.117).

1028 A — A multi-omics and modelling investigation reveals transgenerational, sex-specific effects of the obesogenic compound TBT on hepatic metabolism in mice

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SUB THEME: *Multi-omics/Integrative omics*

Many chemical contaminants, including tributyltin (TBT), are suspected to act as metabolic disruptors, contributing to the development of metabolic diseases (insulin resistance, obesity, ...). Effects of prenatal exposure to these compounds may be transmitted across generations, increasing the susceptibility of subsequent generations to develop metabolic disorders, although the mechanisms involved remain poorly understood. In this study, we investigated the transgenerational effects of prenatal exposure to TBT on liver metabolism, by studying mice at the F3 generation. We conducted a multi-omics analysis, including metabolomic (NMR, LC-MS), lipidomic (GC/LC-MS) and transcriptomic (RNAseq) analyses, performed on liver samples from male and female F3 mice.

Multi-block statistical integration approaches are commonly used for multi-omics data but have limitations. They require that all data be measured on the same samples, which is rare in practice. Also, they often produce reduced signatures, not well-suited for mechanistic insights. To address this, we adopted an original two-step strategy. First, we analyzed each omics-level dataset independently using a combination of univariate and multivariate approaches (AMOPLS), to model the interaction between sex and TBT and identify modulations attributable to each factor. Next, we jointly analyzed these signatures by integrating them into the context of the metabolic network, using a graph-based approach, to identify potential disrupted metabolic pathways.

We highlighted a significant effect of TBT on the transcriptome in males, and on the metabolome and lipidome in both sexes, despite the lack of an observable phenotypic effect in females. We observed a marked sex-specific effect of TBT on the lipidome, with an increase in several phospholipids in males and a decrease in triglycerides and diglycerides in females. Interpreting these signatures within the metabolic network context suggests that ancestral TBT exposure triggers the alteration of distinct metabolic pathways and involve different mechanisms of action between males and females at the hepatic level.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1029 A — BiomiX, a user-friendly bioinformatic tool for democratized analysis and integration of multi-omics data

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SUB THEME: *Multi-omics/Integrative omics*

Interpreting biological system changes requires interpreting vast amounts of multi-omics data. While user-friendly tools exist for single-omics analysis, integrating multiple omics still requires bioinformatics expertise, limiting accessibility for the broader scientific community.

BiomiX tackles the bottleneck in high-throughput omics data analysis, enabling efficient and integrated analysis of multi-omics data using different tools such as Multi-Omics Factor Analysis (MOFA), DIABLO and similarity network fusion (SNF). This tools also allows to carry out some of the data processing stages of different omics workflows, such as metabolomics, transcriptomics or methylomics. Specifically for the metabolomics workflow, BiomiX supports both targeted and untargeted pre-processed data as input, and enables data transformation and scaling, QC pre-visualization and univariate statistical analysis. Additionally, for untargeted metabolomics based on LC-HRMS data, BiomiX supports the annotation step by the CEU Mass Mediator tool and by comparing MS/MS fragmentation spectra using the TidyMass package.

BiomiX fine-tunes MOFA and DIABLO integration to identify shared sources of variation across omics data to optimise factor number selection. BiomiX highlights factors distinguishing between cohorts and provides tools to interpret discriminative MOFA factors using innovative bibliography research on PubMed. The users are guided in the interpretation by the correlation of discriminant factors with clinical data and pathways analysis of the top contributing metabolites and genes. To provide a complete overview, SNF provides a network view of each omics, highlighting structural similarities.

Biomix is fully accessible on GitHub (https://ixi-97.github.io) including examples and tutorials for its use. This R-based, user-friendly tool is compatible with multiple operating systems and aims to make multi-omics analysis accessible to non-experts in bioinformatics. Furthermore, new perspectives are currently shaping the development of the second version, which will include additional omics workflows (e.g., microbiomics, proteomics) and novel data integration methods.

Reference: Iperi, C., Fernández-Ochoa, Á., Barturen, G. et al. BMC Bioinformatics 26, 2025. https://doi.org/10.1186/s12859-024-06022-y

1030 A — Multi-omic data integration approaches at the interface of diet, metabolism, and gut microbiota – case study with plant based protein-rich food products

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CO-AUTHORS: *Veera Houttu, Ville Koistinen, Topi Meuronen, Sara Ferreira Rocha, Jasmin Raita, Leo Lahti, Kati Hanhineva*

SUB THEME: *Multi-omics/Integrative omics*

There is an urgent global need to increase the consumption of plant-based foods for human health and sustainable food production. Novel methods have emerged to manufacture protein-rich plant-based foods to achieve good nutritional and sensory properties, but their effects on human health including microbiota are not yet well studied.

In the Newplant project, we conducted a clinical intervention with 37 participants, who followed three types of diet—whole unprocessed plant-based (e.g., canned beans), mildly processed (e.g., tofu), and refined (e.g., soy nuggets)—over five weeks. We collected metabolomic, metagenomic, clinical, and food frequency data to investigate links between the studied dietary patterns and biological changes.

Within this study, we aim to identify patterns of co-variation between metabolomic and metagenomic profiles to understand whether specific metabolites and microbial taxa show consistent shifts both within and across diets. Furthermore, we will employ machine learning models through the IntegratedLearner R package to identify the metabolites and microbial species most significantly affected by varying diets.

We observed a statistically significant difference in Shannon alpha-diversity of microbial communities between the start and the end of the whole unprocessed and mildly processed diets. Additionally, when considering the study timepoints, a difference in the refined diet was observed in the batch from the first week of the study. Furthermore, we identified a carry-over effect of alpha-diversity across the study timepoints.

Ultimately, our findings contribute to a better understanding of the effect of plant-based products to facilitate the development of more sustainable food solutions.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1031 A — Effective Exploratory Data Analysis Strategies in Metabolomics Studies

PRESENTING AUTHOR: *Justin JJ van der Hooft, Wageningen University & Research, Netherlands*
CO-AUTHORS: *Mitja Zdouc, Justin JJ van der Hooft*
SUB THEME: *Other*

Nature has always been a rich resource of diverse chemistry. Fortunately, in recent years, technological advances in the omics fields have increased our capacity in measuring them. For example, modern mass spectrometers generate information-rich chemical profiles used for untargeted metabolomics studies. Such studies are typical examples of exploratory data analysis, as one does not a priory know what one will discover in the data. However, researchers should have a study goal: whether it is finding out differences in plant tissue metabolic content or associating antimicrobial activity to metabolites in bacterial extracts. However, the current information-rich metabolomics profiles leave researchers with the daunting task to separate valuable signals from everything else.

In this contribution, we will highlight the importance of effective visualization strategies linked to computational metabolomics strategies that together allow researchers to make justified, reliable, and reproducible choices in their exploratory analyses. First, I will explain several key computational metabolomics concepts that underpin these priotirization strategies, including mass spectral organization and annotation, and showcase how visualizations can enhance data interpretation. Then, we will highlight the recently developed triade of tools SpecXplore, msFeaST, and FERMO. After establishing reasonable thresholds for mass spectral grouping and matching, researchers can use statistics and phenotype information such as bioactivity to filter and select for relevant metabolite features. I will show how we can quickly zoom in on specialized mushroom metabolites affeced by changes in their growth substrate. Furthermore, we will showcase how novel variants of known peptides are uncovered using only a few selection criteria. We will finish the contribution with highlighting the important role of Open Science and Software in stimulating community-based science.

Altogether, we expect that the presented progress in computational metabolomics tools will empower metabolomics researchers navigating their increasingly complex datasets to find and annotate relevant and novel chemistry in nature.

1032 A — notame: R/Bioconductor package for untargeted LC–MS metabolomics data analysis

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CO-AUTHORS: *Atte Lihtamo, Retu Haikonen, Ville Koistinen, Leo Lahti, Kati Hanhineva*
SUB THEME: *Statistical methods*

From the perspective of metabolites as the continuation of the central dogma of molecular biology, metabolomics provides the closest link to many phenotypes of interest. Untargeted liquid chromatography-mass spectrometry (LC-MS) attracts many new practitioners in quantitative metabolomics research, largely due to its sensitivity and broad coverage of the metabolome: the small molecules in a biological sample. However, due to experimental reasons and the extensive data analysis, untargeted LC-MS metabolomics data analysis meets challenges with regard to quality and reproducibility.

In an effort to address these challenges in our research, the notame R package was developed in parallel with an associated protocol article published in the “Metabolomics Data Processing and Data Analysis —Current Best Practices” special issue of the Metabolites journal. The main outcome is identifying interesting features for laborious downstream steps relating to biological context, such as metabolite identification and pathway analysis, which fall outside the purview of notame.

To further promote quality and reproducibility, notame has now been substantially upgraded to be included in the next release of Bioconductor, a repository focused on high-quality open software for omics research. The package has also been reworked to facilitate overview of its functionality as per a recognized conceptualization of untargeted LC-MS metabolomics data analysis. Finally, Bioconductor packages providing complementary functionality were explored for the prospect of fully programmatic analyses, enhancing reproducibility.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

1033 A	**MetaProViz R-package: From functional analysis towards mechanistic insights from metabolomics data by integrating prior knowledge**

PRESENTING AUTHOR: ***Christina Schmidt, University Clinics Heidelberg, Germany***
CO-AUTHORS: ***Denes Turei, Dimitrios Prymidis, Macabe Daley, Christian Frezza, Julio Saez-Rodriguez***
SUB THEME: ***Systems biology***

To gain mechanistic understanding of metabolic deregulation in diseases and identify therapeutic targets, the right data analysis strategies and prior knowledge integration are key. Here we present MetaProViz (Metabolomics Processing, functional analysis and Visualization), a free open-source R-package that facilitates metabolomics data analysis and integration with prior knowledge to generate mechanistic hypotheses to explain the metabolic dysregulation and potential downstream consequences (https://saezlab.github.io/MetaProViz/). MetaProViz offers an interactive framework consisting of five modules: Processing, differential analysis, prior knowledge access & refactoring, functional analysis and visualization for both intracellular and exometabolomics. The latter measures changes in cell-culture media by comparing fresh with spent media and requires dedicated support for normalisation and differential analysis compared to standard metabolomics experiments.

We showcase MetaProViz functionalities in the vignettes using publicly available kidney cancer metabolomics data from cell-lines, cell-culture media, and tumour tissue, offering a robust foundation for testing and validating analysis workflows. To improve the integration of metabolomics data with prior knowledge, we created Metabolism Signature Database (MetSigDB), a collection of annotated metabolite sets. MetSigDB not only includes classical pathway-metabolite sets, but also chemical class-metabolite sets, metabolite-receptor and transporter sets, and enables the conversion of gene sets to metabolite sets by using enzyme-metabolic reaction prior knowledge. Moreover, MetaProViz translates between metabolite IDs, analyzes mapping ambiguities and adds potential other IDs to the measured data. The latter is particularly important since analytical evidence usually does not distinguish stereochemical or positional structural information, which leads to uncertainty in assigning metabolite IDs and metabolite IDs with different degrees of ambiguity. In combination with MetaProViz functional analysis toolkit, including enrichment, sample metadata, and biologically informed clustering for exometabolomics, to combine intracellular with exometabolomics, and for multi-condition experiments, MetaProViz helps users to harness the full potential of metabolomics data, ultimately generating mechanistic hypotheses.



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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1050 A — Multi-omics characterization of an advanced platform for in vitro testing using 3D models of young and aged cardiac tissue

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SUB THEME: *Ageing research*

Cell culture techniques have become indispensable tools in biological research, enabling different studies under controlled conditions. While traditional monolayer cultures have long been the cornerstone of in vitro studies due to their simplicity and reproducibility, 3D cultures have emerged as a revolutionary alternative to replicate the structural and functional complexity of in vivo tissues. Here we designed scaffolded 3D models of co-cultured cardiomyocytes and endothelial cells with controlled biomechanical properties mimicking young and aged models. We used a multi-omics approach including metabolomics, proteomics, and transcriptomics to reveal critical variations in cellular behavior across both models, delivering a holistic, multidimensional view of cellular systems and elucidating the molecular mechanisms that distinguish young and aged tissues.

The comparison between 3D young and aged models revealed significant metabolic, structural, and functional differences, highlighting key aspects of cellular aging. The aged model exhibited mitochondrial dysfunction, increased energy demand and impaired fatty acid oxidation, leading to metabolic stress. Additionally, altered calcium homeostasis and decreased protein anabolic processes suggest defects in cellular function and maintenance. Increased kynurenine, serotonin and anserine in aged cells further supports a higher maturation stage, with potential implications for cardiovascular health.

Moreover, the increased presence of branched-chain amino acids indicates an adaptive response to muscle breakdown and energy deficiency, while their association with mTOR activation suggests potential pathological consequences such as cardiac hypertrophy and impaired mitochondrial function. Lipidomic analysis reinforced these findings, revealing a depletion of triglyceride stores and an increase in phosphatidylcholines, sphingomyelins and ceramides, indicating modulation of hypertrophy, cellular stress, membrane reorganization, inflammation, and apoptosis.

Our results suggest that the 3D aged model undergoes substantial metabolic reprogramming, oxidative stress, and structural adaptations, resembling the cellular dysfunctions associated with aging. These findings provide valuable insights into the mechanisms underlying cellular aging and their potential impact on tissue function and disease development.

1051 A — Plasma Hippuric Acid Decline as a marker of Frailty: Insights from Two Longitudinal Italian Cohorts of Older Adults

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SUB THEME: *Ageing research*

Frailty is a geriatric syndrome characterized by increased vulnerability to stressors. Early identification of individuals at risk of frailty may offer opportunities to prevent its progression, thus promoting healthy ageing. This study aimed to investigate the relationship between plasma hippuric acid (HA) concentration -a human-gut co-metabolite derived from the polyphenols intake- and frailty across two longitudinal Italian cohorts.

Plasma HA was quantified using European Medicines Agency-validated mass spectrometry methods in plasma samples from InCHIANTI (n=534; 55.4% females; aged ≥65 years, 1998, 2002, 2004, 2008 follow-ups FU) and InveCe.Ab (n=912; 53.8% females; aged ≥70 years, 2010, 2012, 2014, 2018 FU) participants who were non-frail at baseline. Frailty was assessed through two different classifications: frailty index (FI) and frailty phenotype (FP). The association between HA and frailty within the two cohorts was assessed by linear mixed models adjusting for sex, age, educational status, renal function, and body mass index.

Median values of HA concentrations were significantly lower in frail compared to non-frail participants (measured by FI) in both (i) InCHIANTI study: HAnon-frail =1.59 (interquartile range, IQR, 0.99-2.74) ug/ml vs HAfrail=1.18 (IQR 0.75-1.88) ug/ml in 2004 year follow up; HAnon-frail =1.44 (IQR 0.87-2.50) ug/ml vs HAfrail=1.12 (IQR 0.63-1.99) ug/ml in 2007 year follow up; and (ii) InveCe.Ab study: HAnon-frail=2.19 (IQR 1.26-3.92) ug/ml vs HAfrail=1.81 (IQR 0.87-3.65) ug/ml in 2018 year follow up. No statistically significant association was observed when frailty was assessed using the FP in any investigated follow-ups. Higher plasma HA levels were significantly associated with a reduced risk of frailty ($p=0.027$ InCHIANTI and $p=0.043$ InveCe.Ab). Mediation analysis indicated that fruit and vegetable consumption negatively mediated the relationship between HA levels and FI ($p<0.05$) in the InveCe.Ab cohort.

Our findings suggest that low plasma HA levels may serve as a marker of frailty, potentially linked to reduced fruit and vegetable intake.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1052 A — Changes in Plasma and Fecal Bile Acids due to Diet and Exercise Affect Bone Health

PRESENTING AUTHOR: *Marco Balasso, Medical University of Graz, Austria*
CO-AUTHORS: *Mithra Gruber, Gunter Almer, Hans-Jürgen Gruber, Markus Herrmann, Giovanny Rodriguez-Blanco, Nerea Alonso*
SUB THEME: *Ageing research*

Bile acids (BAs) are signalling lipids and play a role in maintaining bone health. Primary and secondary BAs are quantified in clinics using LC-MS/MS methodologies. However, microbiome-catalyzed reactions produce a diverse group of metabolites that may also influence bone metabolism. Their production and how they regulate bone health can be shaped by diet and physical activity.

In this study we aim to evaluate the effect of exercise on serum and fecal BA diversity in a diet-induced obesity model using 106 female Long-Evans rats. The rats were assigned to high-sugar (HSD), high-fat (HFD), and high-fat/-sugar diet groups (HFSD), and underwent mild treadmill exercise for 8 months. Bone quality was assessed with µCT at endpoint, revealing deteriorations in femoral cortical and trabecular bone health. An untargeted reversed-phase LC-MS/MS approach applying data-dependent acquisition was used to identify novel BAs, aiming to establish correlations with bone loss.

High caloric intake led to elevated BA levels in both serum and stool, regardless of exercise. Microbial amino acid conjugated BAs are prevalently found in stool, while in serum only alanocholic acid was identified. Exercise partially mitigated the sugar-induced increase in unconjugated, glycine-, and taurine-conjugated BAs in stool. Sedentary HSD rats showed significant elevations, whereas exercised animals had levels similar to controls. Microbial conjugates like alanocholic and alanodeoxycholic acid were significantly elevated in sedentary rats ($p < 0.001$, 2- and 4-fold, respectively) and increased even more in HFSD rats when compared to control ($p<0.001$, 70- and 130-fold, respectively). These increased levels negatively correlated with cortical bone area ($p < 0.05$). Conversely, sedentary HSD rats showed reduced levels of sulfated taurodeoxycholic ($p = 0.02$, 0.6-fold) and taurochenodeoxycholic acid ($p = 0.04$, 0.6-fold) in serum, in contrast to the exercised animals. Our results suggest that recently described BAs, like microbially conjugated BAs could play a role in bone homeostasis.

1053 A — Control of Aging-Related Abnormal Protein Accumulation and Clearance Mechanisms in AD mice

PRESENTING AUTHOR: *Jee-yin Ahn, Sungkyunkwan University School Of Medicine, South Korea*
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SUB THEME: *Ageing research*

Aging disrupts normal cellular metabolism, accelerating the accumulation of abnormally modified or aggregated proteins, which can progress into disease. In this study, we identify ErbB3-binding protein 1 (EBP1) as a novel regulator of tau protein accumulation and neurofibrillary tangles, key pathological features of Alzheimer's disease (AD). While EBP1 has been proposed to modulate amyloid-beta (Aβ) accumulation, its role in tau pathology remained unexplored.

We demonstrate that EBP1 directly interacts with tau protein, promoting microtubule stabilization in neurons. In contrast, Aging disrupts normal cellular metabolism, accelerating the accumulation of abnormally modified or aggregated proteins, which can progress into disease. In this study, we identify ErbB3-binding protein 1 (EBP1) as a novel regulator of tau protein accumulation and neurofibrillary tangles, key pathological features of Alzheimer's disease (AD). While EBP1 has been proposed to modulate amyloid-beta (Aβ) accumulation, its role in tau pathology remained unexplored. we demonstrate that EBP1 directly interacts with tau protein, promoting microtubule stabilization in neurons. In contrast, EBP1 depletion leads to cytoskeletal disruption, dissociating tau from microtubules (MT) and resulting in age-dependent phosphorylated tau (p-Tau) accumulation. Additionally, we discovered that tau acts as an acetyltransferase for EBP1, and the decline in EBP1 acetylation with aging contributes to p-Tau aggregation, thereby advancing AD progression. Our findings show that acetylated EBP1 levels decrease with aging and in AD mouse models, resulting in neuronal degeneration and exacerbated p-Tau aggregation. Importantly, enhancing EBP1 expression or preserving its acetylation effectively reduces p-Tau aggregation, highlighting EBP1 as a potential therapeutic target. Collectively, our study reveals that EBP1 regulates microtubule stability through tau binding and modulates p-Tau levels, providing novel insights into molecular mechanisms underlying age-related protein aggregation and AD pathology.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1054 A — In Vitro and In Vivo Assessment of Natural Products for Hair Loss Prevention and Regrowth in Androgenetic Alopecia

PRESENTING AUTHOR: *Ching Peng Wei, Taipei Medical University, Taiwan*

SUB THEME: *Ageing research*

Androgenetic alopecia (AGA) represents the most common form of hair loss, primarily driven by dysregulated androgen metabolism and associated inflammatory signaling cascades. In recent years, natural products (NPs) have garnered increasing attention as potential therapeutic agents, owing to their diverse bioactive constituents and favorable safety profiles. While numerous NPs have been traditionally utilized across Asian regions for the treatment of hair loss, the precise molecular mechanisms mediating their therapeutic effects remain inadequately characterized and warrant further investigation.

In this study, we examined the inhibitory potential of selected herbal compounds on 5α-reductase (SRD5A) and Janus kinase (JAK) two pivotal enzymes involved in the androgenic and inflammatory signaling pathways underlying androgenetic alopecia (AGA). Utilizing an in vivo model, male C57BL/6 mice were administered the herbal formulations over a 21-day period. The treatment resulted in a marked enhancement of hair regrowth in the experimental groups, highlighting the therapeutic promise of these natural compounds in modulating key molecular targets associated with AGA pathogenesis.

Enzyme activity assays demonstrated that Angelica sinensis and Panax ginseng significantly inhibited the activity of both SRD5A and JAK1, highlighting their dual regulatory potential on androgenic and inflammatory pathways associated with hair follicle modulation. To further identify the bioactive compounds responsible for these effects, LC-MS and GC-MS profiling of the herbal extracts was performed. Follow-up investigations, including compound isolation, cell-based functional assays, and Western blot analysis, are warranted to elucidate the specific signaling mechanisms involved.

1055 A — Lipidomic and Proteomic Alterations in Elderly with Major Depressive Episode: A Multi-Omic Study with Prospects for Metabolomic Integration

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CO-AUTHORS: *Adriana Santodo, Licia Costa, Bradley J Smith, Victor Corasolla Carregari, Gustavo HMF Souza, Erica M Vieira, Ana Paula Mendes-Silva, Breno S Diniz, Marcelo A. Mori, Daniel Martins-de-Souza*

SUB THEME: *Ageing research*

Late-life depression (LLD) is a multifactorial disorder associated with systemic and central nervous system dysfunctions, including alterations in oxidative stress, inflammation, and lipid metabolism. To investigate these molecular changes, we conducted exploratory untargeted lipidomic and proteomic analyses in plasma samples from 50 elderly individuals (19 LLD patients and 31 controls).

Our analyses revealed significant differences in lipid species profiles, including oxidized and saturated CoA derivatives, phosphatidylinositols (PIs), and phosphatidylserines (PS). An increase in CoA 12:7;O2, an oxidized lipid, was observed, suggesting heightened lipid peroxidation and oxidative stress in LLD. A decrease in CoA 13:0, a saturated fatty acyl-CoA, was also noted, potentially indicating shifts in lipid metabolism. Additionally, alterations in the levels of PS and PIs were observed, suggesting potential disruptions in intracellular signaling, membrane dynamics, and vesicle-mediated transport.

Proteomic analyses identified dysregulation in pathways related to intracellular lipid transport and membrane integrity, highlighting possible impacts on neuronal health and synaptic function. These alterations in protein pathways align with potential disruptions in lipid metabolism observed in the lipidomic data.

The observed increase in oxidized CoA 12:7;O2 may reflect a response to oxidative stress and inflammation, while the reduction in CoA 13:0 suggests metabolic adaptations. The alterations in PS and PI levels point to possible disruptions in lipid signaling and membrane-associated processes that could affect neuronal communication and synaptic plasticity.

This preliminary study highlights the interaction between oxidative stress, lipid metabolism, and proteomic pathways in LLD. The observed alterations in oxidized lipids, CoA derivatives, and phospholipids suggest a state of oxidative imbalance and metabolic remodeling in LLD. These findings implicate membrane dynamics and intracellular signaling in the pathophysiology of LLD. While the initial findings emphasize the relevance of a multi-omic approach, further studies will integrate deeper metabolomic data to explore the metabolic implications of depression in the elderly.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1056 A — Global skeletal muscle metabolomics reveals mechanisms behind higher response to resistance training in older adults

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CO-AUTHORS: ***Changhyun Lim, Manoel Lixandrão, Dakshat Trivedid, Yun Xu, Konstantinos Prokopidis, Hamilton Roschel, Stuart Phillips, Howbeer Muhamadali***

SUB THEME: ***Ageing research***

To understand the mechaisnm behind high respond (HighR) compared to low respond (LowR) to resistnace training (RT) and whey protein supplementation (20g/day), we analysied vastus laterails muscle biopsies from a total of 50 participants. Utilising the MRI muscle cross-sectional area (CSA) data, we defined responders as those who had hypertrophy exceeding the 1.7% method error. Quadriceps CSA in the lower responder (LowR) (n=25, mean age 69±5 years) and HighR (n=25, mean age 67±4 years) increased from 53.6 ± 12.1 cm2 to 55.4 ± 12.8 cm2 after 10 weeks of RET (3.3 ± 1.7%, $P < 0.001$) and increased the absolute CSA in the higher responders (HighR) from 53.7 ± 12.5 cm2 to 59.2 ± 13.6 cm2 (10.3 ± 2.0%, $P < 0.001$). Muscle biopsies were taken from the vastus lateralis before and after RT. We performed untargeted liquid chromatography-mass spectrometry metabolomics to investigate changes in muscle metabolic regulation. The partial least squares discriminant analysis (PLS-DA) yielded the best results using the polar extracts, achieving a 75% average correct classification rate for predicting HighR and LowR. There was no signifncat differences in metabolomic profile at the basline. Our findings revealed several metabolic pathways, including branched-chain amino acid catabolism, tryptophan metabolism (indole and kynurenine pathways), the TCA cycle, gut-derived metabolites, carnitine shuttle metabolism as prominent pathways disrupted in LowR. We provide new insights and has the potential to identify and enhance interventions targeting muscle metabolism, ultimately improving muscle mass and strength to reduce the risk of sarcopenia and frailty in older age.

1057 A — METABOLOMIC PROFILING FOR RELAPSE-ASSOCIATED BIOMARKER DISCOVERY IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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SUB THEME: ***Biomarker validation and clinical translation***

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, with relapse posing a major challenge to sustained remission and clinical management. This study investigates the application of untargeted metabolomics for identifying biochemical markers associated with relapse in pediatric ALL. Bone marrow samples from pediatric patients were classified into relapse, non-relapse, and benign groups, followed by metabolite extraction and analysis using liquid chromatography–mass spectrometry (LC-MS). Data preprocessing and normalization were performed using MetaboAnalyst 6.0, including zero-value imputation and $\log_{10}$ transformation. Multivariate statistical analyses, including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and sparse PLS-DA (sPLS-DA), were employed to explore metabolic patterns across groups. Preliminary analysis revealed trends suggestive of metabolite-level differentiation associated with clinical outcomes. While full validation is ongoing, these early observations support the potential of metabolomics in distinguishing relapse-related profiles and guiding biomarker discovery. The integration of metabolomic signatures into clinical workflows may ultimately enable non-invasive risk stratification and real-time monitoring of disease progression. This study contributes to the advancement of precision medicine in pediatric oncology by demonstrating the feasibility of metabolic profiling as a diagnostic and surveillance tool in acute lymphoblastic leukemia.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1058 A — Integrating NMR metabolomics into prognostic models for heart transplantation

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SUB THEME: ***Biomarker validation and clinical translation***

Introduction

Primary Graft Disfunction (PGD) is the most significant cause of early morbidity and mortality after heart transplant, with a reported incidence of 28.2% (Smith et al., 2022). Additional complications arise from the use of marginal grafts, obtained by donation after circulatory death (DCD) (Boffini et al., 2025).

Among the parameters considered for donor selection there are the level of serum troponin and the inotropic/vasoactive medication. The graft suitability is checked by sampling endomyocardial biopsies (EMBs) for optical/electron microscopy.

Aims

This study aimed to integrate tissue metabolomics in the multimodal procedure for graft evaluation in heart transplant. The objective was to identify new molecular markers of injury and prognostic markers of PGD.

Methods

EMBs were collected immediately before (T0, T1) and 2 weeks after (T2) graft implantation. The hydrophilic metabolites extracted from EMBs were assessed combining the conventional NMR metabolomics workflow (Beckonert et al., 2007) with ASICS R-package for automated metabolite identification and quantification (Lefort et al., 2021; Tardivel et al., 2017).

The associations between donor/recipient variables and metabolomics features were determined by generalized linear models, correlation analysis and machine learning using MetaboAnalyst 6.0 (Pang et al., 2024).

Results and conclusions

The NMR analysis of 57 EMBs from 21 patients was completed. Despite the microscopic dimension of EMBs, 75 of the 216 metabolites included in the ASICS library were identified and quantified.

The molecular profile of T2 EMBs exhibited an association with PGD classification. Prognostic markers of PGD were not found in T0-T1 EMBs likely due to: 1) the limited sample size of the study combined to the multiple level of the PGD classification; 2) the number of donor/recipient covariates affecting the prediction models. Nevertheless, it was possible to correlate metabolic profile with the serum troponin level, a key indicator of heart damage, and with the medication received by donors.

1059 A — Atopic dermatitis – specific biomarker development in fingernails through lipid profiling

PRESENTING AUTHOR: ***Baegon Kang, Kyungpook National University, South Korea***

CO-AUTHORS: ***Kyung-Sik Song, Kwang-Hyeon Liu***

SUB THEME: ***Biomarker validation and clinical translation***

Atopic dermatitis – specific biomarker development in fingernails through lipid profiling

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Atopic dermatitis (AD) is one of the most common inflammatory dermatologic diseases worldwide and is associated with skin barrier dysfunction. Patients with AD observed significant lipid composition changes in skin samples, reflecting skin barrier dysfunction. The nail, as a skin appendage, is a thin plate of keratinized epithelial tissue derived from the epidermis. Nail dystrophy is observed in approximately 10% of AD patients. Using nails as a non-invasive bio-sample to identify biomarkers responsible for AD has the advantage of repeat sampling with high replicability, and long-term metabolic history from patients. Nail lipid composition has been poorly investigated despite its potential as a biomarker source of AD. In this study, we developed an analytical method for liquid chromatography-tandem mass spectrometry (LC-MS/MS), and gas chromatography-mass spectrometry (GC-MS) based comprehensive lipid profiling in the fingernail plate. Various lipid species were identified, including acyl carnitine, ceramide, neutral lipids, sterol lipids, phospholipids, and free fatty acids. This study presents a methodology for a lipid profiling tool in fingernail plate and future research on discovering lipid alterations in AD. This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HP23C0129).

Keywords: atopic dermatitis, fingernail, lipid profiling, mass spectrometry, non-invasion

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1060 A — Serum Endocannabinoid Lipid Profiling Reveals Candidate Biomarkers for Neuropathy in Type 1 Diabetes

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SUB THEME: ***Biomarker validation and clinical translation***

Objective:

Distal symmetrical polyneuropathy (DSPN) is a common complication of type 1 diabetes (T1D), characterized by sensory impairments due to damage to small and partially myelinated nerve fibers. These changes can manifest as numbness or pain, although not all individuals with DSPN experience pain. Fatty acids (FAs), fatty acid amides (FAAs), fatty acid ethanolamides (FAEs), monoacylglycerols (MAGs), and other bioactive lipids of the endocannabinoid system (ECS) are involved in inflammation and nociception. This study profiled these lipid species in serum from type 1 diabetic individuals with and without DSPN, and from non-diabetic controls, to explore their role and biomarker relevance in DSPN pathophysiology.

Research Design and Methods:

A cross-sectional study was conducted, including 122 participants: 21 non-diabetic controls, 56 T1D patients without DSPN, and 45 with DSPN diagnosed per Toronto criteria. Serum lipids were measured using targeted liquid chromatography–mass spectrometry. Kruskal-Wallis and Spearman correlation tests with FDR correction were used for statistical analysis.

Results:

Five FAAs—Linoleamide, Octadecanamide,Oleamide, Palmitamide, and Tetradecanamide—were significantly elevated in T1D patients with DSPN ($q \leq 0.001$). Linoleamide, Oleamide, and Palmitamide were positively correlated with vibration perception ($q < 0.05$). Octadecanamide showed strong associations with IL-4, IL-12p70, IL-13, IL-17A, TNFα, and E-Selectin, while Linoleamide correlated with MCP-1, IL-17A, IL-12p70, and E-Selectin.

Conclusions:

FAAs may play a role in DSPN pathogenesis through metabolic, inflammatory, and vascular pathways. Their associations with sensory and inflammatory parameters highlight their potential as biomarkers and therapeutic targets. Longitudinal studies are needed to validate their clinical utility.

1061 A — Podocyte metabolic adaptation to lipid overload – application to focal segmental glomerulosclerosis

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CO-AUTHORS: ***Aurore Hecq, Emmanuel Esteve, Vanessa Tagliatti, Jean-Marie Colet, Anne-Emilie Declèves***

SUB THEME: ***Biomarker validation and clinical translation***

Focal segmental glomerulosclerosis (FSGS) is a complex renal disorder marked by diverse glomerular alterations. Podocytes are the main targets of injury, with different stressors—depending on FSGS type—causing their death or detachment, leading to characteristic segmental scarring. Progress in treatment is limited by the difficulty in grouping patients by common podocyte-related mechanisms. Identifying specific biomarkers could enable more targeted therapies. Thus, here, our goal is to identify metabolic signatures and biomarkers of lipotoxicity as a first FSGS-inducing stress observed in the maladaptive form. For this aim, podocytes in vitro will be exposed to palmitate (PA).

Cells used were human immortalized podocytes. To identify metabolic signature, we first characterized the basal metabolism of podocytes using untargeted proton nuclear magnetic resonance (1H-NMR) spectroscopy-based metabolomics.

Podocytes were exposed to either PA or bovine serum albumine (BSA) for 24 hours, and both intracellular and extracellular compartments were analyzed by 1H-NMR spectroscopy.

Multivariate analysis, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were applied to generate scores and loadings plots.

A 24-hour exposure to PA led to a reduction in podocyte metabolic activity at 150 and 300µM. Metabolomic analysis revealed a clear separation between PA-treated and BSA-treated (vehicle control) samples. In the extracellular media, this separation was largely by elevated levels of branched- and unbranched-chain amino acids, as well as glucose, choline, and acetate in PA-treated samples.

In cell lysates, PA-treated podocytes displayed higher levels of myo-inositol, glucose, o- phosphocholine, lactate, and glycine, whereas BSA-treated podocytes have more choline, aspartate and glutamate.

This study enabled the characterization of basal podocyte metabolism using 1H-NMR based- metabolomics and demonstrated that PA induces distinct metabolic signals, making it a potential source of biomarkers. These comprehensive analyses contribute to a deeper understanding of podocyte responses and metabolic alterations in the context of FSGS-related stresses.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1062 A — Fecal metabolomic analysis reveals biomarkers for early diagnosis of colorectal cancer.

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CO-AUTHORS: ***Dinh Hai Le, Phuong Nhung Nguyen***

SUB THEME: ***Biomarker validation and clinical translation***

Early diagnosis unequivocally improves the survival rate and therapeutic intervention for colorectal cancer patients. Despite recent advancements of early screening approaches, the sensitivity, specificity and accuracy of screening results are still limited. In this study, a comparative fecal metabolomic analysis was conducted to identify potential biomarkers between 49 colorectal cancer (CRC) patients and 51 healthy controls (HC) using an UPLC-QToF-MS method. Mass features of both negative and positive ion mode were annotated with LipidBlast 2022 database and integrated lipidomic database in Ms-DIAL version 5.5.250221. Statistical analysis identified 14 metabolites with significant differences (p-value ≤ 0.05 and false discovery rate ≤ 0.05) between the two investigated groups. Interestingly, a plastic derivative product (dioctyl phthalate) has been identified in the distinct profile of two groups emphasizing the danger of long term exposure of plastic product to our health. Another noticeable differential metabolite is dodecylbenzenesulfonic acid which could belong to exposomes from daily life routine products. Remaining 12 metabolites were further enriched by MetaboAnalyst6.0 and 1 metabolite (palmitoylethanolamide) has been significantly enriched with neurotransmission pathways. This study highlights the innovative use of metabolomic profiling in CRC diagnosis and underscores the need for future research to validate these biomarkers.

1063 A — Metabolic phenotyping and patient stratification of solid cancers with quantitative NMR serum spectroscopy

PRESENTING AUTHOR: ***Christoph Trautwein, University of Tuebingen, Germany***

CO-AUTHORS: ***Gyuntae Bae, Sisi Deng, Georgy Berezhnoy, Stefan Kommoss, Alfred Königsrainer, Lukas Mager, Saskia Biskup, Mathias Heikenwälder, Christoph Trautwein***

SUB THEME: ***Biomarker validation and clinical translation***

Introduction

The late diagnosis of solid cancers remains a major reason for poor prognosis and high mortality. Tumor development and progression of hepatocellular, colorectal, high-grade serous ovarian carcinoma (HCC, CRC, and HGSOC), glioblastoma multiforme (GBM), and pancreatic ductal adenocarcinoma (PDAC) are all profoundly implicated in dysregulated metabolism. Knowledge upon disease-specific metabolite concentrations in blood samples could provide a precious diagnostic tool for early-stage prediction or monitoring of disease progression and treatment efficacy.

Methods

We employed quantitative NMR serum spectroscopy [1] to measure absolute concentrations of 38 metabolites, 112 lipoprotein parameters and inflammatory glycoproteins of human HCC, CRC, HGSOC, GBM and PDAC patients and healthy controls to identify carcinoma-associated molecular signatures in a simple blood sample.

Results

We observed that HGSOC [2] and PADC [3] exhibited similar metabolic rewiring, whilst CRC, HCC, and GBM [4] showed increased ketogenesis, gut-microbial, and one-carbon metabolism, respectively. Remarkably, blood glutamine levels were unique for each type of cancer. HGSOC patients showed a hyperlipidemic phenotype that was more pronounced than that of the other carcinoma and healthy cohorts, including elevated levels of HDLs and apolipoproteins A-1 and A-2. With regard to the composition and size distribution of each lipoprotein, we found VLDL1-4 with phospholipids, cholesterol, free cholesterol, and triglycerides to be prominently higher in PDAC, while CRC showed the highest level of VLDL1 with phospholipids and the lowest level of LDL4-5. Moreover, HCC and GBM were characterized by the lowest level of HDL3-4 with cholesterol and the highest level of HDL1-2 with free cholesterol, respectively.

Conclusion

Using quantitative NMR, we uncovered unique metabolic, lipidemic, and inflammatory profiles of distinct solid cancers. These cancer-specific profiles offer great perspective for new biomarker panels that can be used for cancer diagnosis, treatment monitoring and prevention screening programs.

References

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[3] https://doi.org/10.1021/acs.jproteome.3c00611

[4] https://doi.org/10.1172/jci.insight.153526

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1064 A — Exploring Serum Metabolome and Lipoproteome of Elderly Patients with Early and Metastatic Colorectal Cancer

PRESENTING AUTHOR: ***Maria Salobehaj, CERM, Italy***
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SUB THEME: ***Cancer***

Colorectal cancer (CRC) is the third most common cancer and the second cause of cancer-related mortality worldwide, especially in elderly[1]. A major factor in CRC prognosis is stage: nearly 50% of new CRC cases are diagnosed at stage III and IV and it is urgent to find less invasive screening diagnostic tools[2]. High risk stage II and III patient benefit differently from adjuvant chemotherapy: it is necessary to evaluate risk/benefit ratio on an individual basis. It is urgent to find less invasive screening diagnostic tools and new essential biomarker[3]. Metabolomics is a valuable tool for biomarker discovery, including CRC[4].

We propose metabolomics using Nuclear Magnetic Resonance Spectroscopy, coupled to statistical tools, to analyze serum samples from elderly patients with CRC, early (pre-, post-operative and follow-up) and metastatic (before adjuvant chemotherapy and during disease progression).

Comparing metabolic profiles at different time points in early CRC (T0 vs T1 vs T2 vs T3) patients, 18 metabolites and 77 lipoproteins were significantly altered, whereas in metastatic CRC (T0 vs T1) 3 metabolites and 6 lipoproteins changed significantly. Then, T1 of early CRC patients was compared to T0 metastatic CRC patients to identify possible micrometastatic disease: a panel of 7 metabolites and 44 lipoproteins showed significant alterations. Moreover, comparing metabolic profiles in different colon portions (right, left and rectum), 16 metabolites and 75 lipoproteins emerged as significantly altered. To conclude, we focused on metabolomic and lipoproteomic differences between cancer grades and stages at T0 and T1. Our work strongly supports the rising importance of metabolomics for precise health medicine.

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1065 A — Metabolomic profiling of RSL3-induced HT-29 colon adenocarcinoma cell line and tumor resection specimens using 1H NMR spectroscopy to probe the role of ferroptosis in colorectal cancer

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SUB THEME: ***Cancer***

Ferroptosis is a recently discovered regulated cell death modality critically dependent of intracellular iron and characterized by extensive lipid peroxidation, culminating in plasma membrane rupture. Due to its inherent immunomodulatory effects, ferroptosis has been implicated in various pathophysiological conditions, such as in cancer where it exhibits a dual role in tumor development and suppression. To gain insights into the role of ferroptosis in colorectal cancer (CRC), 1H NMR metabolomic profiling was carried out in HT-29 colon adenocarcinoma cell line treated with RSL3, a known ferroptosis inducer, and with the clinical relevance also explored in parallel via profiling of paired tissue samples from CRC patients. RSL3-treated HT29 cells showed reduced cell viability (42% - 70%) after 15 – 20 hours of incubation, which could be reversed by coincubation with the ferroptosis inhibitor, ferrostatin-1, confirming ferroptotic cell death. The pelletized cells and tissues were extracted with cold aqueous methanol, homogenized, and the collected supernate was dried, redissolved in phosphate buffered D2O and subjected to 1H NMR analyses with DSS as reference standard. The obtained 1H NMR spectra, after processing and correction, were binned and used as input data for multivariate analysis. It was also annotated in parallel, using built-in Chenomx database and comparison with literature, with more metabolites being putatively identified in tissues. Multivariate analysis, specifically PLS-DA, clearly differentiated between treated and untreated HT-29 cells, and between normal and tumor tissues. For the former, the greatest contributors to the observed variation were from the downfield region containing signals for formate, hypoxanthine, inosine, and phthalate, while for the latter, in addition to the downfield signals similar to HT-29, upfield signals corresponding putatively to lactate, threonine, isoleucine, and valine also contributed to the observed separation. These similarities and differences could be further probed, ultimately towards exploiting ferroptosis for better management of colorectal cancer.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1066 A — Deciphering the Complexity of Experimental Design for Organoids Metabolism Research

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SUB THEME: *Cancer*

Patient-derived organoids (PDOs) have emerged as powerful in vitro models for studying cancer, as they preserve the genetic and phenotypic heterogeneity of patient tumors. These three-dimensional structures provide a physiologically relevant platform to investigate tumor metabolism, drug responses, and interactions with the tumor microenvironment. By enabling co-culture experiments with autologous tumor-infiltrating lymphocytes (TILs), PDOs also offer a valuable tool for exploring tumor-immune interactions and identifying novel therapeutic strategies. Utilizing this model, we aim to develop an approach to identify patient-specific metabolic interventions that enhance the efficacy of immune checkpoint blockade and prevent resistance in metastatic colorectal cancer (mCRC).

Studying metabolism in PDOs and PDO–TIL co-cultures is challenging due to variability in size, growth, and culture conditions, as well as extracellular matrices and media composition. Low PDO biomass further complicates metabolite extraction, demanding highly sensitive techniques and optimized workflows.

In this study, we optimized key parameters influencing the metabolism of PDOs and TILs to establish robust co-culture conditions for CRC research. PDOs were generated and autologous TILs were expanded from mCRC patient biopsies. Given the relevance of lymphocyte proportion and activation state, TIL populations were characterized by flow cytometry. PDO seeding conditions were refined based on growth kinetics. Different culture media were evaluated by assessing cell viability, growth rate, and metabolite consumption rates. In addition, preliminary metabolomics analyses guided the experimental design and workflow optimization.

Establishing appropriate co-culture conditions will provide a robust platform for investigating metabolic interventions in mCRC, ultimately improving the translational potential of metabolic studies for mCRC treatment.

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1067 A — Sex-dependent effects of diet on metabolic profiles and tumor burden in a colorectal cancer mouse model

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SUB THEME: *Cancer*

Background: Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide. Diet, a key modifiable risk factor, shapes gut microbiota and luminal metabolite composition. Western diet (WD) and Mediterranean diet (MD) represent unhealthy and healthy dietary patterns, respectively, known to modulate CRC risk. This study investigates their impact on CRC initiation and biofluid metabolic profiles in a mouse model.

Methods: FabplCre;Apc15lox/+ mice (35 males, 24 females), a CRC model, were randomized to MD or WD. Mice were euthanized at disease onset (13 weeks for males, 15 for females). Fecal and urinary samples were collected pre-dietary intervention, mid-experiment and at endpoint, along with colon luminal content. Metabolic profiling was assessed using ^{1}H NMR spectroscopy.

Results: Diet did not significantly impact colon tumor count. However, WD-fed females exhibited a significantly higher percentage of ileal tumors compared to WD males ($p=0.010$, OR:15.43, CI:1.61-184.4). ^{1}H NMR analysis revealed distinct metabolic profiles between MD and WD across all biofluids. Principal component analysis showed clear diet-based clustering, with pronounced sex-specific differences in urinary spectra. Orthogonal Partial Least Squares (OPLS)-Discriminant Analysis confirmed significant metabolic differences between MD and WD within each sex ($p<0.001$), with diet also driving sex-linked changes ($p<0.002$), except for fecal WD comparisons. OPLS-Regression Analysis identified significant correlations between colon tumor volume and fecal and colon luminal metabolites, specifically in WD males. Colon tumor volume correlated inversely with colon luminal branched-chain amino acids, 5-aminovalerate and an unidentified acetyl-linked compound (Pearson $r= -0.54$ to -0.61, $p<0.026$).

Conclusion: Our findings highlight a sex-dependent effect of diet on metabolic profiles and ileal tumor burden in FabplCre;Apc15lox/+ mice. While colon tumor count was unaffected, correlations between colon tumor volume and luminal metabolites suggest a link with tumor progression. Future work will evaluate ileal lumen metabolome and the cellular impact of tumor-associated metabolites to clarify their role in tumorigenesis.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1068 A — Comprehensive characterization of volatile organic compounds in urine for the annotation of potential prostate cancer biomarkers

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CO-AUTHORS: *Mark Woollam, Mangilal Agarwal, Joe Binkley, David Alonso*
SUB THEME: *Cancer*

Prostate cancer is a major cause of death for males in the United States. Current screening tests for this type of cancer include digital rectal examination (DRE) and the measurement of prostate-specific antigens (PSA) in blood. Unfortunately, these tests lack specificity and often lead to erroneous results. The development of a selective complementary, non-invasive test for the early detection of prostate cancer would be beneficial for early medical intervention, periodic monitoring, and treatment. The goal of this study was to develop an analytical methodology for the identification of potential prostate cancer biomarkers in urine.

Aliquots (2.5 mL) of urine (20 normal, 20 diseased) were placed in 20 mL headspace vials. The vials were incubated for 1 minute at 60 °C, and then extracted with a tri-phase (DVB/PDMS/C-WR) solid-phase microextraction (SPME) fiber for 45 minutes. The materials on the fiber were then desorbed in the inlet of a gas chromatograph, and data was collected using enhanced chromatography and high-resolution time-of-flight mass spectrometry (GCxGC-HRTOFMS). Complementary electron ionization (EI), positive chemical ionization (PCI), and negative chemical ionization data (NCI) were acquired for the samples using a multi-mode source (MMS). The data was processed using automated, non-targeted Peak Find. Sample constituents were annotated using spectral similarity searches (NIST & Wiley Databases), mass accuracy calculations for formula determinations, and retention index filtering. This analytical methodology not only increased the total number of VOCs annotated but also added confidence to compound characterization. The VOCs included hydrocarbons, aromatics, alcohols, aldehydes, esters, sulfur, and nitrogen-containing compounds, as well as xenobiotics (e.g., phthalates, bisphenols), terpenes and terpenoids. Statistical processing of the data resulted in the annotation of potential VOC biomarkers of prostate cancer in urine.

1069 A — Metabolomic Profiling Identifies Biomarkers Linked to Endometrial Carcinoma Severity Using Nuclear Magnetic Resonance Spectroscopy

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CO-AUTHORS: *ChiaoYun Lin, MengHan Chiang, ChiehNi Kuo, Angel Chao, ChihYung Chiu*
SUB THEME: *Cancer*

Background:

Endometrial carcinoma (EC) is among the three most common female reproductive malignancies, typically occurring after menopause or during perimenopause. EC is classified into three grades based on tumor cell abnormalities, with higher grades indicating greater malignancy and poorer prognosis risk. Metabolomics can highlight metabolic differences across cancer grades, aiding in diagnosis and prediction of disease progression. A comprehensive metabolomics study of EC may provide insights into its pathogenesis, improve diagnostic accuracy, and guide personalized treatment.

Methods:

This study analyzed 130 women with endometrial carcinoma, classifying the tumors into three grades based on the degree of abnormality in tumor cells: Grade 1 (well-differentiated) tumors had cells resembling normal endometrial cells (n=68), Grade 2 (moderately differentiated) tumors had cells less similar to normal cells (n=40), and Grade 3 (poorly differentiated) tumors had cells significantly abnormal (n=22). Plasma metabolomic analysis was performed using nuclear magnetic resonance (NMR) spectroscopy, and partial least squares discriminant analysis (PLS-DA) was applied for further analysis. Metabolites significantly associated with different grades of endometrial carcinoma were identified, and their roles in functional metabolic pathways were assessed.

Results:

In this study, body weight and height, and BMI were observed to be higher in women with grade 1 EC compared to those with different grades ($P < 0.05$). -The PLS-DA score plots of plasma 1H-NMR spectra showed separation among different endometrial carcinoma grade groups. Eleven metabolites were observed related to the grades of EC. Among them, lysine was found to be strongly associated with EC grades ($P < 0.01$). Further functional metabolic pathway analysis revealed that the valine- and leucine-related branched-chain amino acid (BCAA) metabolism ($P < 0.001$), and lysine associated biotin metabolism ($P < 0.05$) appeared to be strongly associated with endometrial carcinoma grades.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1070 A — Global Metabolomic and Lipidomic Profiling of Bone Marrow Plasma Identifies Distinct Signatures of Minimal Residual Disease in Multiple Myeloma Patients

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CO-AUTHORS: *Pegah Abdollahi, Barbora Piskláková, Anne-Marie Rasmussen, Helge Rootwelt, Katja B. P. Elgstøen*

SUB THEME: *Cancer*

Objectives: Multiple myeloma (MM) is the second most common hematologic malignancy, with over 188,000 new cases worldwide and nearly 121,000 deaths annually. Within the bone marrow (BM), interactions between malignant plasma cells and surrounding tissues drive disease progression. While treatments once targeted cancer cells, newer strategies harness the patient's immune system in the BM. However, relapse remains frequent due to minimal residual disease (MRD), underscoring the need for deeper BM understanding. Global metabolomics and lipidomics offer insights into metabolic and lipid signatures underlying MM, potentially revealing novel biomarkers and therapeutic targets.

Methods and patients: Bone marrow plasma samples (n=8) were obtained from the Oslo Myeloma Centre biobank, including patients at diagnosis and after first-line treatment reaching either MRD-positive (MRD+) or MRD-negative (MRD-) state. Metabolites and lipids were extracted using methanol and isopropanol, respectively. Samples were analyzed in triplicate via HPLC coupled to Q-Exactive Orbitrap MS or Fusion Orbitrap Tribrid, operating in full MS and MS/MS modes in both polarities. Compound Discoverer 3.3 was used for peak integration, alignment, QC correction, and statistical analyses (two-tailed Student's t-test, PCA). Metabolites were identified through in-house and public libraries (Human Metabolome Database, mzCloud, ChemSpider).

Results: We identified over one hundred metabolites (Level 1 and 2), including amino acids, purines, pyrimidines, and exogenous compounds, as well as 485 lipids in positive mode and 105 lipids in negative mode from over 20 lipid classes. PCA revealed clustering in patients before and after treatment. Univariate analysis showed multiple differentially abundant metabolites and lipids between MRD- and MRD+ post-treatment, pointing to exogenous and microbial-derived compounds.

Conclusions: This comprehensive approach unveiled distinct metabolic and lipid profiles in the BM of MM, distinguishing MRD+ from MRD- groups. These findings highlight potential biomarkers and therapeutic targets, enabling enhanced disease monitoring, earlier intervention, and the development of more effective and personalized treatment.

1071 A — Optimizing Early Cancer Detection: Innovative Use of Metabolomics Profiling and Ensemble Machine Learning for a Unified Screening Test

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CO-AUTHORS: *Martina Lombardi, Nicole Palmieri, Alessio Trotta, Andrea Ingenito, Vera Abenante, Sean Richards, Steven Symes, Pierpaolo Cavallo, Marcello Persico, Mario Masarone*

SUB THEME: *Cancer*

Current cancer screening methods often suffer from low patient compliance and the burden of multiple tests. Here we use an integrated screening approach that leverages metabolomics profiling from minimally invasive dried blood spot (DBS) samples combined with advanced machine learning for early cancer screening.

A total of 1,616 DBS samples were analyzed, including 873 healthy controls and 743 cancer patients. Cancer cases comprised 149 bladder, 126 endometrial, 100 colorectal, 44 ovarian, 43 prostate, 27 renal, 19 cervical, 17 breast, 13 vaginal, 12 testicular, 8 penile, 5 uterine sarcoma, 5 liver, and 5 neuroendocrine cases. Samples were processed comprising metabolite extraction, purification, and derivatization following lyophilization. Untargeted metabolomic profiling was performed with GC-MS, and chromatograms were pre-processed using a custom Python script that applied dynamic time warping for alignment, normalized data based on internal standard, and computed peak areas after deconvolution. The dataset was then refined through median normalization, logarithmic transformation, and autoscaling.

To address class imbalance, a combination of undersampling and SMOTE was employed. Multiple machine learning classification models were built and optimized via cross-validation, including PLS-DA, linear discriminant analysis, naïve Bayes, decision tree, random forest, k-nearest neighbor, artificial neural network, support vector machine, logistic regression, generalized linear model, and fast large margin. An ensemble model was developed using a voting scheme weighted by cross-validation accuracy and classification confidence.

Separate ensembles were constructed for each screening stage and applied in a sequential fashion: one to first distinguish healthy subjects from cancer patients, then one to identify colorectal cancer against other cancers, and then additional models for bladder, lung, melanoma, and kidney cancers, and gender-specific models to distinguish breast (women) and prostate (men) cancers. All ensembles demonstrated robust performance with positive Q^2 and R^2 values, accuracies ranging from 85% to 100%, statistically significant permutation tests ($p<0.001$), and AUC-ROC values>0.9.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1072 A — Tracking Glioma Progression: Blood-Based Metabolic Markers from Pre-Diagnosis to Surgery

PRESENTING AUTHOR: ***Benny Björkblom, Umeå University, Sweden***
CO-AUTHORS: ***Sebastian Jonsson, Henrik Antti, Beatrice Melin, Benny Björkblom***
SUB THEME: ***Cancer***

Gliomas are highly invasive brain tumors with poor prognosis, particularly glioblastoma, the most aggressive subtype. Late detection significantly worsens prognosis, underscoring the need for early diagnostic markers. This study investigates metabolic alterations in blood samples collected at surgery and up to 20 years before diagnosis to identify potential markers for early glioma detection.

We analyzed blood samples from 52 glioma patients using untargeted UHPLC-MS/MS. Metabolite profiling targeted 773 annotated metabolites and 164 unknown compounds, capturing both established and novel metabolic alterations. Multivariate data analysis, including OPLS Effect Projection and Discriminant Analysis, was applied to compare pre-diagnostic and surgery samples. Subtype-specific (glioblastoma vs. lower grade glioma) and sex-specific analyses were conducted. Our study reveals significant metabolic shifts over time, with 518 detected metabolites showing substantial changes. Amino acid metabolism, particularly N-lactoyl amino acids, was notably elevated at surgery. Glioblastoma patients exhibited distinct metabolic profiles, including reduced long-chain acylcarnitine levels, compared to non-glioblastoma cases. While metabolic changes were largely consistent between sexes, some sex-specific differences emerged. Notably, a panel of eight metabolites demonstrated strong potential for early glioma detection, with levels rising well before diagnosis.

These findings highlight the promise of blood-based metabolic markers for earlier glioma detection. Further validation in larger cohorts could pave the way for a non-invasive tool, improving early diagnosis and patient survival.

1073 A — A Liquid Biopsy Metabolomics and Lipidomics Signature to Distinguish HCC from Chronic HCV

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SUB THEME: ***Cancer***

Early and accurate diagnosis of hepatocellular carcinoma (HCC) is crucial for improving patient outcomes. However, current biomarkers, such as α-fetoprotein (AFP) and micro-RNAs, often fail to meet clinical demands. To address this gap, we integrated a metabolomics and lipidomics liquid biopsy strategy to identify plasma biomarkers capable of distinguishing HCC from chronic hepatitis C virus (HCV)-infected patients.

In the untargeted phase, plasma samples from a cohort of 102 HCV-positive patients with HCC, chronic HCV, and mixed cryoglobulinemia (MC) were analyzed using HILIC and RP chromatography with a DDA-PASEF approach. A supervised PLS-DA model demonstrated that metabolomics and lipidomics had superior predictive accuracy for HCC compared to AFP in the test set. Additionally, when applied to HCC patients with AFP values <20 ng/mL, the model, using only the top 20 variables selected by VIP scores, achieved an Area Under the Curve (AUC) of 0.94. Key discriminant features included short-, medium-, and long-chain acyl-carnitines (CARs) and lysophosphatidylcholines (LPCs).

To enable absolute quantification, we transitioned to a targeted approach in an external cohort. A HILIC-HRMS t-SIM Multiplexing mode was developed and applied to quantify a panel of CARs and LPCs within a 10-minute analysis time. The method demonstrated robust analytical performance: sensitivity (LOD_avg: 0.0558 ng/mL, LOQ_avg: 0.667 ng/mL), accuracy (98.75%), linearity (R^2 = 0.999) stability (intra-day CV: 0.39%, inter-day CV: 1.46%) low matrix effects (10%) and high recovery rates (95.82%) using isotope-labeled standards further confirmed its robustness.

Results showed elevated short- and long-chain CARs in HCC, whereas medium-chain species were more abundant in HCV. LPC levels were markedly reduced in HCC for both saturated and unsaturated species. These distinct metabolic profiles hold promise for developing a novel risk classification index for HCC, representing a significant step toward early diagnosis and personalized patient care.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1074 A — Toward Precision Theranostics: Deciphering the Complexity of Tumor Microenvironment by Quantitative and Spatial Metabolomics

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SUB THEME: *Cancer*

Senescence, an irreversible cell cycle arrest, can be readily induced in tumors by many chemo-, radio- and immunotherapies. The senescence-associated secretory phenotype (SASP) modifies the tumor microenvironment and is further associated with therapy resistance, while the metabolomic role of SASP remains poorly understood. This study aims to quantitatively and spatially characterize senescent-cell metabolome to identify senescence-related metabolic markers relevant to cancer diagnostics and therapy.

Metabolic alterations caused by chemotherapeutic senescence induction were investigated in tumor cell lines and mouse tumor tissue. Senescent and control cells of two hepatocellular carcinoma cell lines, H-Ras and AMp19 were characterized by ^{1}H-NMR spectroscopy-based metabolomics. Mouse AMp19-cell subcutaneous xenograft tumors were subjected to validation of senescence induction by H&E staining, p21, p53 and Ki73 marker staining. Senescence radiotracer uptake areas and spatial metabolomics were analyzed by autoradiography and timsTOF-fleX MALDI-2 imaging MS with particular interest in low-molecular-weight metabolites and lipids.

Senescent H-Ras-cell metabolomics highlighted changes in creatine, myo-inositol, and phospholipid pathways, emphasizing senescence's impact on cellular growth and extracellular matrix degradation [1]. AMp19-cell model further highlighted the dynamic nature of senescence over time. Autoradiography of senescence radiotracer uptake in vivo co-localized with the spatial MALDI imaging-characterized metabolite distribution ex vivo indicated senescent region-specific feature accumulation in the tumor tissue. Further sphingolipid and phosphoethanolamine pathway alterations that are highly relevant for pre- and anti-inflammation regulatory omega-3 and -6 fatty acid balance during the cell cycle arrest where characterized.

NMR spectroscopy-based and spatial metabolomics by MALDI imaging MS alongside in vivo imaging are highly relevant and powerful approaches to pinpoint the senescent tumor metabolic heterogeneity during cellular growth, energy, and repair mechanisms. As certain lipids modulate SASP, lipid and SASP metabolic interactions have a potential to guide therapeutic and diagnostic strategies targeting senescent tumor cells and mitigate their adverse effects.

[1]Knopf P, et al. 2024 https://doi.org/10.1002/nbm.5157

1075 A — Targeted LC/MS Derivatization for Accurate Quantification of Phosphorylated Metabolites in Cancer Research

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SUB THEME: *Cancer*

Phosphorylated metabolites (PMs) serve as crucial biomarkers for diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. However, their quantification in biological matrices presents significant challenges due to matrix effects and ion suppression, stemming from their similar physicochemical properties and ionization behaviors. Additionally, the presence of phosphate groups complicates chromatographic separation, often resulting in poor peak shapes and reduced LC-MS sensitivity. Derivatization offers a solution by enhancing ionization efficiency, improving detection sensitivity, and stabilizing compounds to prevent fragmentation during ionization. While monophosphate derivatization is well-documented, modifying diphosphates and triphosphates remains challenging due to their high polarity, instability, and propensity to form multiple products. Moreover, phosphate groups are pH-sensitive and prone to forming metal ion complexes, further complicating their reactivity. Therefore, developing selective derivatization strategies is critical to overcoming these obstacles and enabling precise quantification in complex biological samples.

Our study focuses on the comparative evaluation of different derivatization strategies for multiple phosphate groups in combination with LC-MS analysis. We assessed four derivatization reagents, each utilizing a distinct reaction mechanism. At the same time, sample preparation with titanium dioxide was optimised to enhance selective derivatisation.

The optimized conditions will be applied in a cancer study, while simultaneously incorporating four-channel chemical isotope labeling [1], which targets the most abundant functional groups, including amine, phenol, hydroxyl, carboxyl, and carbonyl. Furthermore, the implementation of 13C-labeling will serve to enhance the quantitative accuracy of the study by minimising isotopic effects and thereby facilitating improved metabolite tracking in complex biological samples. This methodological approach ensures precise differentiation between endogenous metabolites and labeled standards, thereby increasing the reliability of comparative metabolic studies.

1 Anal. Chem. 2019, 91, 18, 12108–12115

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1076 A — Distinct Metabolic Signatures in Breast Cancer Extracellular Vesicles: Implications for Tumor Progression and Biomarker Discovery

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SUB THEME: *Cancer*

Extracellular vesicles (EVs) are membrane-derived particles that facilitate intercellular communication by transferring diverse cargo, including DNA, proteins, lipids and small molecules. Recent studies highlight the role of EVs in the tumor microenvironment (TME), where they promote tumor growth and survival. Altered metabolism is a hallmark of breast cancer, and metabolites can act as signaling molecules, influencing neighboring cells in the TME. Hence, it is likely that metabolite cargo of EVs contributes to breast cancer progression. However, while advances in EV isolation and characterization techniques have expanded our understanding of proteome and RNA cargo, the metabolic content of cancer-derived EVs remains unexplored.

Here, we integrate mass spectrometry-based metabolomics with proteomics data to characterize the metabolic contents of breast cancer-derived EVs. Metabolic profiling of EVs secreted by different breast cancer cell lines revealed a distinct metabolic signature, composed of nucleotides and amino acids, that differentiates EVs from their parental cells. In addition, EV metabolic profiles reflect characteristics of their originating breast cancer subtype. Notably, we observed an enrichment of immunosuppressive purines in EVs secreted by triple-negative breast cancer cells. To further investigate whether metabolic enzymes in EVs align with these metabolic signatures, we analyzed the abundance of metabolic enzymes across different breast cancer-derived EVs. Our findings show that EVs from different breast cancer subtypes contain distinct metabolic enzyme profiles, associated with specific subcellular locations. For example, triple negative breast cancer-derived EVs contain higher levels of enzymes linked to purine metabolism and cytosol compared to other subtypes.

Collectively, our findings demonstrate that breast cancer-derived EVs carry both shared and subtype-specific metabolic cargo, reflecting the metabolic characteristics of their parental cells. These metabolism-associated EV contents may serve as potential biomarkers or functional regulators in breast cancer development and progression.

1077 A — Pre-Diagnostic Urinary Metabolites and Kidney Cancer Risk

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SUB THEME: *Cancer*

Kidney cancer incidence has increased worldwide in recent decades. While metabolomic studies have shown promise in unveiling mechanisms underlying disease development, few studies have investigated pre-diagnostic urinary metabolites and kidney cancer risk. We conducted a case-control study nested within the Shanghai Women's and Men's Health Studies to prospectively investigate the association between urinary metabolites and cancer risk. Two hundred primary kidney cancer cases and their individually matched controls were included. A total of 1,301 metabolites were evaluated, and 67 metabolites were found nominally associated with kidney cancer using conditional logistic regression. After backward selection, eleven urine metabolites remained significantly associated with kidney cancer: lipids (e.g., picolinoylglycine, odds ratio (OR); 95% confidence interval (CI): 2.01 [1.44, 2.79], and pregnanediol-3-glucuronide, OR; 95% CI: 0.56 [0.39, 0.82]), xenobiotics (e.g., beta-guanidinopropanoate, OR; 95% CI: 1.75 [1.32, 2.32] and 4-vinylphenol sulfate, OR; 95% CI: 0.66 [0.49, 0.90]), and nucleotides (e.g., allantoic acid, OR; 95% CI: 0.71 [0.54, 0.92]). Time lag analysis showed that metabolite-kidney cancer associations were stronger for beta-guanidinopropanoate (OR; 95%CI: 8.22 [1.68, 40.18]) and picolinoylglycine (OR; 95% CI: 6.45[1.28, 32.43]), but weaker for allantoic acid (OR; 95%CI: 0.87 [0.37, 2.06]) and 3-methylglutarate/2-methylglutarate (OR; 95%CI: 0.62 [0.19, 2.00]) when urinary samples were collected within 3 years between urine sample collection and cancer diagnosis (Pinteraction <0.05 for all). Future metabolomics studies with large sample sizes, particularly from multiple ancestry populations, are needed to validate our findings.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1078 A — Serum Metabolic Signatures of Chronic Limb-Threatening Ischemia in Patients with Peripheral Artery Disease

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SUB THEME: *Cardiovascular diseases*

Background:

Peripheral artery disease (PAD) is characterized by the atherosclerotic narrowing of lower limb vessels, leading to ischemic muscle pain in older persons, known as intermittent claudication (IC). Some patients experience progression to advanced chronic limb-threatening ischemia (CLTI) with poor long-term survivorship and 5-year mortality of 50%.

We aimed to perform a comprehensive serum metabolomics study to reveal the mechanisms of PAD pathophysiology that may improve its diagnosis and prognosis to CLTI complementary to the ankle–brachial index (ABI) and clinical presentations.

Methods:

Non-targeted metabolite profiling of serum was performed by multisegment injection–capillary electrophoresis–mass spectrometry (MSI–CE–MS) from age and sex-matched, non-diabetic, PAD participants who were recruited and clinically stratified based on the Rutherford classification into CLTI (n = 18) and IC (n = 20), and non-PAD controls (n = 20). Overall, 85 authentic metabolites were consistently detected (CV < 30%) in the majority of serum samples (>75%) when applying stringent quality control (QC), with high confidence (level 1) identification of 77 out of the 85 metabolites.

Results:

Compared to the non-PAD controls, PAD patients had lower serum concentrations of creatine, histidine, lysine, oxoproline, monomethylarginine, as well as higher circulating phenylacetylglutamine ($p < 0.05$). Importantly, CLTI cases exhibited higher serum concentrations of carnitine, creatinine, cystine and trimethylamine-N-oxide along with lower circulating fatty acids relative to well matched IC patients. Most serum metabolites associated with PAD progression were also correlated with ABI ($r = \pm 0.24–0.59$, $p < 0.05$), whereas the ratio of stearic acid to carnitine, and arginine to propionylcarnitine differentiated CLTI from IC with good accuracy (AUC = 0.87, $p = 4.0 \times 10–5$).

Conclusion:

This work provides new biochemical insights into PAD progression for the early detection and monitoring of high-risk patients to improve prognosis and direct vascular interventions. Future work aims at further validation for clinical translation of candidate biomarkers.

1079 A — Uncovering the shared lipidome-wide markers of carotid artery atherosclerotic plaque and metabolic associated fatty liver disease (MAFLD) in non-overlapping Individuals: The Young Finns study

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SUB THEME: *Cardiovascular diseases*

Background: Metabolic associated fatty liver disease (MAFLD) and carotid artery atherosclerotic plaque diseases, are both linked to circulatory lipid and lipoprotein metabolism. However, shared lipidomic-wide mechanisms underlying these diseases in non-overlapping populations remain unexplored.

Objectives: To identify plasma lipid species associated with both MAFLD and carotid artery plaque diseases in order to uncover shared metabolic pathways.

Subjects and Methods: We analyzed data from the Young Finns Study cohort from 2007 and 2018 follow-ups (n=1496, aged 41-56 years, 56.3% females). Ultrasound was used to determine the prevalence of both carotid atherosclerosis plaques and MAFLD during 2018 follow-up. Participants were divided into two subsets of not overlapping subjects: 693 with only carotid plaque but without MAFLD and 586 with only MAFLD but without carotid plaque. Lipidomic profiling of 437 lipid species from plasma was performed during 2007 follow-up (30-45 yrs) using liquid-chromatography-tandem mass spectrometry. Logistic regression models, unadjusted and adjusted for age, sex, physical activity, alcohol consumption, and smoking, were used to separately assess lipid associations with both disease outcomes. Odds ratios (OR) and confidence intervals (95% CI) were calculated for each lipid species, and multiple testing corrections were performed using the false discovery rate (FDR) method (< 0.05).

Results: In unadjusted and adjusted models, 80 and 4 lipids respectively, were significantly associated with carotid plaque. In adjusted model; 202 lipids were linked to MAFLD. In unadjusted model, we found 51 lipids significantly associated with both diseases. Notably, in the adjusted models only one lipid, PC 40:4, was significantly associated with both diseases, and significantly increased risk of disease in both conditions OR 2.59 (95% CI, 1.57- 4.32) and OR 5.26 (95% CI, 2.81- 9.85) for carotid plaques and MAFLD, respectively.

Conclusion: Our findings identify PC 40:4 as a shared lipid signature between MAFLD and carotid plaque disease, suggesting common metabolic pathways.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1080 A — May the post-translational process of succination be involved in cardiotoxicity?

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CO-AUTHORS: *Julien De Winter, Anne-Emilie Declèves, Jean-Marie Colet*
SUB THEME: *Cardiovascular diseases*

Abstract

Cardiovascular diseases (CVDs) are one of the leading causes of morbidity and mortality worldwide. CVDs can be caused by different conditions including heart failure, atherosclerosis, and ischemic cardiomyopathy. The impact of CVD on patients' quality of life is significant, highlighting the urgent need for better management, including the development of new therapies that request deeper molecular understanding. One possible target could be SUMOylation, a post-translational modification (PTM) in which Small Ubiquitin-like Modifier (SUMO) proteins are covalently attached to lysine residues on target proteins, has emerged as a key regulatory process in cardiovascular development and function. In certain pathological cases, the SUMOylation of proteins that are critical for cardiac function can be significantly modified. It is particularly the case for SUMO1, one of the members of the SUMO family, which is essential for regulating SERCA2a, a calcium pump that plays an essential role in cardiac muscle relaxation by regulating calcium reuptake after contraction. In this study we investigated how succination, a PTM caused by the reaction of fumarate with cysteine residues following its accumulation in cells, impacts SUMO1 function of SUMOylating SERCA2a. Using AC16 cardiomyocytes treated with sodium fumaric acid, we detected some metabolic shifts following the elevation of fumarate concentration via 1H-NMR spectroscopy. Moreover, Western blot analysis suggests that succinated SUMO1 has a lower capacity to SUMOylate SERCA2a. This finding indicates that succination could potentially impair calcium fluxes, thus contributing to cardiotoxic effects. By exploring the link between both PTMS, succination and SUMOylation, this research reveals a novel regulatory mechanism in cardiac physiopathology, offering potential new avenues for therapeutic intervention in cardiovascular diseases.

1081 A — The influence of diabetes on the lipidomic signature of abdominal aortic aneurysm

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SUB THEME: *Cardiovascular diseases*

An abdominal aortic aneurysm (AAA) is an abnormal enlargement of the aortic wall which is life-threatening due to risk of rupture. Besides type-2 diabetes (T2D) being a major risk factor for cardiovascular disease, recent studies suggest that patients with T2D have a lower risk of AAA development, growth and rupture. Moreover, metformin, the first-line therapy for T2D, has been associated with decreased AAA growth rates. The aim of this study is to investigate differences in the plasma lipid profile between non-diabetic (ND) and diabetic (D) patients with AAA to explore the potential underlying effects of metformin.

Lipids from 25 plasma samples (5 healthy controls and 11 ND-AAA and 9 D-AAA patients undergoing elective open aortic surgical repair) were extracted with butanol:methanol (1:1, v/v) and analysed by an untargeted C18-RP-LC-MS/MS lipidomic approach. Lipostar and R software were used for data and statistical analysis, respectively.

Comparing ND-AAA with controls, ND-AAA patients have decreased levels of ether-linked phosphatidylcholines (PC) and phosphatidylethanolamines (PE) bearing polyunsaturated fatty acids (PUFA), saturated ceramides and sphingoid bases. Reduced PUFA are considered a cardiovascular risk factor. Comparing ND-AAA with D-AAA, saturated ceramides and sphingoid bases are reduced in ND-AAA, suggesting that T2D or metformin use raises their levels to normality. However, acylcarnitines show reduced levels only in D-AAA patients, which is associated with lower cardiovascular risk. D-AAA patients have also significantly lower levels of lyso-PC, likely due to reduced fatty acid oxidation since metformin decreases mitochondrial activity, enhancing glucose uptake and use as energy source. Compared to controls, cholesteryl esters are significantly diminished in both ND-AAA and D-AAA, possibly attributed to statin use or an aneurysm-induced alteration.

The modulation of the circulating lipid profile in D-AAA patients may be attributed to metformin intake. Further studies are needed to better understand the isolated effects of diabetes and metformin-induced lipid alterations.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1082 A — Metabolic profile of human bone marrow hematopoietic stem and progenitor cells upon differentiation, aging and leukemia

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SUB THEME: *Cellular metabolism*

Metabolic cues are crucial for regulating hematopoietic stem and progenitor cells (HSPCs). However, the metabolic profile of human HSPCs remains poorly understood due to the limited number of cells and the scarcity of bone marrow samples. Here, we present the integrated metabolome, lipidome and transcriptome of human adult HSPCs (lineage-, CD34+, CD38-) upon differentiation, aging and acute myeloid leukemia (AML). The combination of low-input targeted metabolomics with our newly optimized low-input untargeted lipidomics workflow allows us to detect up to 219 metabolites and lipids from a starting material of 3,000 and 5,000 HSPCs, respectively. Among other findings, we observe elevated levels of the essential nutrient choline in HSPCs compared to downstream progenitors, which decline upon aging and further decrease in AML. Functionally, we show that choline supplementation fuels lipid production in HSPCs and enhances stemness. Overall, our study provides a comprehensive resource identifying metabolic changes that can be utilized to promote and enhance human stem cell function.

1083 A — Isotope tracing reveals metabolic mechanisms of medium-chain fatty acid anaplerosis in malate dehydrogenase 2 deficiency

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CO-AUTHORS: *Jolita Ciapaite, Fried Zwartkruis, Nanda Verhoeven-Duif, Judith Jans*

SUB THEME: *Cellular metabolism*

Malate dehydrogenase 2 (MDH2) deficiency is an ultra-rare inborn metabolic disease characterized by severe early-onset encephalopathy, psychomotor delay and muscle hypotonia. Anaplerotic therapy with the odd-chain triglyceride triheptanoin has shown clinical benefit in one patient, yet its precise metabolic mechanisms remain unclear. To investigate this, we generated MDH2-knockout HEK293T and A549 cell models. Using these models, we aimed to characterize the metabolic phenotype of MDH2 deficiency and elucidate the effects of heptanoate, the free fatty acid form of triheptanoin and octanoate, a key component of the medium-chain triglyceride (MCT) diet. MDH2-deficient cells exhibited severely impaired TCA cycle flux and a decreased NAD+/NADH ratio, resulting in disrupted glycolysis and serine biosynthesis and an increased lactate/pyruvate ratio. Isotope tracing with 13C3-heptanoate and 13C2-octanoate revealed that direct anaplerosis by these fatty acids in MDH2-deficient cells was minimal. Despite this, they effectively restored the NAD+/NADH ratio, decreased the lactate/pyruvate ratio and restored serine biosynthesis. Furthermore, heptanoate was able to partially normalize decreased levels of odd-chain acylcarnitines in MDH2 knockout cells. These results suggest that medium-chain fatty acids may exert their therapeutic effects not only through citric acid cycle replenishment, but also by enhancing fatty acid oxidation and hereby modulating the redox balance. Therefore, this could be an effective treatment strategy for several inborn metabolic diseases affecting the cellular redox state.

1084 A — Blueberry Extract Modulates Lipid and Amino Acid Metabolism for Cytoprotection in IEC-6 Cells

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SUB THEME: *Cellular metabolism*

Vaccinium myrtillus L. is widely recognized for its high content of secondary metabolites which exhibit significant antioxidant and anti-inflammatory activities (1). Although these bioactive compounds are well documented for their health benefits, the specific metabolic pathways that drive their cytoprotective effects under oxidative stress remain unclear. To address this, we performed an untargeted high-resolution mass spectrometry (HR-MS) metabolomic approach to explore how a blueberry extract (BLUBE) influences key biochemical pathways in IEC-6 small intestine epithelial cells exposed to hydrogen peroxide (H_2O_2).

Oxidative stress—induced here by H_2O_2 exposure—is a pivotal factor implicated in multiple degenerative disorders, reflecting pathophysiological processes observed in vivo (2). Rather than relying solely on cellular antioxidant assays, our HR-MS metabolomic workflow provided a comprehensive snapshot of the intracellular metabolite landscape, allowing detection of shifts in cellular biochemistry. Following data acquisition, chemometric procedures were applied to enhance data quality. Multivariate analysis via Partial Least Squares Discriminant Analysis (PLS-DA) clearly distinguished control, H_2O_2-treated, and BLUBE-treated samples, achieving 98.75 ± 2.31% classification accuracy. This robust separation underscores the power of metabolomics to capture distinct metabolic fingerprints associated with oxidative injury and to identify specific perturbations that BLUBE counteracts. Pathway enrichment analysis highlighted modulation of sphingolipid metabolism, taurine and hypotaurine metabolism, and glycerophospholipid metabolism (3). BLUBE's impact on these pathways suggests its protective role extends beyond neutralizing reactive oxygen species (ROS). By influencing lipid and amino acid metabolism, BLUBE may maintain redox balance, and promote cell survival.

By integrating untargeted metabolomic profiling with pathway enrichment analysis, our HR-MS–based study provides a clearer picture of BLUBE's cytoprotective action, revealing effects beyond direct ROS neutralization. BLUBE modulates lipid and amino acid pathways crucial for cellular stress responses, highlighting potential regulatory targets. These findings underscore the broad-spectrum role of anthocyanins and phenolics in preserving redox balance, stabilizing energy metabolism, and fostering cell viability.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1085 A — Towards a combined phenotypic and metabolic profiling framework for studying the regulation of cell metabolism by the cell cycle machinery in cancer cell quiescence

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CO-AUTHORS: *Eva Schuler, Peter Jessernig, Lorena Michaelis, Karin Ortmayr*
SUB THEME: *Cellular metabolism*

A hallmark feature of cancer cells are pathologically high proliferation rates, as evidenced by a large number of anti-proliferative therapies. Nonetheless, non-dividing cell states in cancer are increasingly recognized as a therapeutic problem due to therapy resistance and a relative lack of knowledge about key mechanisms allowing cancer cells to access and survive in a quiescent state (G_0) for long periods. In earlier work, we have identified a characteristic adaptation of cell metabolism during the transition from fast proliferation to quiescence in several cancer cell lineages, including a prominent shift towards fatty acid oxidation and the importance of functional mitochondria. However, it remains an open question whether and how this metabolic adaptation to a non-dividing cell state is coordinated by the cell cycle machinery. To address this gap, we are establishing a systematic framework for perturbing cell cycle regulation using genetic interference by CRISPR-Cas9 knockouts combined with phenotypic and metabolic profiling by LC-MS based non-targeted metabolomics in cultured human cancer cells.

Here, we present an optimized procedure for effective interference with cell cycle regulation using CRISPR-Cas9 technology. We find that knockouts of several cell cycle regulators individually often do not show effects on proliferation, likely due to functional redundance and/or compensation by other factors. Nonetheless, we show that such effects can be bypassed by performing double-knockouts, i.e. parallel knockouts of two functionally linked genes, to effectively perturb cell cycle progression and induce detectable growth phenotypes. This methodology paves the way for assessing links between cell cycle regulation and metabolism to foster a mechanistic understanding of cancer cell quiescence and uncover potential new therapeutic targets.

1086 A — Glycine: the missing link between carbohydrate and xenobiotic metabolism in iPSC-derived hepatocyte maturation

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SUB THEME: *Cellular metabolism*

Modulation of cellular metabolism is a recognized driver in pluripotent stem cell (PSC) development and differentiation. Cellular differentiation is associated with decreasing glycolytic rates and the enhancement of oxidative metabolism. Glycine has been identified as an essential nutrient driving the maturation of liver cell models, including hepatocyte-like cells (HLCs) differentiated from induced PSCs (iPSCs). HLCs acquire xenobiotic CYP3A4 activity under a glycine-supplemented nutritional regimen.

In this study, we unravel the link between carbohydrate and xenobiotic metabolism induced by glycine in the differentiation process of HLCs. Using semi-targeted LC-MS-based metabolomics and 13C isotope-resolved metabolomics, we identified not only an expected increase in oxidative metabolism during differentiation but also the enhancement of various other biosynthetic pathways important for liver functionality. A glycine-rich environment boosted various metabolic pathways: collagen and bile acid biosynthesis, one-carbon metabolism, and heme biosynthesis, all characteristics of a hepatic phenotype. The increase of heme synthesis is particularly striking, as CYP3A4 is a heme-containing enzyme.

This study highlights the metabolic plasticity of in vitro iPSC models, undergoing metabolic reprogramming as a result of altering nutrient availability. It also demonstrates how modulation of central metabolic pathways may be crucial in the assembly of cytochrome P450 enzymes as key players in drug metabolism. This research suggests the possibility to influence cell behavior for personalized therapeutical purposes.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1087 A — 13C Metabolic Tracing in Human Adipocytes Provides a Potential NAM for Assessing Metabolism-Disrupting Properties of Environmental Chemicals

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SUB THEME: *Endocrine and metabolic diseases*

Exposure to certain environmental chemicals, including phthalates, may promote the development of metabolic diseases, including obesity. Since specific regulatory methods to identify metabolism-disrupting chemicals are currently lacking, the assessment of safer alternatives is hampered. The plasticizer DINCH was introduced as a replacement for banned phthalate plasticizers. However, previous studies have shown that its primary metabolite MINCH promotes adipogenesis of human adipocytes. To assess the effects of MINCH on adipocyte metabolism, human SGBS preadipocytes were exposed for 12 days to MINCH and subsequently incubated with different 13C tracing sources. The 13C labeling pattern of central carbon metabolites was analyzed using a targeted MRM approach and the effects were compared to cells differentiated with the PPARG agonist rosiglitazone and untreated control cells. After 24 h incubation with [U-13C]glucose, analysis of the 13C enrichment indicated a higher glycolytic and pentose phosphate pathway (PPP) flux for energy production and increased acetyl-CoA generation for lipid formation in MINCH-treated and rosiglitazone-differentiated cells. Analysis of the labeling pattern after [1,2-13C]glucose incubation confirmed the upregulation of glycolysis and the PPP cycle in MINCH- and rosiglitazone-treated cells, but showed an increase in the PPP/glucose metabolism ratio only in rosiglitazone-differentiated cells. The [U-13C]glutamine labeling pattern revealed reduced cycling through the TCA cycle and transient upregulation of reductive glutaminolysis for lipid production in MINCH and rosiglitazone-treated cells. Comparison of the 13C enrichment from [U-13C]glucose and [U-13C]glutamine showed increased enrichment from glucose compared to glutamine in MINCH- and rosiglitazone-treated cells, suggesting a switch to glucose for TCA cycle replenishment.

In conclusion, our 13C tracer data indicate that MINCH leads to a rewiring of metabolism towards lipid accumulation and adipogenesis similar to the PPARg agonist rosiglitazone, but they also reveal subtle differences in metabolic pathway activity. This emphasizes the applicability as a potential New Approach Method for the assessment of adipogenic effects.

1088 A — Uncovering Thiazolidinedione-Induced Metabolic Changes in Stem Cells Using a Click Chemistry Approach

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SUB THEME: *Endocrine and metabolic diseases*

Thiazolidinediones (TZDs), a class of insulin-sensitizing drugs, are widely used in the management of type 2 diabetes. While effective in improving insulin sensitivity, these drugs have adverse effects, including increased bone fracture risk. This study explores the molecular mechanisms underlying the action of TZDs using a novel bioorthogonal click-chemistry approach to distinguish mitochondrial and cytosolic pyruvate pools. The methodology combines metabolic flux analysis with stable isotope tracers and mitochondria-targeted probes to measure subcellular metabolite profiles under physiologically relevant conditions.

The findings reveal that next-generation TZD MSDC-0602K and rosiglitazone are more effective inhibitors of mitochondrial pyruvate transport than pioglitazone. These compounds stimulate alternative acetyl-CoA sources, activate glutamine utilization, and enhance mitochondrial malic enzyme activity, generating pyruvate from malate. Rosiglitazone also promotes de novo fatty acid synthesis, while pioglitazone predominantly stimulates cytosolic pyruvate synthesis via phosphoenolpyruvate carboxykinase (PEPCK), contributing to glyceroneogenesis.

This study highlights the potential of bioorthogonal click chemistry for compartment-specific metabolite tagging and provides valuable insights into TZD-induced metabolic alterations. The isotopologue profiles generated offer a deeper understanding of mitochondrial and cytosolic contributions to cellular metabolism, supporting the development of safer and more effective therapeutic strategies for type 2 diabetes.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1089 A — Identifying metabotypes of insulin resistance severity in children with metabolic syndrome

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CO-AUTHORS: ***Álvaro González-Domínguez, Otto Savolainen, Jesús Domínguez-Riscart, Alfonso Lechuga-Sancho, Rikard Landberg, Raúl González-Domínguez***

SUB THEME: ***Endocrine and metabolic diseases***

Insulin resistance is a frequent precursor of typical obesity and metabolic syndrome complications. However, accurate diagnosis remains elusive because of its pathophysiological complexity and heterogeneity. Herein, we have explored the utility of insulin secretion dynamics in response to an oral glucose tolerance test as a surrogate marker to identify distinct metabotypes of disease severity. The study population consisted of children with obesity and insulin resistance, stratified according to the post-challenge insulin peak timing (i.e., early, middle, and late peak), from whom fasting and postprandial plasma and erythrocytes were collected for metabolomics analysis. Children with late insulin peak manifested worse cardiometabolic health (i.e., higher blood pressure, glycemia, and HOMA-IR scores) than early responders. These subjects also showed more pronounced changes in metabolites mirroring failures in energy homeostasis, oxidative stress, metabolism of cholesterol and phospholipids, and adherence to unhealthy dietary habits. Furthermore, delayed insulin peak was associated with impaired metabolic flexibility, as reflected in compromised capacity to regulate mitochondrial energy pathways and the antioxidant defense in response to glucose overload. Altogether, these findings suggest that insulin resistance could encompass several phenotypic subtypes characterized by graded disturbances in distinctive metabolic derangements occurring in childhood obesity, which serve as severity predictive markers.

1090 A — Distinct Metabolic Alterations in Type 2 Diabetes and Diabetic Cardiomyopathy: Insights from Targeted and Untargeted Metabolomics

PRESENTING AUTHOR: ***Yejin Jung, Keimyung University, South Korea***

CO-AUTHORS: ***Jae-Hyung Park, Hochan Cho, Sooyeun Lee***

SUB THEME: ***Endocrine and metabolic diseases***

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that disrupts glucose, lipid, amino acid, and energy metabolism, primarily through insulin resistance and mitochondrial dysfunction. A severe complication of T2DM, diabetic cardiomyopathy (DCM), is characterized by impaired diastolic and systolic cardiac function. Recent metabolomic studies have highlighted mitochondrial dysfunction and altered energy metabolism in T2DM, with branched-chain amino acids strongly associated with the disease's pathogenesis. However, the metabolic differences that distinguish T2DM from DCM remain poorly understood. This study aimed to characterize the metabolic alterations between T2DM and DCM and to identify potential biomarkers. Plasma samples from three groups, healthy individuals, T2DM patients, and DCM patients (n = 15 per group), were analyzed using comprehensive targeted metabolomics (focusing on amino acids, polyamines, and tricarboxylic acid [TCA] cycle metabolites) and untargeted approaches. Multivariate analysis revealed distinct metabolic profiles that effectively differentiated the three groups. Targeted analysis showed that suppression of the TCA cycle was closely associated with altered amino acid levels, underscoring impaired energy metabolism as a key feature in the pathogenesis of DCM. These findings offer valuable insights into the distinct metabolic alterations between T2DM and DCM, providing a foundation for the development of potential biomarkers that could facilitate early diagnosis and inform targeted therapeutic strategies for DCM in T2DM patients.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1091 A — Metabolomic signature of Alström and Bardet-Biedl syndromes

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CO-AUTHORS: ***Ewa Zmyslowska-Polkowska, Krzysztof Solowiej, Sandra Chmielewska, Katarzyna Miniewska, Sebastian Skoczylas, Julia Grzybowska-Adamowicz, Michal Ciborowski, Adam Kretowski, Agnieszka Zmysłowska***

SUB THEME: ***Endocrine and metabolic diseases***

Background: Alström syndrome (ALMS) and Bardet-Biedl syndrome (BBS) are rare genetic ciliopathies characterized by multisystemic involvement, with overlapping clinical features such as early-onset obesity, vision loss, and progressive neurodegeneration. Given the absence of effective causal therapies, investigations focused on understanding the molecular processes characteristic of ALMS and BBS are needed to develop effective therapeutic strategies. Metabolomics is a powerful tool for revealing metabolic pathways affected by a disease and proposing drug targets. The present study used untargeted metabolomics to evaluate metabolic pathways perturbed by ALMS and BBS.

Materials and methods: The study group included ALMS and BBS patients (n=30), obese subjects (n=20), and healthy controls (n=30). From each participant, gingival crevicular fluid (GCF), saliva, and serum samples were collected and forwarded for GC-MS analysis. Obtained metabolic profiles were analyzed using the Kruskal–Wallis test with the Conover-Iman post hoc analysis ($p<0.05$). Pathway enrichment analysis was performed for significant metabolites. Additionally, metabolomics data was correlated with clinical parameters.

Results: A total of 21, 50, and 52 significant metabolites were identified in GCF, saliva, and serum, respectively. These metabolites were predominantly related to amino acid (AA), fatty acid, and carbohydrate metabolism. In GCF, disrupted pathways included alanine, aspartate and glutamate metabolism, arginine biosynthesis, and glutathione metabolism. Similar disruptions were observed in saliva, along with alterations in arginine and proline metabolism and beta-alanine biosynthesis. More extensive dysregulations were detected in serum, involving unsaturated fatty acid biosynthesis, glycerolipid metabolism, and AA metabolism. In GCF, alanine levels positively correlated with triglycerides, creatinine, insulin, and HOMA-IR, whereas 3-hydroxybutyric acid positively correlated with HDL cholesterol and negatively with triglycerides. AAs were positively associated with markers of insulin resistance in both saliva and serum.

Conclusions: Metabolic perturbations observed in ALMS and BBS mainly involve AA and lipid metabolism.

1092 A — Metabolic Signature of Graves' Orbitopathy: A Multiplatform Metabolomic Study

PRESENTING AUTHOR: ***Sandra Chmielewska, Medical University of Bialystok, Poland***

CO-AUTHORS: ***Patrycja Mojsak, Joanna Godzien, Diana Leszczynska, Julia Zelkowska, Adrian Godlewski, Michal Ciborowski, Katarzyna Siewko, Adam Kretowski***

SUB THEME: ***Endocrine and metabolic diseases***

Background: Graves' orbitopathy (GO) is a rare autoimmune disease characterized by inflammation of the orbital tissues. Diagnosis largely relies on subjective clinical and imaging assessments, with no sensitive or specific biomarkers currently available. The limited understanding of GO pathogenesis has led to predominantly symptomatic treatment approaches. Metabolomics represents a powerful tool for identifying biomarkers and elucidating disease-related pathways. This study employed metabolomics to gain deeper insights into the metabolic mechanisms underlying GO.

Materials and Methods: This is the first study to perform comprehensive multiplatform metabolic profiling of serum samples from GO patients using both untargeted and targeted approaches. Untargeted metabolomics was conducted using liquid chromatography–mass spectrometry (LC-QTOF-MS) and capillary electrophoresis–mass spectrometry (CE-MS), while targeted analysis was performed using LC-MS with a commercially available kit for metabolites quantification. For untargeted analysis, serum samples were collected from 18 GO patients and 30 healthy controls; for targeted analysis, 28 GO patients and 24 controls were included. Statistical evaluation was performed using the Mann–Whitney U test with Benjamini–Hochberg correction ($p < 0.05$).

Results: Comparison between patients with active (moderate-to-severe) GO and healthy controls revealed a distinctive serum metabolic signature capable of discriminating GO cases from controls. A total of 67 metabolites showed statistically significant differences, including those detected by LC-QTOF-MS (57), CE-MS (3), and targeted metabolomics (12), with the majority showing reduced levels in GO patients. These metabolites belong to several classes, predominantly glycerophosphocholines (42), glycerophosphoethanolamines (6), sphingomyelins (7), and acylcarnitines (5).

Conclusion: This study reveals distinct metabolic alterations in Graves' orbitopathy, particularly in lipid metabolism. These findings may contribute to the development of improved diagnostic methods and novel therapeutic strategies for this autoimmune disease.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1093 A — The application of GC-MS for the analysis of metabolic changes induced via activation of brown adipose tissue by cold exposure

PRESENTING AUTHOR: ***Katarzyna Miniewska, Medical University of Bialystok, Poland***
CO-AUTHORS: ***Patrycja Mojsak, Paulina Choinska, Sandra Chmielewska, Joanna Godzien, Katarzyna Maliszewska, Adam Kretowski, Michal Ciborowski***
SUB THEME: ***Endocrine and metabolic diseases***

Introduction: Brown adipose tissue (BAT) has recently been considered a potential target to fight against obesity and type 2 diabetes. However, neither the mechanisms underlying its activation nor its associations with clinical parameters are well understood. The capability of metabolomics to simultaneously analyse multiple classes of compounds seems to be a perfect tool to study this scientific topic.

Aim of the study: The aim of this study was to explore alterations during cold exposure associated with the activation of BAT.

Methods: Twenty-five healthy young male adults underwent PET/MR imaging after two hours of cold exposure (CE). Based on the obtained results, they were divided into two groups, depending on BAT presence (15 possessing BAT, BAT(+); and 7 lacking BAT, BAT(-)). The level of metabolites was measured in plasma samples at the three time points: before as well as one and two hours after CE. Measurements were performed using a standard GC-MS method for metabolite profiling.

Results: In the BAT(+) group, we noticed that during CE, the level of several fatty acids (elaidic, linoleic, oleic, palmitic and palmitoleic) was significantly decreased after 120 minutes of CE compared to the baseline. These changes were not observed in BAT(-) group. While comparing BAT(+) and BAT(-) groups, we observed that the BAT(+) group exhibited significantly lower levels of succinic acid. After 60 minutes of CE, BAT(+) group showed significantly increased levels of elaidic, linoleic, oleic and trans-13-octadecenoic acid. Furthermore, the differences in elaidic and oleic acid levels persisted for another hour.

Conclusions: Between-group differences in the level of fatty acids during CE, and the fact that alterations during CE affected only the BAT(+) group, suggest that the activation of BAT affects lipid metabolism.

1094 A — Comprehensive Multi-Omic Analysis of Non-Invasive Skin Samples: Unveiling Connections Between Skin Health and Metabolic Syndrome

PRESENTING AUTHOR: ***Paolo Bonini, oloBion, Spain***
CO-AUTHORS: ***Sanae Benabou, Sajjan Singh Mehta, Ron Kingma, Neus Pou, Yeray Gabaldon, Raquel Cumeras, Oliver Fiehn***
SUB THEME: ***Endocrine and metabolic diseases***

Metabolic syndrome (MetS) is a global health concern characterized by abdominal obesity, hypertension, insulin resistance, and dyslipidemia. Emerging evidence links MetS with various skin disorders, suggesting that cutaneous manifestations may serve as early indicators of underlying metabolic disturbances. Insulin resistance and chronic low-grade inflammation are believed to underpin both MetS and these dermatological conditions. A multi-omic analysis of the skin may offer insights into MetS pathophysiology, identify novel biomarkers, and drive innovative therapeutic approaches1.

In a double-blind, randomized, parallel-group, placebo-controlled study, 64 subjects were divided into four groups (n=16 each): three groups received varying concentrations of a natural active dietary supplement, and one group received a placebo. Non-invasive stratum corneum samples were collected at baseline and subsequent time points. Metabolomic and lipidomic analyses utilized a dual-phase extraction method, while proteomic profiling employed the PreOmics ENRICH-iST kit. Data acquisition was performed using Zeno SWATH MS. Proteomic data was processed with DIA-NN software, and metabolomic and lipidomic data with oloMAP 2.0.

Our platform enables the identification of over 1,100 distinct lipids across 18 lipid classes, 1,400 proteins, and 200 metabolites across all samples. Notably, subjects receiving active formulations exhibited a significant increase in long-chain ceramides compared to the placebo group. These ceramides are essential for maintaining skin barrier function. Additionally, metabolomic analysis revealed alterations in metabolites associated with glucose metabolism, reduction in fatty acid oxidation intermediates and inflammatory cytokines, suggesting a systemic effect of the supplement on metabolic pathways.

This multi-omic study demonstrates that supplementation with the active ingredient leads to significant changes in the skin's lipid and metabolite profiles, particularly an increase in long-chain ceramides, which are crucial for skin barrier integrity. These findings provide mechanistic insights into how dietary interventions can modulate skin composition and function, offering potential therapeutic strategies for enhancing skin health and managing MetS.

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1095 A — A comprehensive untargeted metabolomics evaluation of lobular carcinoma

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CO-AUTHORS: *Erdem Karadeniz, Julia Kuligowski, Guillermo Quintás Soriano, Semih ÖZLİ, Mehmet Emrah YAMAN*

SUB THEME: *Epidemiology*

Invasive lobular carcinoma is one of the most common breast cancer subtypes, which could be diagnosed in around 15% of breast cancers. Although it shows a good prognosis relative to invasive ductal carcinoma, diagnosis and long-term management of patients are very hard to follow. Therefore, new biomarker discoveries are vital for this subtype. In our study, we have performed an untargeted metabolomics assay to understand the metabolic alterations in invasive lobular carcinoma patients. 50 healthy controls and patients were eligible for the analysis. Agilent Q-TOF MS/MS 6530 was used to generate a metabolome profile of each patient. Iterated data-dependent acquisition was evaluated. Bioinformatic analyses were performed via MATLAB and appropriate toolboxes.

Acknowledgement : This project was funded by TUBİTAK-2519 COST Working Group Support Program and Lobular Breast Cancer: Discovery Science, Translational Goals, Clinical Impact (LOBSTERPOT)

1096 A — Metabolites linking diet, persistent organic pollutants and microbiota composition to cardiovascular disease

PRESENTING AUTHOR: ★ *Yingxiao Yan, Chalmers Univ. of Technology, Sweden*

CO-AUTHORS: *Tessa Schillemans, Gael Toubon, Anton Ribbenstedt, Agneta Åkesson, Ingegerd Johansson, Ingvar Bergdahl, Carl Brunius*

SUB THEME: *Epidemiology*

Metabolomics provides opportunities to investigate mechanisms linking exposures to health outcomes. We aimed to discover metabolic regulation patterns that could link complex exposures (diet, persistent organic pollutants and gut microbiota) to future cardiovascular diseases (myocardial infarction (MI) and stroke), using meet-in-the-middle analysis.

We used data from 13309 participants in four Nordic cohorts (SIMPLER-Uppsala Clinical [SIMP-UC]; SIMPLER-Västerås Clinical [SIMP-VC]; the BioDiVa subcohort of the Västerbotten Intervention Program and the MAX subcohort of the Diet, Cancer and Health-Next Generations cohort) to identify metabolite features from untargeted LC-MS analysis that associated with exposures and outcomes. Outcome-related metabolite features were selected using Elastic Net-analysis (MUVR2 R package) and replicated using logistic regression in a cross-cohort discovery-replication design (SIMP-UC and SIMP-VC), adjusting for sex, age, education and smoking status. Associations of the outcome-related features with exposures were then investigated using partial spearman correlation, adjusting for sex, age, education, BMI (for organochlorine pollutants) and total energy intake (for food items) using data from all four cohorts for discovery and replication.

A total of 33 metabolite features associated with either MI or stroke in both discovery and replication cohorts. Among the 19 annotated features, pipecolate associated with lower risk of MI and correlated with nuts and dry fruit, and ascorbic acid (vitamin C). ω-hydroxydodecanoic acid associated with lower risk of MI and correlated with alcohol and red wine. Additionally, phosphatidylethanolamine PE(P-16:0/22:6) associated with lower risk of MI and correlated with per- and polyfluoroalkyl substances. Features correlating with microbiota species remain unannotated, suggesting microbiota involvement through pathways that could not yet be elucidated.

Using a robust discovery-replication scheme, only few exposure-metabolite-outcome associations could be fully reproduced, highlighting that metabolite associations observed in single-cohort studies may be either cohort-specific or spurious. Interpretation of the results is ongoing and further discussions of their implications will be presented.

1097 A — A Pilot Study on the Influence of Maternal Diet and Lifestyle on the Human Milk Exposome in the Context of Prematurity

PRESENTING AUTHOR: *Julia Kuligowski, Health Research Inst. La Fe, Spain*

CO-AUTHORS: *Alba Moreno-Giménez, Cristina Zapata de Miguel, Laura Campos-Berga, Eirini Bathrellou, Maria Gormaz, Anna Parra-Llorca, Ian Oesterle, Sabrina Berger, Kola Ayeni, Isabel Ten-Doménech, Vinicius Verri-Hernandes, Guillermo Quintás, Benedikt Warth*

SUB THEME: *Exposome research*

Human milk (HM) is the primary source of nutrition for preterm infants and plays a crucial role in their growth and development. However, its composition is influenced by various maternal factors, including diet and lifestyle. This pilot study aimed to assess the relation of maternal dietary habits and lifestyle on the HM exposome, focusing on xenobiotics such as mycotoxins and polyphenols, and their potential transfer to preterm infants. A cohort of mothers of preterm infants was classified into two clusters based on dietary intake, physical activity, and sleep patterns. Biofluid samples, including HM and infant urine, were collected and analyzed using both targeted and untargeted metabolomic approaches. A total of 17 polyphenols and 6 mycotoxins were detected in HM, while 14 polyphenols and 4 mycotoxins were present in infant urine. Despite differences in maternal diet and lifestyle between both clusters regarding (i.e., fewer sleeping hours and higher intakes in 24h recall questionnaires except for amino acids in cluster 1 vs cluster 2), no significant variations in xenobiotic concentrations were found between the two clusters. Correlation analysis between urine and HM profiles revealed three distinct xenobiotic groups, indicating complex metabolic interactions rather than direct dietary transfer. This study provides insights into the complex interplay between maternal factors and the HM exposome, highlighting the need for further large-scale investigations. While no direct link was established between maternal diet, HM composition, and infant xenobiotic exposure, the observed metabolic and developmental patterns underscore the necessity for a deeper understanding of early-life exposures in preterm infants.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1098 A — An innovative liquid chromatography-high-resolution mass spectrometry based technique for detecting micro/nanoplastics in human inflammatory bowel disease tissue

PRESENTING AUTHOR: ***Radmila Pavlovic, IRCCS San Raffaele Scientific Institute, Italy***
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SUB THEME: ***Exposome research***

Background

Inflammatory Bowel Disease (IBD) is a chronic disorder characterized by inflammation of the gastrointestinal tract (GT) and has a complex, multifactorial etiology. Emerging evidence suggests that environmental pollutants, including micro/nanoplastic particles (MNPs), may play a role in IBD pathogenesis. MNPs are widespread contaminants that can enter the GT through ingestion of contaminated food. Traditionally, MNPs have been analyzed using microscopic techniques, which pose limitations in precise identification and quantification. This study utilizes a newly developed liquid chromatography-high-resolution mass spectrometry (LC-HRMS) method to detect and analyze MNPs-related compounds in human intestinal tissues, addressing these challenges.

Materials & Methods

Human intestinal tissue samples were homogenized and subjected to monomer extraction using an organic solvent mixture, followed by incubation and high-speed centrifugation. Blank samples were processed alongside tissue samples to account for potential procedural contaminants. The supernatants underwent liquid chromatography separation and high-resolution mass spectrometry analysis. The analytical workflow incorporated: 1) A targeted approach using known standards to identify MNPs-related monomers. 2) An untargeted tactic to identify a broader range of MNPs-related compounds through suspect screening methodology.

Results

The analysis identified multiple plastic-associated compounds, including terephthalic acid, bisphenol A, and various phthalates. Notably, some polyethylene terephthalate (PET) oligomers were detected exclusively in tissue samples from IBD patients, suggesting a possible connection between MNP exposure and disease pathology. Caprolactam, a nylon degradation product, was found in both tissue and blank samples but exhibited significantly higher concentrations in tissues, indicating potential in vivo polymer degradation.

Conclusions

This study successfully applied LC-HRMS to detect and quantify MNP-related compounds in human intestinal tissues. The identification of unique plastic-related compounds in IBD-affected samples supports the hypothesis that MNP exposure may contribute to disease development and progression. Further research is essential to understand the broader health implications of MNPs and refine detection methodologies for future clinical investigations.

1099 A — Human breast milk metabolome and exposome: exploring the key markers and their associations infant growth

PRESENTING AUTHOR: ***Wei-Hsiang Chang, National Cheng Kung University, Taiwan***
CO-AUTHORS: ***Yung-Chieh Lin, Wei-Jeng Chao***
SUB THEME: ***Exposome research***

Background and objective: Human breast milk (HBM) is a highly complex biological fluid essential for achieving developmental milestones in early life. Previous studies on HBM monitoring have typically focused either on its metabolic profile or chemical exposures. However, few have simultaneously assessed both metabolites and chemical exposures to explore their combined effects on infant growth in terms of benefits and risks.

Material and Methods: A total of 178 mother-child pairs were recruited from the iFamily Project – Early Childhood Health at National Cheng Kung University Hospital. Mature lactation milk samples were collected within 30 ± 5 days postpartum to characterize HBM before the infant reached one year of age. Metabolite profiling and chemical exposome analysis were conducted using LC-QTOF-MS. Student's t-test was used to identify significant differences in metabolites and chemical compounds between normal and abnormal development groups. PLS-DA was employed to identify metabolite fingerprints and chemical compounds in mature milk across both groups. Additionally, multiple linear regression models were used to explore associations between individual metabolomic and exposomic components, while weighted quantile sum regression assessed their mixture effects.

Result: A total of 5,703 metabolites and 3,522 chemical compounds were identified in 178 samples using LC-HRMS suspect screening. Semi-quantification of HBM metabolites, including phospholipids, bile acids, flavonoids, carotenoids, and dihydroxylutein, ranged from 15.8 to 2,049.7 ng/mL. These metabolites were predominant in colostrum, transitional milk, and mature milk, reflecting metabolic changes across lactation stages. After adjusting for potential confounders, three specific metabolites and four chemical compounds showed significant associations with infant body weight, body length, and head circumference.

Conclusion: A non-targeted metabolomics and exposomics platform (MEPH) has been successfully established using LC-QTOF-MS coupled with in-house libraries to characterize HBM metabolomic and exposomic profiles. Maternal nutrition and chemical exposures play a crucial role in lactogenesis, influencing infant growth throughout breastfeeding.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1100 A — Simultaneous Quantitation and Discovery (SQUAD) workflow using multiple fragmentation techniques for quantitation and characterization of trace level nitrosamines in biological matrices.

PRESENTING AUTHOR: ***Sunandini Yedla, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Brandon Bills, Rahul Deshpande, Bashar Amer, Susan Bird, Min Du, Vlad Zabrouskov***

SUB THEME: ***Exposome research***

Nitrosamines are carcinogenic compounds that cause harm through water, food, cosmetics, and by nitrosation in gastrointestinal tract etc. An accurate, reliable analytical method is critical for the detection and quantification of these trace-level, highly polar compounds for their exposure, and related disease studies.

An optimized SQUAD workflow is presented that discovers unknown compounds in the matrix by using high resolution orbitrap detector, and a sensitive linear ion trap detector that quantifies the low intensity nitrosamines in a single run. The workflow uses multiple fragmentation types – HCD, CID and UVPD help characterize isomeric structures.

SQUAD experiments were performed on dilution series (1.6fg to 25ng) of EPA 521 Nitrosamine Mix (Sigma-Aldrich), aromatic nitrosamines, NIST1950 and Urine diluent matrices spiked with calibration standards, through analytical and nano liquid chromatography using Orbitrap IQ-X Tribrid mass spectrometer connected to Vanquish Horizon and Neo UHPLC with C18 analytical and nano columns.

Spectra were evaluated for nitrosamines and quantified using FreeStyle and TraceFinder and annotated using Mass Frontier software.

Optimized fragmentation was achieved by adjusting precursor specific collision energy dynamically. Peaks with six or more data points that have analyte of interest across all the data points were identified for detection and quantification. The limit of detection range was 1ppb to 2.5ppb (analytical) and 25ppt to 0.5ppb (nano). Spiked matrices data identified nitrosamines through discovery analysis in the Orbitrap MS and quantified in the ion trap simultaneously. Targeted analysis of 5-Nitrosalicylic acid yielded unique UVPD fragments relative to HCD and CID spectra.

Future work includes further optimization for gentle ionization, sensitivity and orthogonal fragmentation techniques for characterizing isomers and unknowns.

Novel Aspect

Parallel ion trap analysis for quantifying trace level compounds and untargeted analysis method in a single run.

1101 A — An untargeted GCxGC-MS approach to investigate exposome in healthy aging

PRESENTING AUTHOR: ***Ameya Mahesh Barde, University Of Eastern Piedmont (upo), Italy***

SUB THEME: ***Exposome research***

Diseases that have significant effects on public health are closely and intricately connected to both genetic and environmental variables, such as stress, diet, lifestyle, and chemical contaminants. Individual reactions to these exposures are still not well known, though. This study introduces an innovative untargeted two-dimensional gas chromatography mass spectrometry method for investigating the molecules of exposomes in healthy people. Exposome molecules were extracted from 500 µL of serum using solid phase extraction performed on C18 column. Blanks were also evaluated using vacutainer, tubes and syringe. A LECO Pegasus BT 4D GC×GC-TOFMS instrument (Leco Corp., St. Josef, MI, USA) was used. One hundred serum samples from healthy volunteers exposed to the same working environment (hospital) were prepared and analyzed.

A comprehensive untargeted analysis identified a total of 98 exposome molecules based on mass-to-charge ratio (m/z), fragmentation, retention indices and standards. In addition, twelve known exposomes (Benzophenone, Ph.3,1-methylethyl, Methylenebis, Benzyl alcohol, Biphenyl, Decanedioic acid, 2-ethylhexyl salicylate, 6-tert-butyl, p,p-DDE, Fluoranthene, Anthracene and Pyrene) were also quantified with calibration curves. Serum samples were stratified based on age (young, middle, and old) and sex. The molecules were categorized into eight functional groups, including household chemicals, personal care products, pesticides (e.g., p.p.-DDE, an organochlorine pesticide), industrial chemicals, phthalates, and food additives, with further subclassifications (e.g., volatile organic compounds (VOCs), poly-fluoroalkyl compounds, organophosphorus compounds). Stratification by sex and age revealed that household chemicals (22%), personal care products (21%), pesticides (18%), and industrial chemicals (17%) were the most significant contributors. This study highlighted the diverse nature of exposomes and their differential distribution across population groups. Moreover, this preliminary investigation demonstrates that untargeted metabolomics is an effective approach for exposomic studies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1102 A — Multiomic profiling of patients with inflammatory bowel disease and other autoimmune diseases

PRESENTING AUTHOR: ***Jakub Rozhon, Palacký University Olomouc, Czech Republic, Czechia***
CO-AUTHORS: ***Aleš Kvasnička, Eliška Ivanovová, Radana Brumarová, Jaroslava Friedecká, René Lenobel, Eva Karásková, David Friedecký***
SUB THEME: ***Gastrointestinal diseases***

Introduction:

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic autoimmune disorders affecting the gastrointestinal tract. Despite their increasing incidence and clinical complexity, diagnostic and prognostic tools remain limited. Systems-level approaches such as lipidomics, metabolomics, and proteomics offer new opportunities to identify molecular signatures associated with disease progression and immune-mediated pathology.

Main aim:

To characterise the molecular landscape of IBD and related autoimmune diseases by integrating lipidomic, metabolomic, and proteomic analyses of pediatric and adolescent patient serum samples.

Methods:

A comprehensive multiomic study of serum from patients with UC and CD, alongside healthy controls, was conducted. Semi-targeted lipidomic and targeted metabolomic profiling were performed using RP LC-MS/MS and HILIC LC-MS/MS, respectively (Sciex QTrap 6500+), covering > 700 lipid species and > 110 metabolites. Untargeted proteomics carried out via high-resolution nanoRS LC-MS (Bruker TimsTOF Pro 2) employing the PASEF DDA with ion mobility separation, identifying > 150 proteins. All data were processed using multivariate statistical modelling and pathway enrichment analyses.

Results:

Integrated analysis revealed distinct multiomic signatures for each autoimmune condition. In IBD, a downregulation of plasmalogens and sphingomyelins was observed, accompanied by elevated levels of FA 22:6. Metabolomics indicated perturbations in compounds associated with the citrate cycle, arginine metabolism, vitamin pathways, and microbial-associated metabolites. Proteomics uncovered differential abundance of inflammation-related proteins, including acute-phase reactants and components of the complement cascade. Multiomic correlation analysis revealed coordinated inflammatory responses linking lipid peroxidation, immune activation, and metabolic stress.

Conclusions:

This integrative study highlights disease-specific molecular alterations in pediatric autoimmune diseases. The multiomic strategy offers a comprehensive view of IBD pathophysiology and supports the identification of improved biomarkers and therapeutic targets for immune-mediated inflammatory diseases.

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Practice - New Omic Technologies (CZ.02.01.01/00/23_021/0009224).

1103 A — Metabolomic Shifting across Disease Stages: Unveiling Pathogenesis of Hepatic Encephalopathy in Acute-on-Chronic Liver Disease

PRESENTING AUTHOR: ***Surender Surender, PGIMER, India***
CO-AUTHORS: ***Madhumita Premkumar, Savita Attri, Jasvinder Nain, Rohit Mehtani, Arnab Pal***
SUB THEME: ***Gastrointestinal diseases***

Introduction:Hepatic Encephalopathy(HE) is a neuropsychiatric condition emerges as a complication of cirrhosis which hampers health-related quality of life and decreases survival of patients. Pattern of metabolome seems to vary with the complication of cirrhosis, from stable decompensated cirrhosis(DC) to Acute-on-Chronic Liver disease(ACLF) and HE development. The study was hypothesized to show a change in neuro-metabolome in HE at any stage of cirrhosis complication, indicating pathogenesis and progression of the disease.

Methods: We prospectively enrolled alcohol etiology liver disease patients from hepatology departments(OPD and IPD). A total of 149 patients were included of which 21 were DC, 40 were ACLF without-HE and 75 with ACLF with-HE & 13 healthy control(HC) were also enrolled. Methanolic extraction of serum samples were derivatized with BSTFA. Random samples were analyzed with Agilent(GC-(SQ)-MSD) with HP-5MS column in full scan mode. MZmine ver-4.5 was utilized for data preprocessing and metabolite annotation, while MetaboAnalyst 6.0 was employed for statistical analysis and enrichment.

Results:Unsupervised and supervised multivariate analysis showed intersecting clusters of disease conditions. ACLF with HE is the advancement of ACLF, so it showed the least differentiation on OPLSDA (R2Y= 0.76, Q2=0.53, p<5e-04), compared to DC (R2Y= 0.896, Q2=0.814, p<5e-04) and HC(R2Y= 0.92, Q2=0.91, p<5e-04) showed maximum discriminant ability, after 2000 permutations. Amino acids, carbohydrates, ureas, fatty acids, and alcohol were common metabolites upregulated metabolites. VIP features>1.5 supports the classical mechanism of HE pathogenesis as involves urea & ammonia recycle and BCAA dysregulation but also unveiled new pathways of energy failure, amino-acid metabolism, and transporters-defects of bile & amines.

Conclusion:Our data reveal metabolome changes corresponding to increasing liver disease severity compared to healthy controls. Beyond the classical ammonia and urea cycle mechanisms, new pathways involved in disease pathogenesis and progression were identified. However, since the study was conducted using low-resolution MSD, further validation with high-throughput instruments is required.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1104 A — Profiling Gut Microbiome-derived Metabolites to Identify Biomarkers for Non-alcoholic fatty liver disease Diagnosis

PRESENTING AUTHOR: ***Jieun Choi, Seoul National University, South Korea***
CO-AUTHORS: ***Daehwan Kim, Hyun Ho Song, Ki-Tae Suk, Do Yup Lee***
SUB THEME: ***Gastrointestinal diseases***

Non-alcoholic fatty liver disease (NAFLD) is a metabolic disorder characterized by excessive hepatic fat accumulation in the absence of significant alcohol consumption. This disorder arises from genetic predisposition and various external factors such as microbial imbalances and unidentified dietary components. Most NAFLD patients exhibit no distinct clinical symptoms, except for variations in specific clinical parameters. Additionally, the lack of definitive treatments for NAFLD has led to an increasing demand for early diagnosis and the development of biomarkers. Recent research has highlighted gut dysbiosis as a key contributor to NAFLD, which can lead to intestinal barrier dysfunction and increased gut permeability. The communication between the gut microbiome and the liver through the gut-liver axis has been discovered, and the evaluation of the intestinal microenvironment has been suggested to identify precise biomarkers for NAFLD. To investigate gut environment alterations associated with NAFLD, we performed untargeted metabolomic profiling and targeted analysis of short-chain fatty acids (SCFAs) in fecal samples from both healthy individuals and NAFLD patients using LC-Orbitrap mass spectrometry.

To characterize gut microbiome-driven metabolic changes in NAFLD patients compared to healthy controls, we conducted both univariate and multivariate statistical analyses. Furthermore, a machine learning–based predictive model was developed using prioritized metabolites, and its diagnostic performance was evaluated through receiver operating characteristic (ROC) curve analysis. These results may contribute to the development of non-invasive biomarkers for NAFLD diagnosis and prognosis through gut metabolic signatures.

1105 A — Expanding the MR1 ligandome by multi-platform chromatography-mass spectrometry

PRESENTING AUTHOR: ***John Sidda, University of Oxford, United Kingdom***
CO-AUTHORS: ***Rita Szoke-Kovacs, Rich Suckling, Mariolina Salio, James McCullagh***
SUB THEME: ***Immunometabolism***

The major histocompatibility complex class 1-related protein-1 (MR1) has a key role in presenting self- and non-self antigens to T cells and was first shown to present a range of microbial-derived metabolites and intermediates from the riboflavin biosynthetic pathway as a proxy to detect infection. More recently, a subset of T cells (MR1T cells) have been shown to respond to a variety of different cancers cells in an MR1-dependent manner, targeting these cells for destruction. MR1 exhibits very little polymorphism, it is therefore an attractive target for pan-population T-cell receptor-based anticancer immunotherapies. However, a major current bottleneck to exploiting MR1 in cancer immunotherapy is the unknown nature of the small molecule MR1 ligands that mediate MR1:T-cell receptor interactions.

By spiking in known concentrations of MR1:ligand mixtures to cell lysates, capturing the MR1:ligand complexes using an anti-MR1 antibody and submitting the eluates to multi-platform analysis (reverse phase liquid chromatography mass spectrometry, RPLC-MS and anion-exchange chromatography-mass spectrometry, AEC-MS) we have demonstrated we can capture 10 previously reported MR1 ligands and 6 novel MR1 ligands. We demonstrate this novel workflow using multi-platform discovery metabolomics can sensitively and selectively recover MR1:ligand complexes. Crucially, we detect ~50% at lower limits of detection by AEC-MS compared to RPLC-MS, highlighting the advantage of a multi-chromatographic platform to expand the chemical detection space, and AEC-MS in particular for high-polarity MR1 ligands.

We have applied the workflow to cancer cells, capturing MR1 ligands from acute monocytic leukaemia (THP1) cells and confirming previous reports that pyridoxal and pyridoxal phosphate can be pulled down and identified. We have also identified eight novel MR1 ligands from these cells using the same methodology, with structural elucidation and in vitro validation of these putative new ligands ongoing.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1106 A — AI-based non-targeted metabolomics analysis reveals novel immunomodulatory checkpoints of cancer regulation and mTOR signalling

PRESENTING AUTHOR: ***Wolfram Weckwerth, University Of Vienna, Vienna Metabolomics Center, Austria***

SUB THEME: ***Immunometabolism***

The fusion of untargeted metabolomics and AI-powered metabolic modeling provides a robust method for unraveling the complexity of biological systems. This research expands on integrating metabolomics, proteomics, and RNA sequencing with AI-based approaches to investigate macrophage metabolism, emphasizing the significance of mTOR signaling in diverse biological scenarios. Through a mathematical-statistical analysis rooted in dynamical systems theory, the Weckwerth lab pinpointed PHGDH as a pivotal metabolic checkpoint for macrophage polarization [1]. Subsequent research confirmed this role by demonstrating that PHGDH deletion in a mouse model reversed the immunosuppressive properties of tumor-associated macrophages (TAMs), triggered α-ketoglutarate and mTORC1 signaling, and suppressed solid tumor growth [2]. Moreover, investigations into natural compounds like hesperetin and norbergenin highlight their potential to influence mTORC1 signaling, mitophagy, and apoptosis, thereby mitigating inflammatory responses and lipotoxicity in NAFLD [3,4]. By harnessing high-throughput metabolic and proteomic data alongside sophisticated AI methodologies, this study aims to decode the metabolic pathways involved in immune modulation, cancer dynamics, and the therapeutic impact of natural substances. The findings underscore the immense potential of combining metabolomics with AI to reveal critical metabolic disruptions and identify valuable biomarkers for disease diagnosis and therapeutic evaluation. This multidisciplinary strategy offers a promising future for advancing both precision medicine and natural therapeutic applications.

[1] Wilson et al. Cell reports 30, 1542-1552. e1547 (2020).

[2] Cai et al. Cell Mol Immunol doi:10.1038/s41423-024-01134-0 (2024).

[3] Li et al. Front Immunol 14, 1117638, doi:10.3389/fimmu.2023.1117638 (2023).

[4] Li et al. J Agric Food Chem 72, 13039-13053, doi:10.1021/acs.jafc.3c09132 (2024).

1107 A — THE ROLE OF DETOXIFICATION IN ORGANIC ACIDEMIAS

PRESENTING AUTHOR: ***Gabriel Sanjo Aruwajoye, North-West University, South Africa***

CO-AUTHORS: ***Rencia van Der Sluis***

SUB THEME: ***Inborn errors of metabolism and neonatal screening***

Organic acidemias (OAs) are a group of rare inherited metabolic disorders characterized by enzyme deficiencies leading to toxic accumulation of organic acids. Isovaleric Acidemia (IVA), Propionic Acidemia (PA), and Methylmalonic Acidemia (MMA) are examples of OAs. Symptoms caused by the accumulation of toxic metabolites in these disorders are alleviated through the manipulation of detoxification pathways.

Very little is currently known regarding the detoxification enzymes responsible for the detoxification of the toxic metabolites as well as to what extent interindividual variation influences detoxification efficiency. This suggests that there is a need for more personalized treatment strategies.

This study investigated the different detoxification enzymes that are involved in detoxifying toxic metabolites produced in IVA, PA, and MMA. The genetic variants that can influence the enzyme activity of these enzymes were also summarised. Understanding the contribution of individual genetic variants on the variation in detoxification ability may lead to significant advances in the clinical management of OAs.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1108 A — Red blood cell lipidomics highlights metabolic plasticity in mitochondrial fatty acid β-oxidation disorder MCADD

PRESENTING AUTHOR: *Inês Guerra, University of Aveiro, Portugal*

CO-AUTHORS: *Pedro Domingues, Helena B. Ferreira, Sónia Moreira, Sara Ferreira, Luísa Diogo, Stefano Bonciarelli, Laura Goracci, Tânia Melo, M. Rosário Domingues, Ana S. P. Moreira*

SUB THEME: *Inborn errors of metabolism and neonatal screening*

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most prevalent mitochondrial fatty acid β-oxidation disorder, characterized by the accumulation of medium-chain acylcarnitines. Despite the therapeutic approach based on frequent feeding, changes in the lipid homeostasis have been reported in plasma of MCADD patients. While plasma lipidome is susceptible to short-term fluctuations due to dietary intake, the lipid composition of red blood cells (RBC) is less influenced by dietary changes. Thus, RBC may be an alternative to monitor lipid metabolic plasticity in MCADD, offering a more stable prognosis biomarker for MCADD. This study aimed to assess the RBC lipidome plasticity of MCADD and control individuals and identify potential prognostic biomarkers.

The study analyzed RBC samples from 73 participants (35 MCADD patients and 38 controls). Lipid extraction followed the Bligh and Dyer method, with lipid species characterized by untargeted C18-RP-LC-MS/MS lipidomic approach. Data analysis utilized Lipostar software, and statistical interpretation was conducted using R Statistical Software.

A total of 240 lipid species were significant altered in MCADD. Notably, an upregulation of sphingolipids – including ceramides and sphingomyelins (SM) – as well as lysophosphatidylcholines (LPC) and lysophosphatidylethanolamines was observed, together with increased LPC/PC ratio. These changes suggest a possible association with oxidative stress, inflammation and neurological complications. Simultaneously, the depletion of PUFA-containing phospholipids - phosphatidylcholines (PC) and phosphatidylethanolamines (PE) - and plasmalogens may compromise the antioxidant defences and potentially exacerbate oxidative stress. Furthermore, the altered PC/PE and PC+SM/PE+PS ratios observed in MCADD patients could be associated with changes in RBC membranes properties, such as fluidity and asymmetry.

All these changes may have systemic implications for lipid metabolism in MCADD patients and cause long-term complications in older age. This study highlights the utility of RBC lipidomics as a robust tool for understanding the pathophysiology of MCADD and suggest its potential for monitoring disease progression.

1109 A — Neonatal Dried Blood Spot Metabolomics Reveals Signatures of Early-Onset Myeloproliferative Neoplasms

PRESENTING AUTHOR: *Domenica Berardi, Yale University, United States*

CO-AUTHORS: *Caroline Johnson, Nikolai Podoltsev, Xiaomei Ma*

SUB THEME: *Inborn errors of metabolism and neonatal screening*

Myeloproliferative Neoplasms (MPN) are a group of hematopoietic malignancies generally seen in older adults, but more cases are being diagnosed in younger patients (ranging from 0 to ~30 years) for whom the aetiology is poorly understood. Assessing early-life exposures and their potential effects on the biology of the individual is complex. However, dried blood spots (DBS) which are collected at birth, years preceding the development of cancer, provide a resource to retrospectively analyze this critical window of exposure. Metabolomics analysis of these DBS offers a unique opportunity to measure both exposures and biological effects, through the examination of metabolites, including those derived from environmental sources, the microbiome, and endogenous processes.

This study investigates the role of early-life exposures in the pathogenesis of early-onset MPN (EO-MPN) through untargeted metabolomics-based mass spectrometry on neonatal DBS samples. We combine a broad analysis of cases versus matched controls with a focused investigation of cohort-specific factors within the cases. This multi-step approach allows for the identification of potential confounding factors related to the general population and isolates those that are likely to contribute to the disease.

Multivariate statistical analyses such as PCA, PLS-DA and Uniform Manifold Approximation and Projection (UMAP), revealed subtle metabolic differences between cases and control particularly when stratifying by age of diagnosis and birth cohort. Despite the modest fold changes resulting from the comparison, key metabolic alterations in the cases were measured for fatty acids, amino acids and metabolites involved in redox pathways.

This study represents the first application of a comprehensive metabolomics analysis in the context of early-onset MPN. The findings from this preliminary analysis suggest that early-life metabolic shifts may play a role in MPN development, and lay the groundwork for future research to explore these metabolites as potential biomarkers and therapeutic targets for EO-MPN.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1110 A — Endo-metabolomic profiling of Rotavirus infected intestinal cells reveal potential host metabolic agonists utilized by the virus for successful replication

PRESENTING AUTHOR: ***Suvrotoa Mitra, ICMR - NIRBI (formerly ICMR-NICED), India***

CO-AUTHORS: ***Shreya Banerjee, Rakesh Sarkar, Mamta Chawla-Sarkar***

SUB THEME: ***Infectious diseases***

Background

Omics-based technologies, including 'metabolomics', is being increasingly explored nowadays for better understanding of viral pathogenesis. No prior metabolomics-based study has been conducted on host cells infected with the diarrheagenic ds-RNA virus, Rotavirus(RV). Our present study is a first of its kind involving profiling of the host cellular endo-metabolic landscape on RV infection.

Methodology

UHPLS-based mass spectrometry for endo-metabolomics of mock and RV-infected HT-29 cells at 6,12 hr post infection (h.p.i.). Data analysis using Metaboanalyst. Mechanistic studies done using cell-based biochemical assays, qRT-PCR, immuno-blotting & immuno-fluorescence; targeted upregulation or inhibition of metabolites using synthetic molecules.

Results

>100 metabolites were altered in RV infected HT-29 cells at both timepoints. The citric-acid cycle, alanine-aspartate-glutamate metabolism and the nucleotide metabolism were commonly altered pathways in RV-infected cells at both 6 and 12 h.p.i.. At 6 h.p.i., the steroid-hormone biosynthesis, glycolysis/gluconeogenesis pathways were also found significantly altered. The beta-alanine metabolism, glutathione metabolism, and butanoate, sphingolipid, glycerophospholipid metabolism pathways were found exclusively altered at 12 h.p.i($p<0.05$). Based on metabolomics results, the glutathione metabolism and the alanine-aspartate-glutamate metabolism pathways were chosen for further validation. RV-infected HT-29 cells caused progressive decrease in glutathione(GSG) levels at 6 and 12h.p.i.. Estimation of cellular glutamine levels governing both nucleotide and aspartate-glutamate metabolism revealed reduced glutamine at both 6 and 12h.p.i. in RV-infection. Intracellular glutamate and aspartate levels were higher in RV-infected cells at 6h.p.i. and decreased at later timepoint. Liproxstatin-1 treatment, restoring GSH levels in RV-infected cells reduced RV infection. L-DON (glutamine-antagonist), CB-839 and AOAA (inhibitors to enzymes glutaminase and glutamate oxaloacetate transaminase respectively) also demonstrated potent anti-RV effects in-vitro.

Conclusion

Endo-metabolic profiling of RV-infected human intestinal cells revealed significant alteration in glutathione metabolism pathway and the glutamate-aspartate metabolism pathway. Agonists and antagonist molecules to these pathways significantly abrogated RV-infection and might find application as potent broad-spectrum, host-directed, antiviral therapeutic.

1111 A — Global LC-MS Metabolomics and LASSO-regression Identify Affected Pathways in Sepsis Patients in the Emergency Department

PRESENTING AUTHOR: ***Sander Guttorm, Oslo Metropolitan University, Norway***

CO-AUTHORS: ***Per Flemmen, Christian Prebensen, Niclas Karlsson, Helge Rootwelt, Erik Amundsen, Katja Elgstøen***

SUB THEME: ***Infectious diseases***

Sepsis is one of the leading causes of death worldwide, with a mortality rate upwards of 20%. Rapid and accurate diagnosis of sepsis is crucial for effective sepsis management, yet the current diagnostic tools lack sufficient sensitivity and specificity. Understanding metabolic dysregulation in sepsis could provide insights into disease mechanisms and reveal novel diagnostic and therapeutic targets. This study aimed to identify and characterize metabolic pathways affected in patients with sepsis at time of presentation in the emergency department (ED) using global LC-MS metabolomics.

The study cohort consisted of 70 patients presenting to the ED with suspected sepsis: According to post hoc adjudication by an infectious disease clinician, 35 had confirmed sepsis (Sepsis-3 criteria) and 35 non-sepsis patients (11 with infection without new-onset organ failure, and 24 with organ failure but no infection). EDTA-plasma was extracted using methanol and analyzed by global LC-MS metabolomics in positive and negative ionization mode. Pathways specifically affected in sepsis patients were identified based on differentially abundant metabolites and features (DAMs) selected using differential analysis and least absolute shrinkage and selection operator (LASSO) regression, comparing (A) sepsis against infection-only patients, (B) sepsis against organ failure-only patients and (C) sepsis against non-sepsis patients.

Differential analysis identified 183 DAMs (63 from comparison (A), 145 from comparison (B)) (adjusted p-value < 0.1, foldchange >1.5), and the LASSO-regression selected features of interest based on comparison (C). N-acetylputrescine (level 1 of identification) was found to be elevated in sepsis patients compared to non-sepsis patients in both DA and LASSO (bootstrapped LASSO-repeatability of 0.829). GABA biosynthesis (eukaryote specific) was one of the pathways significantly affected in patients with sepsis. Ongoing studies are assessing pre-analytical stability and biological changes of these markers over time in sepsis patients. In conclusion, these findings indicate that metabolic dysregulation in sepsis may involve GABA biosynthesis.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1112 A — The mode of actions of T. gondii tissue cyst inhibitors

PRESENTING AUTHOR: *Deborah Maus, Robert Koch-institute, Germany*

CO-AUTHORS: *Martin Blume*

SUB THEME: *Infectious diseases*

Toxoplasma gondii, a widespread parasite infecting 25-30% of humans, persists in a chronic bradyzoite form within tissue cysts, making it difficult to eradicate. While current treatments target the rapidly replicating tachyzoite form, they fail to eliminate the dormant bradyzoite stage. The metabolic processes of T. gondii, especially in bradyzoites, are poorly understood. In this study, we employed a human myotube-based culture system to generate drug-tolerant bradyzoites and screened the MMV Pathogen Box for compounds with dual activity against both tachyzoites and bradyzoites. Metabolic profiling, including stable isotope-resolved analysis, identified several compounds that target the mitochondrial bc1-complex, such as MMV1028806 and buparvaquone, causing a depletion of ATP levels in bradyzoites. These findings highlight the crucial role of mitochondrial metabolism in bradyzoite survival. Additionally, our data revealed distinct metabolic signatures linked to specific drug mechanisms, expanding our understanding of parasite metabolism and drug action. This study demonstrates that bc1-complex inhibitors could be promising candidates for developing therapies to target chronic T. gondii infections and contribute to filling the gaps in the metabolic understanding of this under-researched parasite.

1113 A — Spatially-divergent metabolic impact of experimental toxoplasmosis: immunological and microbial correlates

PRESENTING AUTHOR: *Caitlyn E. Middleton, San Diego State University, United States*

CO-AUTHORS: *Mahbobeh Lesani, Tzu-Yu Feng, Jan Carlos Urban Arroyo, Eli Casarez, Sarah E. Ewald, Laura-Isobel McCall*

SUB THEME: *Infectious diseases*

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite Toxoplasma gondii. The parasite initially infects the small intestine and disseminates to nearly every organ of the body during acute infection, before establishing chronic infection in skeletal muscle, cardiac muscle and brain. In this study, we used toxoplasmosis, as a prototypical example of a disease regulated by strong type I immune responses, to assess the relative roles of local parasite burden, local tissue inflammation and the microbiome in shaping local tissue metabolism during acute and chronic infection. We compared metabolism in eleven sampling sites in C57BL/6 mice during the acute and chronic stage of T. gondii infection. In the acute stage of infection, the location of the highest metabolic perturbation was the liver, followed by skeletal muscle, and duodenum. In the chronic stage, the highest metabolic perturbation was in the large intestine, cecum, and cecum contents. This pattern was unexpected, since these organs are not associated with T. gondii persistence. Overall spatial mismatches were observed between parasite location and metabolic perturbation, indicating a low correlation between parasite burden and metabolism in both acute and chronic timepoints. In contrast, a stronger association with indicators of type I immune responses was observed, indicating a tighter relationship between metabolic perturbation and local immunity, than with local parasite burden. In addition, we observed significant changes in microbiota composition with infection, and candidate microbial origins for multiple metabolites impacted by infection. This study sheds light on metabolic perturbation as a result of T. gondii infection across organs, and its determinants.

1114 A — Multi-omics analysis of a prebiotic intervention with pectin in lipid transfer proteins (LTPs) allergic patients

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CO-AUTHORS: *Francisca Gómez, Natalia Pérez-Sánchez, Rafael Núñez, Domingo Barber, Stefan Vieths, Stephan Scheurer, Alba Rodríguez-Nogales, Cristobalina Mayorga, María José Torres, Alma Villaseñor, Coral Barbas*

SUB THEME: *Microbiome research*

Imbalances in the composition of the gut microbiota are implicated in the development of inflammatory diseases, including food allergy (FA). In the Mediterranean region, the most prevalent form of FA in adults is allergic reactions to plant foods caused by sensitization to lipid transfer proteins (LTPs). Currently, there is great interest in using dietary fibers like pectins for prebiotic treatment of FA. Therefore, the potential of pectin to prevent LTP allergy was evaluated in this interventional study using a multi-omics approach.

34 LTP-allergic patients received placebo or one of two different esterified pectins for two months: Placebo (n=9), Active-1 (n=13) and Active-2 (n=12). Serum and stool samples were collected before and after the intervention to assess the effects of pectin on the systemic immune response and gut microbiota. Serum targeted proteomics was used to analyze immune response changes, while gut microbiota composition was examined using 16S rRNA gene sequencing. Additionally, short-chain fatty acids (SCFAs) and bile acids (BAs) were quantified using liquid chromatography coupled to triple quadrupole mass spectrometry (LC-QqQ-MS) with Agilent 6470 and 6460 QqQ-MS instruments, respectively. Lastly, integrated models using mixOmics were built to correlate the different platforms.

Prebiotic treatment with pectin induced a significant clinical increase in allergen tolerance in over 50% of pectin-treated patients. Furthermore, patients treated with both pectins showed a systemic decrease in Th2-type (IL4, IL13) and Th17-type (IL17C) cytokines compared to placebo. Pectin treatment also increased the abundance of anti-inflammatory (i.e. Bifidobacterium, Faecalibacterium prausnitzii) and pectin-fermenting bacterial populations (i.e. Bacteroides, Roseburia intestinalis). Additionally, fecal levels of microbial-derived branched SCFAs (i.e. isobutyric, isovaleric, 2-methylbutyric acids) and secondary BAs (i.e. GCDCA, GDCA) were reduced after pectin administration compared to placebo. Finally, mixOmics integration revealed the unique signature of pectin-treated patients.

Overall, this study suggests that pectin may be a promising treatment for FA to LTPs.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1115 A — Gut Microbiome-Related Metabolites Mediate Kidney Function in Obesity

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CO-AUTHORS: *Manyi Jia, Kanta Chechi, Fiona Newberry, Marina Cardellini, Rossella Menghini, José-Maria Moreno-Navarrete, Jordi Mayneris-Perxachs, Zhaojie Wang, Ulrike Löber, Sofia Forslund, Julian Marchesi, Miriam Moffatt, Massimo Federici, José Manuel Fernández-Real, Lesley Hoyles, Marc-Emmanuel Dumas*

SUB THEME: *Microbiome research*

Obesity is a complex and increasingly prevalent condition associated with comorbidities such as metabolic dysfunction-associated steatotic liver disease (MASLD), posing major public health challenges. Alterations in the gut microbiome and its metabolites have been shown to contribute to the development of these diseases and may occur long before clinical onset. However, the mechanisms underlying host-microbiome interactions in obesity remain underexplored.

To address this gap, we utilised data from the FLORINASH project, which aims to elucidate the biological processes mediating host-microbiome crosstalk in MASLD. Participants were recruited from Italy and Spain with varying degrees of obesity and MASLD. Expanding on our previous work, we conducted an integrative metagenomic and metabolomic analysis to investigate a potential causal role of microbial aromatic amino acid metabolism in modulating kidney function in obesity. We analysed data from 662 non-diabetic obese individuals (Spain: n = 416; Italy: n = 246; male: 31%) with matched metagenomic, metabolomic, and clinical features.

Untargeted metabolomic profiling was performed using UHPLC-MS, identifying 1,271 metabolites. Metagenomic data were assembled and annotated to generate individual-specific taxonomic and functional profiles. Using gutSMASH, we predicted microbial metabolic gene clusters and identified six bacterial genera with gene clusters potentially involved in the pathway of hydroxybenzoate to phenol – a step in aromatic amino acid metabolism.

The abundance of Phascolarctobacterium showed a significant positive correlation with concentrations of phenol sulfate and phenol glucuronide. Furthermore, mediation analysis demonstrated that both Phascolarctobacterium abundance and associated KEGG orthology (KO) gene counts showed mediation effects on kidney function-related clinical features - including uric acid, urea, creatinine, and glomerular filtration rate – via phenol-associated metabolites.

These findings suggest host-microbiome interactions mediated by metabolites in obesity, with potential implications for biomarker discovery and the development of microbiome-targeted therapies.

1116 A — Metabolite richness indicates improved glycaemic homeostasis in an obese non-diabetic population

PRESENTING AUTHOR: *Manyi Jia, Imperial College London, United Kingdom*

CO-AUTHORS: *Francesc Puig-Castellvi, Kanta Chechi, Petros Andrikopoulos, Romina Pacheco-Tapia, Inés Castro Dionicio, Mickaël Chevalier, Ema Mocsonokyova, Amelia Yuan Wang, Zhaojie Wang, Amélie Bonnefond, Philippe Froguel, Abbas Dehghan, Lesley Hoyles, José-Manuel Fernández-Real, Massimo Federici, Remy Burcelin, Sofia Forslund-Startceva, Ulrike Loeber, Sara Vieria-Silva, Gwen Falony, Marc-Emmanuel Dumas*

SUB THEME: *Microbiome research*

Gut microbiome species and gene richness has emerged as a marker of metabolic health which can be modulated by dietary and lifestyle interventions. Likewise, we have introduced the notion of metabolite richness which is decreased in severe obesity. Metabolite richness is defined as the number of metabolites detected in the human circulating metabolome, which captures both endogenous and exogenous (i.e., microbiome, environment, food and medication) metabolome. Here, we evaluated whether metabolome richness reports on host glycaemic homeostasis utilising the FLORINASH (N=925) population, whilst adjusting for country, sex and age.

Our analyses suggest that metabolite richness associates with improved oral glucose tolerance. Given the intricate chemical composition of the human metabolome, we partitioned metabolite richness by metabolite clusters identified using Tanimoto coefficient-based chemical similarity and characterised cluster-specific metabolomic signature for glucose tolerance. Metabolite clusters correlated with impaired glucose tolerance were also linked to the Bact2 enterotype previously associated with dysregulated metabolic health. Moreover, we demonstrated the population variance in metabolite richness explained by the host genetics and the gut microbiome varied across different chemical similarity clusters. Our findings revealed that metabolite richness associates with microbiome and host genetics. Metabolite richness is also a clinically modifiable trait that can track host glycaemic control. This insight would enhance the utility of the human metabolome in elucidating the molecular intricacies of insulin resistance driven by the host-microbiome-environment interactions and pave the way for therapeutic targets.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1117 A — LC-MS/MS Metabolomics Reveals Altered Gut Microbial Metabolites in Thai Peritoneal Dialysis

PRESENTING AUTHOR: ***Weerawan Manokasemsan, SiMPC, Thailand***

CO-AUTHORS: ***Narumol Jariyasopit, Kwanjeera Wanichthanarak, Patcha Poungsombat, Alongkorn Kurilung, Suphitcha Limjiasahapong, Kajol Thapa, Yongyut Sirivatanauksorn, Sukit Ruksasuk, Thatsaphan Srithongkul, Chagriya Kitiyakara, Sakda Khoomrung***

SUB THEME: ***Microbiome research***

End-stage kidney disease (ESKD) patients experience the accumulation of gut microbiota-derived uremic toxins (UTs), which contribute to disease progression. This study developed and validated an LC-MS/MS metabolomics approach to identify and quantify gut microbial metabolites (GMMs) in both fecal and plasma samples from ESKD patients undergoing peritoneal dialysis. We quantified 16 uremic toxins and 17 secondary bile acids in plasma and fecal samples from 31 peritoneal dialysis patients and 60 healthy controls. Our findings revealed significant differences in plasma GMM profiles between PD patients and HC, whereas fecal metabolite profiles remained largely similar.

Notably, we identified alterations in both known and novel GMMs, including indoxyl sulfate, phenyl sulfate, hippuric acid, and imidazole propionate (ImP), which were the most significantly altered plasma-specific metabolites. Interestingly, plasma ImP concentrations in ESKD patients were three times higher than in healthy controls and correlated with five kidney-related markers, a finding reported for the first time in this study.

These findings highlight the role of gut-derived metabolites in systemic metabolic dysfunction and their potential as biomarkers or therapeutic targets for ESKD management.

1118 A — Simultaneous Quantitation and Discovery (SQUAD) of fecal bile acids and their conjugates in adults with autism spectrum disorder (ASD)

PRESENTING AUTHOR: ***Bashar Amer, Thermo Fisher Scientific, United States***

CO-AUTHORS: ***Alexey Melnik, James B. Adams, Rosa Krajmalnik-Brown, Khemlal Nirmalkar, Brandon Bills, Rafael Melani, Susan S. Bird***

SUB THEME: ***Microbiome research***

Bile acids (BAs) are synthesized from cholesterol and play a key role in lipid metabolism and gut microbiota interactions. Altered microbiomes can modify bile acid composition, leading to the production of conjugated bile acids and structurally related metabolites, potentially influencing disease states. Here, we developed a metabolomics workflow for the simultaneous quantitation and discovery (SQUAD) of fecal BAs and their conjugates using a Tribrid mass spectrometer. This workflow employs Real-Time Library Search (RTLS) to enhance spectral similarity measures and confidence in molecular identification during method execution.

Fecal samples from adults with and without ASD were analyzed. BA and microbially conjugated bile acid (MCBA) standards from Bileomix Inc. were separated on a reversed phase column within an LC system. Data were acquired using PRM quantitation in the linear ion trap and high-resolution Orbitrap MS1 scanning for enhanced annotation.

SQUAD metabolomics enabled absolute quantitation of BA targets over a dynamic range of 5–6 orders of magnitude, with a lower limit of quantification (LLOQ) of 10 femtomoles and detection (LLOD) at 0.25 femtomoles. The Orbitrap MS3 approach improved isomer discrimination, particularly for leucine/isoleucine-conjugated BAs.

We identified several microbially conjugated bile acids previously determined to be associated with gut conditions, including IBD. In particular, we found high amounts of leucine/isoleucine, tyrosine, and phenylalanine-conjugated bile acids. It's important to note that neither chromatography nor MS2-level fragments were able to distinguish Isoleucine from leucine-conjugated bile acids. The MS3 level on the Ascend system was required to detect specific fragments and quantify levels of Leu/leu conjugates.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1119 A — Unveiling the Interplay Between Gut Microbiome and Metabolic Phenotype in Anorexia Nervosa Through Multi-Omics Analysis

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CO-AUTHORS: *Laura Mayo-Martínez, M. Paz Lorenzo, Gabriel Á Martos-Moreno, Montserrat Graell, Coral Barbas, Jesús Argente, Antonia García, Francisco Javier Rupérez*

SUB THEME: *Microbiome research*

Anorexia nervosa (AN) is a complex mental disorder characterized by severe metabolic and psychological consequences, with a poorly understood etiology. The gut-brain axis plays a crucial role in AN, as alterations in gut microbiota may influence metabolic and neurological functions. Despite its multifactorial nature, the interplay between the gut microbiome and metabolic phenotypes in AN remains underexplored, necessitating further research to elucidate disease mechanisms and identify potential therapeutic targets.

This study employs a multi-omics approach to investigate the intricate relationships between the gut microbiome and metabolic phenotypes in AN patients. Our cohort consisted of AN patients and healthy controls, from whom plasma and fecal samples were collected for comprehensive analysis. We applied a combination of untargeted metabolomics techniques, including liquid chromatography-mass spectrometry (LC-QTOF-MS), gas chromatography-mass spectrometry (GC-Q-MS), and capillary electrophoresis-mass spectrometry (CE-TOF-MS), to uncover distinct metabolic phenotypes associated with AN. Additionally, targeted methodologies were developed and validated for the quantification of specific microbial metabolites, such as short-chain fatty acids (SCFAs) and D- and L-amino acids (D/L-AAs), using liquid chromatography-tandem mass spectrometry (LC-QqQ-MS).

Shotgun sequencing of fecal samples revealed significant differences in microbial composition between AN patients and controls, with distinct microbial profiles within the AN cohort. Notably, one subgroup (M3) showed a bacterial composition and metabolomic profile similar to that of healthy controls. Integrative analysis combining metabolomics and metagenomics data highlighted key metabolites and bacterial taxa associated with appetite regulation, energy metabolism, and satiety signaling. Our findings underscore the importance of the gut microbiome in shaping metabolic phenotypes in AN, offering new insights into disease pathophysiology and potential therapeutic targets. This research underscores the value of a holistic, multi-omics approach in understanding complex disorders like AN.

1120 A — Lipidomics in mild traumatic brain injury as potential prognostic biomarkers

PRESENTING AUTHOR: *Henrique Ribeiro, Örebro University, Sweden*

CO-AUTHORS: *Ilias Thomas, Alex Dickens, Jussi Posti, Endre Czeiter, Daniel Duberg, Tim Sinioja, Matilda Kråkström, Isabel Helmrich, Kevin Wang, Andrew Maas, Ewout Steyerberg, David Menon, Olli Tenovuo, Tuulia Hyötyläinen, András Büki, Matej Orešič, CENTER-TBI Participants and Investigators*

SUB THEME: *Neurological disorders*

Traumatic brain injury (TBI) has the highest incidence among all common neurological disorders, resulting in long-term disability and consequent societal and public health burden. TBI patients are classified as mild, moderate, or severe TBI, based on the Glasgow Coma Scale (GCS). However, a more detailed characterization of the disease phenotype is crucial for TBI management and for predicting disease outcomes in individual patients. We have previously shown that lipids alterations have potential as prognostic markers and might also reflect different pathophysiological mechanisms in the brain.

Here we measured the circulating lipids in a selection of serum samples from CENTER-TBI cohort, a large multi-center European study. In total there were 905 mild TBI cases with different degrees of recovery after 6 months of injury. We also evaluated the patients based on findings in computed tomography (CT) scans by using the Marshall CT classification. Blood samples were taken within 24 h of admission to the clinic, and the serum molecular lipids were measured comprehensively by using UHPLC coupled to Agilent 6545 QTOF mass spectrometer, using authentic lipid class-based reference and internal standards for quantification.

For the prognostic markers' evaluation, we compared different types of variable selection methods based on machine learning, such as penalized logistic regression (LR), XGBoost and random forest-based methods. After variable selection, an iterative LR model was built to determine an optimal lipid panel with the best performance, which was validated in an independent test set. Prediction of patient outcomes, classifying favorable (Glasgow Outcome Scale - extended [GOSe] = 5-8) vs. poor (GOSe = 1-4) recovery outcomes, presented AUC values between 0.711 and 0.737 in the validation series, with noteworthy presence of LPC(20:5) and PC(O-40:5) in all tested methods.

In conclusion, lipidomics holds promise in predicting outcomes in patients with mild TBI, an area of unmet medical need.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1121 A — A Multi-Layer Network-SEM (MLN-SEM) approach to elucidate the metabolic reprogramming of Extracellular Vesicles (EVs) in primary microglia for uncovering the etiology of Alzheimer's disease.

PRESENTING AUTHOR: *Hyun Ho Song, Seoul National University, South Korea*

CO-AUTHORS: *Do Yup Lee*

SUB THEME: *Neurological disorders*

Neuroinflammation and metabolic dysregulation in microglia triggered by amyloid beta (Aβ) are pivotal in the progression of Alzheimer's disease (AD). The propagation of AD pathology is associated with the extracellular matrix of microglia, which includes extracellular vesicles (EVs). Microglia-derived EVs have been recognized as crucial players in AD progression, yet their metabolic mechanisms remain elusive.

A previous study revealed significant changes in 39, 18, and 28 metabolites in microglial cells, culture media, and EVs, respectively. Notably, EVs exhibited shifts in purine metabolism suggestive of oxidative stress. Among these, N-acetyl aspartic acid decreased by 2.3-fold and 2.0-fold in microglial cells and media, respectively, but increased by 3.5-fold in EVs under Aβ exposure. Furthermore, triangular structural equation model-based mediation analysis linked cysteic acid upregulation in EVs to elevated IMP in microglia, and the downregulation of EV 1–16:0-lysoPE to changes in stearoyl-L-carnitine in microglial cells.

In the current study, I advance this previous analysis by applying Multi-Layer Network-SEM (MLN-SEM), an enhanced framework designed to model complex metabolic cascades in the form of Aβ → Microglia (met1 → met2 → met3, ...) → EV (met1). Building upon our prior mediation analysis that identified key metabolic pathways involved in AD progression, MLN-SEM integrates network-based principles to map multi-step intracellular metabolite interactions within microglia that mediate Aβ-induced metabolic reprogramming in EVs.

This approach enables a higher-resolution view of dynamic metabolic relationships, capturing sequential alterations that define the immune-metabolic axis of AD. These findings highlight the power of MLN-SEM to uncover latent mechanistic pathways and propose novel biomarkers and therapeutic targets in the context of AD.

1123 A — Metabolomics and Lipoproteomics in age-related diseases: studying Alzheimer's Disease via a serum omic NMR-based approach

PRESENTING AUTHOR: *Alessia Vignoli, University Of Florence, Italy*

CO-AUTHORS: *Giovanni Bellomo, Federico Paolini Paoletti, Claudio Luchinat, Leonardo Tenori*

SUB THEME: *Neurological disorders*

Alzheimer's disease (AD) is the most common irreversible neurodegenerative disorder in the elderly population. The high variability in AD progression makes it difficult to predict when a patient will convert to dementia. We hypothesize that metabolic alterations in the brain and cerebrospinal fluid [1] may also be reflected at a systemic level in blood serum and that these alterations could serve as prognostic biomarkers.

This study investigates serum biomarkers using ^{1}H-NMR spectroscopy in a population that covers the entire spectrum of cognitive impairment [2]: 57 patients with Alzheimer's disease at the dementia stage (AD-dem), 45 patients with AD at the mild cognitive impairment stage (MCI-AD), and 31 patients with MCI not associated with AD (MCI).

A ^{1}H-NOESY 1D-presat pulse sequence was applied to detect signals from both metabolites and lipoproteins. A panel of 26 metabolites and 112 lipoprotein-related parameters was quantified. The logistic LASSO regression algorithm was employed to identify the optimal combination of metabolite-lipoprotein features and their ratios for group discrimination.

In the training set, our model distinguished AD-dem from MCI with 81.7% accuracy, which was reproduced in the validation set (75.0% accuracy). The progression of MCI-AD patients was evaluated over time, with those showing a ≥1.5-point MMSE (Mini–Mental State Examination test) decline per year classified as fast progressors. Our model, built on four metabolic features, differentiated fast- and slow-progressing MCI-AD patients with 73.3% accuracy.

The identification of potential novel peripheral biomarkers of AD paves the way for an innovative and minimally invasive method to identify AD in its early stages. Moreover, our model appears to be able to sub-stratify MCI-AD patients identifying those associated with a faster rate of clinical progression.

1. Vignoli et al., J. Proteome Res. 19, 1696–1705 (2020).

2. Vignoli et al., J. Transl Med. 23, 119 (2025).

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1124 A — Lipidomics and small molecules mass spectrometry imaging in investigation of lipopolysaccharide-induced inflammation in rat brain

PRESENTING AUTHOR: *Ilia Evstafev, University Of Turku, Finland*
CO-AUTHORS: *Matilda Kråkström, Yvonne Couch, Alex Dickens, Matej Orešič*
SUB THEME: *Neurological disorders*

Neuroinflammation is a key component to a wide range of brain injuries and as such the fundamental metabolic changes following an acute inflammatory challenge are poorly understood. There have been reports of both central and peripheral changes to lipid and other metabolism during the acute phase response after a brain injury. The aim of this study was to characterise the changes in lipid composition and small molecules in the plasma and brain of rats injected intracerebrally with lipopolysaccharide (LPS). This LPS injection mimics an acute inflammatory event in the CNS.

Samples were analysed using high-performance liquid chromatography-high resolution mass spectrometry (HPLC-TOFMS) and MALDI-TOF imaging (MSI). Plasma and brain samples were subjected to liquid-liquid extraction, and extracts were analysed using untargeted HPLC-TOFMS. MALDI imaging was performed using sDHB matrix sublimation and analysis on a Bruker timsTOF fleX MALDI2. The resulting images were analysed in Bruker Scils Lab Pro to assess differences in the distribution of the detected compounds between the hemispheres of the test brains.

Based on the results, a list of compounds of interest was obtained, among which several candidates for further study were identified. MS/MS spectra were recorded to confirm the structure and identify these compounds. candidates that have been found to be discriminated by the LPS injections are: 344.019, 346.001, 404.359, 465.238. In the blood and brain sections the PCA showed a clear separation between the brains injected with LPS and saline. Interestingly, the injected hemispheres of the brain also showed clear differences to the contralateral side showing that the surgery induces a metabolic disturbance which is then exacerbated with the LPS.

In conclusion there is clear metabolite and lipid changes following an LPS injection into the striatum of a rat. This study will provide the mechanistic insights into how these metabolite changes are communicated to the periphery.

1125 A — Impact of the Ketogenic Diet on Volatilomics Prrofile in Exhaled Breath Condensate of Women with Overweight and Obesity – KETO-MINOX study

PRESENTING AUTHOR: *Natalia Drabińska, Institute Of Animal Reproduction And Food Research, Poland*
CO-AUTHORS: *Jerzy Romaszko*
SUB THEME: *Nutrimetabolomics & dietary biomarkers*

Ketogenic diet (KD), known for its high fat and low carbohydrate content, has gained interest for obesity management. KD induces ketosis, where the body burns fat for energy, potentially leading to weight loss and improved metabolic health. Recent research shows that KD can reduce body weight, enhance insulin sensitivity, and decrease inflammation. However, the impact of KD on metabolism is still being explored. This study examined how KD affected the profile of volatile organic compounds (VOCs) in exhaled breath condensate (EBC) in women with overweight and obesity, which may reflect changes in metabolism.

A randomized, single-center, controlled trial involved 80 adult, non-menopausal women with a BMI of 25.5-35, without chronic diseases. Participants received food catering with approximately 1750 kcal daily, containing either KD or a standard diet (SD). Body composition was measured, and EBC samples were collected at baseline (T0), after 4 weeks (T1), and at the end of the study (T2). VOCs were analyzed using GC×GC-ToFMS.

The study was completed by 71 women, with data from 66 participants analyzed (SD = 34, KD = 32). Both groups experienced similar weight loss (KD avg. = 6.42 kg vs. SD avg. = 4.85 kg). In the women following KD, a higher abundance of acetone was observed in EBC, confirming the ketosis state. Otherwise, there were no significant differences between the study groups.

In conclusion, KD is as effective in weight loss as the standard diet. The profile of EBC varied between individuals; however, it was noted that women following KD had a higher abundance of acetone in their breath, confirming ketosis. Based on our study, KD did not lead to any harmful effects on metabolism.

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1126 A — Longitudinal associations of diet quality with serum biomarkers of lipid and amino acid metabolism from childhood to adolescence: the PANIC study

PRESENTING AUTHOR: *Suvi Laamanen, University Of Eastern Finland, Finland*
CO-AUTHORS: *Saara Heinonen, Eero Haapala, Ursula Schwab, Sonja Soininen, Taisa Sallinen, Aino-Maija Eloranta, Timo Lakka*
SUB THEME: *Nutrimetabolomics & dietary biomarkers*

Studies on longitudinal associations between diet quality and lipid and amino acid metabolism in children and adolescents are limited. We studied associations between diet quality and serum markers of lipid and amino acid metabolism in the Physical Activity and Nutrition in Children (PANIC) study. These analyses included 403 children aged 6–9 years at baseline, 360 re-examined two years later at age 9–11 years, and 219 eight years later at age 15–17 years. Food intake was recorded over four days, and diet quality assessed using the Finnish Children Healthy Eating Index (FCHEI). Fasting serum fatty acids, amino acids, apolipoproteins, and lipoprotein particle sizes were analyzed via nuclear magnetic resonance spectroscopy. Linear mixed-effects models, adjusted for sex, age, body fat percentage, pubertal stage, and physical activity, were used to analyze the associations. Better diet quality was linked to increased serum polyunsaturated fatty acids and reduced saturated and monounsaturated fatty acids, alanine and very low-density lipoprotein (VLDL) particle size. Consuming more vegetables, fruits, berries, vegetable oils and margarine with at least 60% fat, fish, and whole grains associated with higher serum polyunsaturated fatty acids and lower saturated fatty acids and smaller VLDL particles. Conversely, consuming higher-fat dairy products and sugary products associated with higher saturated and monounsaturated fatty acids, branched-chain and aromatic amino acids, and larger VLDL particles. A diet rich in fruits, vegetables, unsaturated fats, and fiber, with reduced processed meat and sugar consumption, promotes favorable metabolic changes relevant to cardiometabolic health.

1127 A — Use predictive models built upon annotated and validated intake biomarkers in urine and plasma to accurately classify cranberry juice consumers in a randomized, double-blinded, placebo-controlled trial

PRESENTING AUTHOR: *Liwei Gu, University Of Florida, United States*
CO-AUTHORS: *Timothy Garrett*
SUB THEME: *Nutrimetabolomics & dietary biomarkers*

Introduction and Aim: Clinical trials assessing the effectiveness of cranberries in preventing urinary tract infections have yielded inconsistent results, partly due to unknown participant compliances. The astringency of cranberry products often reduces compliance. This research aimed to develop predictive models using validated intake biomarkers to objectively classify cranberry juice consumers through an untargeted metabolomics approach.

Methodology: Plasma and urine samples were collected from 18 healthy women before and after 3-day consumption of cranberry or apple juice using a randomized, open-labeled, controlled crossover design. A second cohort of 16 women followed a similar protocol in a randomized, double-blinded, placebo-controlled crossover trial, with additional sampling after 21 days. Metabolic profiling was performed on UHPLC-Q-Orbitrap-HRMS.

Findings and Significance: Thirty-six plasma and 27 urinary discriminant metabolic features identified from the open-label study were re-discovered in the double-blinded trial. These metabolites included exogenous (quinic acid) and endogenous (hippuric acid) compounds. Orthogonal partial least-square discriminant analysis (OPLS-DA) models built upon shared plasma or urinary metabolites accurately classified cranberry juice consumers with 84.4% to 100% correction rates. These predictive models provided an objective approach to assess participant compliance in future cranberry consumption trials.

Contribution to Discipline: The research advanced the metabolomics applications in nutrimetabolomics and dietary biomarker discovery.

1128 A — Chemical biology tools for dietary biomarker discovery, including microbiota-derived metabolites

PRESENTING AUTHOR: ★ *Ioanna Tsiara, Uppsala University, Sweden*
CO-AUTHORS: *Daniel Globisch*
SUB THEME: *Nutrimetabolomics & dietary biomarkers*

The human body clears xenobiotics through processes like phase I and phase II biotransformations. These modifications are important across diverse research fields such as toxicology, nutrition, and microbiome metabolism. The two major phase II modifications, sulfation and glucuronidation have been associated with microbiota-host co-metabolism. Furthermore, the importance of the microbiota on the conversion of dietary components has been revealed to impact human physiology. We have previously developed a selective enzymatic treatment of metabolites in human samples for the simplified analysis of sulfated and glucuronidated metabolites. This approach led to the detection of 206 sulfates and 191 glucuronides, including more than 70 microbiota-derived compounds.

Next, we introduced a new chemical biology tool to overcome the challenge of parallel investigation of these phase II modifications and their corresponding unconjugated aglycons in a single sample. For this new methodology, we utilized immobilized recombinant enzymes for the treatment of human urine samples obtained from a dietary intervention. After the selective enzymatic conversion of sulfated and glucuronidated metabolites, the samples were subjected to UHPLC-MS/MS analysis. The obtained raw data were processed using XCMS in R with an optimized script to selectively identify phase II metabolites. The metabolite structure was validated via authentic standards or MS/MS fragmentation. This separate investigation of each metabolite class in a single sample was successfully applied to obtain the dietary glucuronidation and sulfation profile of about 100 compounds. We have now upscaled our investigation of the sulfatome and glucuronide metabolome to analyze 200 samples, revealing significant changes post-intervention in 91 sulfates and 94 glucuronides. Six key metabolites were identified with a high predictive accuracy (AUC = 0.97) for the dietary intake. Our analysis also underscores the individual variability in dietary response and highlights how our advanced metabolomics strategies provide valuable insights into the metabolic biomarkers linked to gut microbiota- host interaction.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1129 A — Establishing The Taiwan Biobank Metabolomics Resource – Planning, Execution and Future Perspectives

PRESENTING AUTHOR: *Chung-ke Chang, Academia Sinica, Taiwan*
CO-AUTHORS: *Ming-Wei Su, Hou-Wei Chu, Yi-An Hsueh, Chien-Wei Lin, Yun-Lun Wang, Sing-Huei Wu, Ching-Yu Kuo, Hwai-I Yang*
SUB THEME: *Other*

Large-scale collection of human metabolomic data is required for establishing the baseline metabolome of a population, which serves as the basis for the study of complex diseases. Population biobanks have become an increasingly important source of high-quality large-scale metabolomic data. The Taiwan Biobank started planning for the collection of metabolomics data in 2015 and has since established a growing resource for population metabolomics. The resource currently consists of nuclear magnetic resonance-based metabolite measurements from 2,469 data subjects, among which 1,624 have follow-up measurements available, and is growing at ca. 1,000 data subjects per year. Relationships between the metabolomic data and genomic data or other phenotypic data can be quickly visualized through a web interface. However, establishing and maintaining a large-scale metabolomic data collection is resource-intensive, time-consuming, and demands thorough planning and precise execution. Here we review the technical, scientific and resource considerations during the planning and execution stages of our program in the past 10 years. We shall describe our pilot experiments, our attempts at automation and our final data collection procedure. We hope that this disclosure will provide the community with helpful suggestions on how to plan and run similar large-scale metabolomics projects.

1130 A — Untargeted exploration of the metabolomic coverage of blood microsampling: A comparison between Mitra®, Capitainer®, and Whatman™ 903 cards

PRESENTING AUTHOR: *Dennisse Avella, Afekta Technologies Oy - University Of Eastern Fin, Finland*
CO-AUTHORS: *Soile Turunen, Glykeria Avgerinou, Anatoli Petridou, Iman Zarei, Vassilis Mougios, Kati Hanhineva, Olli Kärkkäinen*
SUB THEME: *Other*

Blood microsampling (BμS), which collects less than 100 μL of blood, offers a less invasive and cost-effective alternative to venipuncture. However, its metabolomic comparability to conventional samples remains unclear, and standardized BμS metabolomic workflows are lacking. This study applied untargeted metabolomics to compare BμS extraction methods and assess the metabolic coverage of three BμS devices-Mitra®, Capitainer®, and Whatman™ 903 cards against whole blood and plasma to optimize BμS-based human biomonitoring.

Venous blood from 10 adults (5 males, 5 females) was sampled onto the three devices. Using Whatman samples, we first evaluated three extraction techniques (ultrasound, shaker, and homogenizer) at three concentrations (1.5%, 5.5%, and 11.0%). The optimized method was then used to compare the metabolomic profiles across BμS devices, whole blood, and plasma. LC-MS analysis employed both reverse-phase and hydrophilic interaction chromatography, coupled with a Q-TOF mass analyzer in positive and negative ionization modes.

All extraction methods and concentrations proved suitable for BμS untargeted metabolomic analysis. BμS-derived metabolite profiles aligned more closely with whole blood than plasma, with Capitainer showing the highest similarity to whole blood. Metabolomic signatures varied among devices, with Whatman and Capitainer profiles being more like each other than Mitra. Some metabolites were more characteristic of a sample type, whereas others were common across sample types. Additionally, to test suitability for biomonitoring, we investigated how well the devices separated the participants by sex. All five sample types enabled sex-based differentiation, with metabolites such as testosterone sulfate and leucine driving the separation.

These findings enhance our understanding of BμS metabolomics and highlight its potential in human biomonitoring. The choice of the optimal device depends on the metabolites of interest, offering flexibility for clinical and research applications.

This work has been funded by the European Union under the HORIZON-MSCA-2021 project 101073062: HUMAN Harmonising and unifying blood metabolic analysis networks.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1131 A — Deciphering Lipid Alterations in Alport Syndrome Using an Optimized Untargeted Urine Lipidomics Approach

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SUB THEME: ***Other***

Alport syndrome (AS), a hereditary nephropathy caused by collagen IV mutations (COL4A3–5), is characterized by progressive podocyte injury. Emerging evidence suggests a link between lipotoxicity and podocyte dysfunction. However, the lipid metabolism contribution to AS pathogenesis remains insufficiently understood, necessitating comprehensive lipidomic analyses to elucidate these metabolic alterations[1].

In this study, we integrated analytical advancements with translational lipidomics to investigate AS. We developed a streamlined monophasic lipid extraction method (MeOH:EtOH, 1:1,v/v) for untargeted lipidomics, systematically compared with established protocols (Folch; MeOH:MTBE; EtOAc:EtOH) using pooled healthy urine. Coupled with UHPLC (Agilent 1290 Infinity II) and high-resolution QTOF-MS (Agilent 6545), this optimized workflow demonstrated broad lipid coverage, encompassing 315 lipid species across sphingolipids, phospholipids, glycerolipids, and fatty acyls, with enhanced sensitivity and reproducibility[2].

Applying this method, we performed urinary lipidomic profiling from autosomal dominant (ADAS, n=51) and X-linked (XLAS, n=12) AS patients, compared to healthy controls (n=20), alongside paired plasma samples to assess systemic lipid perturbations. Advanced statistical analyses (univariate and multivariate methods, volcano plots, and ROC-curve assessments), identified significant alterations in 76 urinary lipid species in ADAS and 57 in XLAS, with corresponding changes in 181 and 91 plasma lipids. Notably, HexCer(18:0(3O)/24:0(2OH)) and CAR(12:0) emerged as key AS biomarkers.

Integrative lipidomic analysis revealed hallmark metabolic disturbances in AS, including impaired sphingolipid catabolism, FA β-oxidation, and cholesterol--phospholipid ratios. Elevated ceramide levels and dysregulated sphingomyelin metabolism emerged as central mediators of lipotoxicity, highlighting the ceramide pathway as a potential therapeutic target.

By integrating cutting-edge analytical methodology with metabolic insights, this study refines lipotoxicity understanding in AS pathogenesis and establishes a foundation for lipidomics-driven precision medicine. These findings offer potential avenues for targeted therapeutic interventions and underscore the value of tailored analytical strategies in elucidating complex metabolic disorders.

[1]C.E. Kashtan., Am J Kidney Dis (2021)77(2):272-279

[2]B. Fernandez Requena et al., Anal. Chim. Acta (2024)1299:342433

1132 A — Tissue-Nonspecific Alkaline Phosphatase as a Key Regulator of Choline Metabolism: Insights from NMR-Based Metabolomics

PRESENTING AUTHOR: ***Eva Drevet Mulard, Claude Bernard University Lyon 1, France***

CO-AUTHORS: ***Laurence Bessueille, Gilles Rautureau, David Magne***

SUB THEME: ***Other***

Tissue-nonspecific alkaline phosphatase (TNAP), an enzyme attached to cell membranes and released into the bloodstream, is primarily known for its crucial role in skeletal mineralization through the hydrolysis of inorganic pyrophosphate (PPi), the major mineralization inhibitor. However, TNAP is believed to dephosphorylate a broad spectrum of extracellular substrates, thereby contributing to various physiological functions. Using NMR-based metabolomics, we recently identified phosphocholine as a potential new in vivo substrate, suggesting a hypothetical link between TNAP and hepatic phosphatidylcholine production, as well as lipid metabolism.

We show that TNAP-knockout mice develop liver steatosis and reduced serum triglyceride levels, mirroring choline deficiency. In wild-type mice, acute TNAP inhibition by SBI-425 leads to a significant decrease in choline levels in both blood and liver, as revealed by untargeted NMR metabolomics. Hence, metabolomics helped us identify two new potential TNAP substrates: phosphocholine and phosphoethanolamine. Further real-time NMR experiments demonstrate that TNAP inhibition prevents phosphocholine and phosphoethanolamine hydrolysis into choline in both mouse and human serum, reinforcing its role in extracellular choline metabolism. In human hepatocytes, TNAP inhibition blocks phosphocholine hydrolysis and impairs proliferation when phosphocholine is the sole choline source, suggesting a direct impact on cellular lipid synthesis.

To further investigate TNAP's ability to process alternative substrates and characterize its enzymatic activity and structure, we determined that the recombinant enzyme hydrolyses phosphocholine and phosphoethanolamine with similar or even greater efficiency than PPi. Determination of TNAP's 3D structure by X-ray diffraction and cryo-EM experiments show how substrates and inhibitor could interact with the active site.

Finally, this study highlights the power of NMR-based metabolomics in identifying new enzymatic functions and metabolic pathways. Collectively, these findings indicate that TNAP is the extracellular phosphatase enabling cellular choline production and uptake during fasting, and that its absence or inhibition disrupts lipid metabolism and could contribute to metabolic disorders.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1133 A — Metabolome Richness, its Determinants and its Relevance to Obesity

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SUB THEME: *Other*

Obesity is largely influenced by lifestyle, dietary patterns, physical activity and gut microbiome alterations. In particular, the Bact2 enterotype, a distinct microbiome ecology that has been linked to obesity, is characterized by a proinflammatory functional profile and poor gene richness.

Here, we introduce the concept of metabolome richness and show that the loss in microbiome richness in obese individuals mirrors a reduced number of detected metabolites in blood plasma in a METACARDIS study subset (N=859), which we replicated in FLORINASH (N=920). To uncover the determinants of metabolome richness, we applied gradient-boosting decision trees models to predict metabolome richness using demographic, clinical, nutritional, and metagenomics data. Feature attribution analysis using SHAP suggested that the inter-individual variability of metabolite richness was mostly influenced by age, diet, kidney function, and the gut microbiome. Correlation analyses also revealed that metabolite richness is primarily driven by host-, diet-, and microbial-metabolites.

We then stratified metabolic richness by origin (i.e., diet, drug), and tested associations with lifestyle, clinical and metagenomic profiles, identifying significant contributions from pulse intake, kidney function and KEGG pathways. We further examined the structural similarity of the 1061 annotated metabolites using clustering derived from Tanimoto coefficients calculated from chemical fingerprints, and we observed that the metabolites disappearing in obesity shared structural similarities, as most of them were found in the same cluster. The cluster most strongly associated with metabolome richness was characterised by a large number of gut microbial and dietary compounds that contain a phenol group (i.e. polyphenols and phenylpropanoids).

Altogether, our results suggest that metabolome richness depicts the loss of a constellation of dietary and microbial protective metabolites in severe obesity, mirroring the collapse in microbiome gene richness, suggesting a gradual loss in bio- and chemodiversity in metabolic diseases.

1134 A — The genetic architecture of the metabolome across several organs illuminates responses to nutritional and pharmacological challenges

PRESENTING AUTHOR: *Marc-Emmanuel Dumas, CNRS & Imperial College London, France*

SUB THEME: *Other*

Genetic variants disrupting metabolic homeostasis play an important role in the pathogenesis of complex disorders. However, how genetic variants may alter metabolic traits in a tissue-specific manner remain elusive. Here, we deploy a systems genetics strategy to untargeted metabolomics to dissect the tissue-specific genetic architecture of metabolomic traits. Using a rat recombinant inbred panel as a model of human metabolic syndrome, we profiled the metabolome in liver, peritoneal fat, heart, aorta and brain by untargeted ultra-performance liquid chromatography coupled to mass spectrometry generating >16,764 metabolomic features per tissue, among which 2,072 were replicated across all 5 tissues. Tissue metabolites are highly heritable (H2>27%), mostly polygenic. We identified 4,131 metabolomic features under genetic control (mQTLs), with >48% of these being tissue-specific and >200 mapping to the Abcb4 transporter locus acting a master regulator of liver and serum phospholipids. Metabolite-phenotype correlation networks predicted the response to high-fat diet and anti-hypertensive drugs, opening avenues in understanding the genetic basis of metabolic flexibility and their application in systems medicine.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1135 A — Metabolomic Signatures of Habitual Physical Activity in Childhood: A Longitudinal Analysis

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SUB THEME: *Other*

Physical activity (PA) supports health at all life stages, including childhood, where it contributes to improved long-term health outcomes. However, the molecular mechanisms through which PA benefits health remain poorly understood. A promising approach to explore this relationship is through metabolome-wide analysis. In this study, we aimed to investigate the association between habitual PA and metabolic profiles of 515 children from the EU Childhood Obesity Project.

The SenseWear Armband 2 was used to measure habitual PA, allowing us to capture the overall activity and time spent at different intensity levels. Plasma metabolites were measured using targeted LC–MS. Associations between PA and metabolites were assessed longitudinally at three ages (5.5, 8, and 11 years) using linear mixed models and metabolite set enrichment analyses.

Among the 272 metabolites analyzed, 25 were associated with total PA, capturing activity across all intensity levels but predominantly reflecting low-intensity movement. 19 metabolites were associated with moderate-to-vigorous PA, 10 of which overlapped with those linked to total PA. Among these shared metabolites were several docosahexaenoic acid-containing phosphatidylcholines (PC aa C38:6, PC aa C40:6, and PC aa C42:6), which were lower in more active children. Sex-stratified analysis of the PC metabolite group showed negative association with PA in girls, but positive association in boys. Furthermore, BMI interacted with several associations, particularly for free fatty acids, which were higher in more active children with higher BMI, but lower in those with high activity and lower BMI.

Our findings suggest that habitual PA influences metabolic pathways already in early life, and that these effects may vary by exercise intensity, sex, and body composition. To conclude, this study provides insights into how PA impacts the metabolome of children, which, together with other studies, could help identify PA biomarkers and improve the understanding of its role in overall health.

1136 A — METABOLOMIC PROFILE OF PATIENTS WITH BENIGN PROSTATIC HYPERPLASIA: AN INTEGRATED ANALYSIS USING LC-HRMS AND GC-MS

PRESENTING AUTHOR: *Ana Valéria Colnaghi Simionato, Institute of Chemistry - UNICAMP, Brazil*

CO-AUTHORS: *André Luiz Melo Camelo, Hans Rolando Zamora-Obando, Aline Cristina Dias, Thaís de Assis Lopes, Júlia Maria Souza Rossi, Alessandra de Sousa Mesquita, Regina Vincenzi Oliveira, Marina Franco Maggi Tavares, João Pedro Simon Farah, André Matos de Oliveira, Alberto Azoubel Antunes*

SUB THEME: *Other*

Benign Prostatic Hyperplasia (BPH) is a common condition in elderly male patients, leading to urethral compression and urinary flow obstruction. Its diagnosis relies on clinical assessments and invasive examinations.1 Metabolomics represents a promising approach for understanding biological pathways and may provide insights into an individual's phenotype. The aim of this study was to investigate the metabolic profile of plasma samples by a multiplataform analysis to identify potential diagnostic and prognostic biomarkers of BPH. Plasma samples from 30 clinically diagnosed patients with enlarged prostate and 30 individuals from the control group (normal prostate) were subjected to untargeted metabolomic analysis. Analyses were conducted using LC-HRMS and GC-MS platforms to enhance metabolite coverage. Data preprocessing was performed using MS-DIAL software. Chemometric models were obtained using MetaboAnalyst 5.0 platform. The PCA and HCA analyses revealed clustering of the quality control samples, indicating that the observed differences within groups are primarily attributed to biological variability. However, the constructed supervised models based on prostate size were not sufficient to achieve a clear distinction between both groups. This limitation highlighted the need to explore self-organizing maps (a machine learning tool)2, allowing the identification of three distinct groups within the metabolic profile of the samples. Based on the sample classification generated by neural networks, the discriminant models exhibited satisfactory statistical parameters, with fit (R^2) and discrimination (Q^2) coefficients exceeding 0.70 and 0.90, respectively. The suggested compound classes include fatty acids, amino acids, and organic acids, which have strong evidence of association with pre-existing conditions such as diabetes and obesity. The suggested compounds are also related to oxidative stress, energy metabolism, and inflammatory pathways, indicating potential impacts on BPH development. Thus, the combination of multiple analytical platforms and neural networks provides a promising approach for metabolic profile analysis, contributing to the investigation of complex conditions such as BPH.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1137 A — Longitudinal metabolome data reveals aromatic amino acids metabolism linked to differential weight loss after sleeve gastrectomy

PRESENTING AUTHOR: *Hyeon-Seong Lee, KIST, South Korea*
CO-AUTHORS: *Jaeyoung Kwon, Yeongkeun Kwon, Sungsoo Park*
SUB THEME: *Other*

Circulating aromatic amino acids (AAAs) and their derivatives have been highlighted as potential biomarkers of obesity-related medical conditions. Nevertheless, little is known about the longitudinal changes in AAA metabolite levels after bariatric surgery and their clinical implications. In this prospective single-arm longitudinal study, 44 patients with obesity underwent sleeve gastrectomy. Twelve AAAs metabolites were quantified before and 3 and 6 months after surgery. First, we observed characteristic changes in AAA metabolites after sleeve gastrectomy and examined the relationships among AAA metabolites. Second, we investigated how changes in weight and exercise after sleeve gastrectomy were associated with changes in AAA metabolites. Our results revealed disruptions in the AAA-derived metabolic pathways within 6 months after sleeve gastrectomy, with characteristic trajectories identified for different metabolic pathways (indole, serotonin, and dopamine pathways). Among the tryptophan-derived indole compounds, indole-3-propionic acid (IPA) was significantly associated with weight loss 3 and 12 months after surgery. Interaction analyses indicated that the relationship between IPA and weight loss at 3 months was more pronounced in patients who engaged in more exercise after surgery. Our findings suggested that AAA-derived metabolic pathways undergo changes over time after sleeve gastrectomy. Additionally, IPA, a tryptophan-derived microbial metabolite, could be useful as a marker for predicting postoperative weight loss.

1138 A — The Effect of Weight Loss on Depressive Symptom and Serum Dopamine after Sleeve Gastrectomy

PRESENTING AUTHOR: *Jaeyoung Kwon, Korea Institute of Science Technology, South Korea*
CO-AUTHORS: *Hyeon-Seong Lee, Yeongkeun Kwon, Sungsoo Park, Jaeyoung Kwon*
SUB THEME: *Other*

Monitoring mental health and weight loss is crucial after bariatric surgery. The relationship between weight loss and depressive symptoms remains unclear after surgery. The changes between Beck Depression Inventory (BDI) and serum dopamine and serotonin metabolism were investigated based on the degree of weight loss in severely obese patients after sleeve gastrectomy. This study was included 44 participants with severe obesity. Patients were enrolled between January 2019 and December 2020, undergoing sleeve gastrectomy (SG). Data were collected within 12 months after SG. Participants were divided into two groups according to weight loss rate, and changes between BDI and serum dopamine and serotonin metabolism were assessed. A total of 44 participants were divided into high (HWL, n=24) and low (LWL, n=20) weight loss groups based on a total body weight loss of 25%. BDI scores showed a significantly larger decrease in the HWL group after SG ($P<0.001$). At 12 months postoperatively, the HWL group demonstrated a 1.7 times greater improvement in severe depressive symptoms, with significant increases in serum dopamine metabolism at 6 months ($P=0.01$) and 44% higher dopamine levels compared to the LWL group ($P=0.02$). An increase in dopamine (OR=4.26, $P=0.025$) at 6 months and reductions in BDI (OR=0.19, $P=0.02$) at 12 months were more likely in the HWL group, with dopamine showing strong predictive power for assessing the likelihood of greater weight loss (AUC=0.736). Serum dopamine increase following bariatric surgery occurred alongside improvements in BDI severity and demonstrated its utility in distinguishing individuals who achieved greater weight loss.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1139 A — Metabolomic Profiling Reveals Subtle but Distinct Differences in Injury-Prone Youth Athletes

PRESENTING AUTHOR: ***Joanna Godzien, Medical University of Bialystok, Poland***
CO-AUTHORS: ***Katarzyna Miniewska, Julia Zelkowska, Wioleta Gosk, Adam Kretowski, Michal Ciborowski, Tomasz Guszczyn***
SUB THEME: ***Other***

Metabolomic Profiling Reveals Subtle but Distinct Differences in Injury-Prone Youth Athletes

Introduction:

Musculoskeletal injuries are a significant concern in both professional and recreational athletes. Their prevalence is influenced by biological factors such as connective tissue strength and mechanical factors like type and intensity of physical activity. While injuries are more common in contact sports, they also significantly impact the general population, with an estimated 30–75% of recreational runners experiencing injuries annually. Genetic predisposition and metabolic traits may explain why specific individuals are more injury-prone, despite similar external exposures.

Aim of the Study:

This study aimed to investigate metabolic and lipidomic profiles in youth athletes with a history of ligament injuries, compared to injury-free athletes, to identify biomarkers of injury susceptibility.

Methods:

Peripheral blood plasma was collected from 42, currently not injured athletes, with a confirmed history of ligament ruptures and 42 matched controls with no history of ligament injuries. All participants were overall healthy and actively involved in sports such as football, volleyball, gymnastics, and dance. Metabolomic analysis was performed using reversed-phase liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) in positive and negative ionization modes.

Results:

Metabolic differences between injured and non-injured athletes were subtle but detectable, particularly in positive ion mode. Alterations were distributed across various metabolite and lipid classes, including amino acids, amines, phospholipids, and N-acyl amines. Notably, there was a sex-specific pattern: the majority of statistically significant metabolites were altered in male athletes, while fewer differences were observed in females. This suggests potential sex-related metabolic predispositions to ligament injuries.

Conclusions:

The findings suggest that metabolic profiling may aid in identifying athletes at increased risk of ligament injuries. These biomarkers could support personalized prevention strategies and serve as potential therapeutic targets for injury treatment or recurrence prevention.

1140 A — Metabolomics and Genomics of Common Disorders – shared pathways or independent?

PRESENTING AUTHOR: ***Guangju Zhai, Memorial University Of Newfoundland, Canada***
CO-AUTHORS: ***Ming Liu, Jingyi Huang, Andrew Furey, Proton Rahman***
SUB THEME: ***Other***

Background: It is known that an individual could be affected by multiple common chronic disorders such as obesity, type 2 diabetes, cardiovascular disorders (CVD), and osteoarthritis (OA), but question remains to be answered whether the concurrence of multiple chronic diseases in one individual is due to shared pathological pathways or independent. We utilized a well-phenotyped cohort with both metabolomic and genomic data available to answer this important question.

Methods: The Newfoundland Osteoarthritis Study (NFOAS) was a well-phenotyped cohort with a total of 1086 study participants collected, which initially was set up for studying OA. In addition to the OA status, we also collected clinical epidemiological on a total of 88 factors. For the purpose of the current study, we used data on OA, CVD, obesity, and diabetes as they are the most common chronic diseases seen in a population. Genome-wide genotyping data with a total of 9 million variants and fasting plasma metabolomic data with a total of 630 metabolites were available for the current study.

Results: Genome-wide association (GWAS) analysis identified two independent variants associated with OA at GWAS significance ($p<10^{-8}$), but no GWAS significant findings for obesity, diabetes, and CVD. When examining the suggestive significant variants with $p<10^{-5}$, none of them were overlapped among the four common diseases, suggesting these four diseases don't share genetic pathways. However, our metabolome-wide association analyses revealed 9 phosphatidylcholines with long chain glycerophosphoric acids were associated with all four common diseases with metabolome-wide significance (all $p<0.0008$). Mendelian randomization analysis showed that these phosphatidylcholines had a causal relationship with all four diseases.

Conclusions: The concurrence of four most common chronic disorders could be due to shared phosphatidylcholine metabolic pathway alteration, which could be a potential target for developing therapeutic strategies for these common chronic diseases.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1141 A — Bridging Metabolomic Analysis in Blood Microsamples with Venous Whole Blood and Plasma: Comparison of 4D Global Metabolic Profiles of Dried Blood Specimens with Liquid Blood and Plasma

PRESENTING AUTHOR: ***Marlene Nyambura Thaitumu, Greece***

CO-AUTHORS: ***Daniel Marques de Sá e Silva, Georgios Theodoridis, Helen Gika***

SUB THEME: ***Other***

The aim of our study is to investigate whether metabolic information acquired by global profiling of blood microsamples (BμS) is equal with that obtained by conventional blood matrices. Capillary blood collected on the Mitra BμS device was compared to venous liquid blood and plasma in terms of metabolic coverage by untargeted 4D liquid chromatography-quadrupole time of flight-trapped ion mobility (LC-QTOF-TIMS).

Three matrices from six healthy volunteers were collected: finger capillary blood on a Mitra BμS device, venous liquid blood, and plasma. Samples were extracted using methanol (MeOH) followed by vortexing, centrifugation, evaporation, and reconstitution with mobile phase spiked with a 5 ppm internal standards mix (phenylalanine-c13, butyrylcarnitine-d3, and octanoylcarnitine-d3). A mix from all the samples was used for within-run quality control (QC). Samples were analyzed using an untargeted reverse phase LC-QTOF-TIMS assay in positive electrospray ionization (ESI) and data dependent acquisition (DDA)-Parallel Accumulation Serial Fragmentation (PASEF) modes. An Acquity UPLC HSS-T3 (C18), 1.8 μm particle size, 2.1 × 100 mm column was used for metabolite separation over a 17 minute gradient.

Data was analyzed using Metaboscape (version 2021) to determine the number of features and annotations based on in-house build reference standards libraries. Abundances of identified metabolites in the three matrices extracts were compared and further evaluation of the acquired metabolomes performed.

Our study using 4D LC-QTOF-TIMS analysis provides information on the ability of BμS, specifically the Mitra to provide equal if not superior information on circulating metabolites compared to venous liquid blood and plasma. Additionally, it showcases the viability of capillary blood in metabolomic analysis.

Keywords: LC-MS, 4D LC-QTOF-TIMS, BμS, Untargeted Metabolomics, Metabolic Profile, Metabolome.

The work has been Funded by the European Union under the HORIZON-MSCA-2021 project 101073062 : HUMAN – Harmonising and unifying blood metabolic analysis networks.

1142 A — A unified metabolomics and proteomics protocol applied to formalin-fixed paraffin-embedded (FFPE) human brain tissue to characterize granulomas in tuberculous meningitis

PRESENTING AUTHOR: ***Abisola Isaiah, North-West University. Potchefstroom Campus, South Africa***

CO-AUTHORS: ***Du Toit Loots, Aurelia Williams, Marceline Tutu van Furth, Martijn van der Kuip, Shayne Mason***

SUB THEME: ***Other***

Background: Tuberculous meningitis (TBM) is a severe condition caused by Mycobacterium tuberculosis; the leading infectious agent responsible for the highest number of deaths from a single pathogen. The challenges in diagnosing this disease have sparked renewed interest in research. The use of current technological advances, namely metabolomics and proteomics, has provided some new insights into the immune response (granulomas) in TBM. The co-application of the two approaches to characterize granulomas is limited.

Methods: We therefore extracted formalin-fixed paraffin-embedded (FFPE) brain tissue/granuloma from humans (n=68) and applied a new protocol that combines metabolomics (GCxGC-TOFMS) and proteomics (LC-MS/MS) in a single workflow. This eliminated the biases that arise from the use of several samples for different omics workflows.

Results: Numerous metabolites and proteins were identified in this study that characterize granulomas in tuberculous meningitis cases. Moreover, a direct correlation could be made between metabolites and proteins to confidently identify impacted metabolic pathways associated with tuberculous meningitis

Conclusion: To our knowledge, this is the only study to apply this protocol to a cohort of human tissue. We believe this novel approach will take us a step closer to understanding TBM pathogenesis (granuloma characterization) and finding biomarkers to aid in diagnosing TBM.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1143 A — Metabolomics-Inspired Biomaterials: Amorphous Calcium Phosphate with Targeted Metabolic Enhancement for Bone Repair

PRESENTING AUTHOR: *Jingzhi Fan, Riga Technical University, Latvia*
CO-AUTHORS: *Abhishek Indurkar, Annija Vaska, Sophie Verrier, Antons Sizovs, Janis Locs, Kristaps Klavins*
SUB THEME: *Other*

Bone tissue engineering aims to mimic the structural and functional properties of natural bone, promoting tissue regeneration. The comprehending the impact of implantable materials on healing processes is pivotal. Our study employed animal models to identify metabolites in bone healing and explored the development of implants infused with metabolite to facilitate bone recovery.

We used rat and sheep bone defect models to identify systemic metabolic changes during bone healing. Metabolites identified through bioinformatics analysis were used in cell experiments to evaluate mineralization capacity. Amorphous Calcium Phosphate (ACP) and ACP with glutamate (ACP-G) were synthesized from calcium glutamate and characterized. These materials, alongside hydroxyapatite and control, were assessed in vitro to assess osteogenesis. Cold sintered ACP-G were implanted into rat calvaria with critical defect, along with ACP to compare. In vivo results were evaluated through tissue section staining. Metabolomic analysis of serum from animal models with critical bone defects showed decreased glutamine and glutamate levels during bone regeneration. Glutamate and glutamine supplementation enhanced the mineralization capacity of pre-osteoblasts. ACP-Glu was synthesized from calcium glutamate and trisodium phosphate. Glutamate release in media followed a linear trend. Osteoblasts cultured on ACP-Glu exhibited higher osteopontin and osteocalcin levels, along with improved mineralization (Alizarin Red staining), compared to ACP. Metabolite analysis revealed significant changes in energy metabolism, particularly in the TCA cycle. Metabolomics profiling of rat calvaria and sheep tibia revealed systemic metabolic changes during bone regeneration, particularly the significant downregulation of glutamate and glutamine. ACP-Glu demonstrated exceptional efficacy in enhancing energy metabolism and osteogenic properties. The enhanced energy metabolism, especially the TCA cycle, could compensate for anaerobic glycolysis and provide the energy needed for tissue repair processes. The methodology of biomaterial design and evaluation, inspired by animal model data analysis, and the application of metabolomics aspects, is of great practical value in biomaterials engineering.

1144 A — Examining the additive effect of Metformin and High-Intensity Exercise on Lipid Metabolism

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CO-AUTHORS: *René Neuhaus, Thomas Meikopoulos, Ana Gradillas, Carolina González-Riaño, Georgios Theodoridis, Stefanos Nikolaidis, Vassilis Mougios, Helen Gika, Coral Barbas*
SUB THEME: *Other*

Maintaining adequate levels of physical exercise is an optimal way to keep good health. Prolonged and intense physical exercise is known to produce large changes in multiple different lipid changes. However, these changes over multiple time points are not fully understood. It has been suggested that the effects of exercise together with the consumption of the antihyperglycemic drug metformin have an additive effect on the metabolism and function of different organs.

Thus, this study aims to find differences between plasma lipidomic profiles of 9 healthy male individuals after performing high-intensity interval exercise under metformin treatment.

Individual participates in two sessions lasting 24 h where they received 1000 mg of metformin. In one of the sessions, they performed a high-intensity interval exercise test with an average intensity of 67% HRmax and a total duration of 76 min (Session A). In the other session, they rested (Session B). Plasma samples were collected before taking metformin and during each session up to 13-time points spanning 24 h. 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. In addition, 10 iterative-MS/MS runs were performed to assist in the annotation process. Paired Wilcoxon tests between each session at each time point were performed for the statistical analysis.

The observed variations in the lipid profiles -particularly in fatty acids, acylcarnitines, glycerophospholipids, sphingolipids, and triacylglycerols-, underscore the dynamic and complex physiological adaptations to high-intensity interval exercise.

Altogether, these changes provide crucial insights into understanding the complex physiological response of humans to intensive exercise under an antidiabetic drug and pinpoint the main biological processes implicated in maintaining a healthy status.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1145 A — Quantitative and qualitative analysis of bile acids by high-resolution mass spectrometry using electron-based fragmentation

PRESENTING AUTHOR: ***Paul Baker, SCIEX, United States***
CO-AUTHORS: ***Rebekah Sayers, Jason Causon, Robert Proos, Maxim Seferovic, Thomas Horvath***
SUB THEME: ***Other***

Analyzing bile acids using nominal mass instruments is challenging due to the high chemical background in several precursor ions, which can interfere with multiple reaction monitoring (MRM) transitions. High-resolution mass spectrometry (HRMS), which generates a full product ion spectrum for each targeted bile acid, can reduce background interferences by extracting fragment ions within a narrow mass-to-charge (m/z) window, improving signal-to-noise (S/N) ratios. While detecting individual bile acid isomers relies on chromatographic resolution when using collision-induced dissociation (CID), electron-activated dissociation (EAD) provides structurally diagnostic fragment ions that can differentiate bile acid isomers and reduce isomer resolution time. Here, HRMS was used to sensitively quantify bile acids in human plasma with concurrently acquired qualitative data to ensure specificity. Bile acids were detected and quantified in human plasma using an ultra-sensitive HRMS system capable of both CID- and EAD-based fragmentation. Using the longer chromatographic gradient with CID-based fragmentation resulted in a limit of quantitation (LOQ) approximately 10x lower than previously reported with HRMS analysis, which rivals, if not exceeds, the sensitivity of high-end triple quadrupole instruments. In addition, a high-quality MS/MS spectrum was acquired for each targeted bile acid during analysis, which can improve confidence in the assay's specificity. Using EAD-based fragmentation resulted in identifying unique, diagnostic fragments for some bile acid isomers, and when appropriate, this fragmentation mode was used to allow for faster gradients to improve sample throughput.

1146 A — Cellular metabolomics to unravel the molecular mechanisms of the LIRAFLAME intervention trial

PRESENTING AUTHOR: ***Simon Kienle, SDCC, Denmark***
CO-AUTHORS: ***Tommi Suvitaival Suvitaival, Martin Bæk Blond, Joachim Størling, Martin Giera, Peter Rossing, Cristina Legido-Quigley***
SUB THEME: ***Other***

The dramatic increase in diabetes over the past decades poses a major challenge to our health systems. While the exact etiology of diabetes and the underlying cellular mechanisms are not fully understood, dyslipidemia—a condition characterized by abnormal levels of lipids in the blood—has been identified as a major contributing factor. This makes lipids a promising target for intervention and a potential diagnostic tool. Leveraging this potential could significantly improve diabetes healthcare.

Our project elucidated both the clinical effect on the plasma lipidome of the randomized intervention trial LIRAFLAME as well as the cellular mechanisms underlying our clinical observations. Using a targeted lipidomics approach with over 500 transitions of lipid molecules, we found that several lipids are significantly regulated by the GLP1-RA liraglutide in this trial. To further investigate these observations at a cellular level, we conducted a discovery-based metabolomics and lipidomics studies. Therefore, we treated β-cells with the lipids identified from our LIRAFLAME analysis as well as liraglutide and subsequently analyze their metabolic profile via mass spectrometry. This allowed us to better understand the underlying biological processes and provided a holistic view of our data. Overall, the results of this project yielded important insights about how diabetes, lipids, and liraglutide are interconnected.

1147 A — Full-Spectrum Cannabis Oil or Isolated Cannabinoids? Metabolomic Clues from a Neurodegeneration Model

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SUB THEME: ***Other***

Neurodegenerative diseases are characterized by progressive neuronal dysfunction, leading to severe cognitive and motor impairments. Given the limited efficacy of current treatments, cannabis-derived compounds, including full-spectrum oil (FSO) and isolated cannabinoids such as cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC), have attracted considerable attention for their potential therapeutic effects. To elucidate the molecular mechanisms underlying these effects, this study employed untargeted lipidomics and metabolomics to investigate how FSO and isolated cannabinoids influence the cellular metabolome in an in vitro neurodegeneration model using SH-SY5Y neuroblastoma cells.

Lipidomics analysis was performed after 24 hours of treatment with either artisanal or commercial FSO (containing 3.9 and 5.5 µg/mL CBD, respectively) using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) on an Orbitrap platform. For isolated cannabinoids, metabolomics analysis was conducted following 24-hour treatments with CBD (2.5 µmol/L), THC (5.0 µmol/L), or a CBD/THC combination (2.5/5.0 µmol/L), using UHPLC-HRMS on a quadrupole time-of-flight (QTOF) instrument.

FSO treatment significantly altered lipid metabolism, with changes observed in triglycerides, fatty acids, ceramides, phospholipids, and sphingolipids, although no substantial differences were detected between artisanal and commercial formulations. Metabolomics analysis of isolated cannabinoids revealed that CBD modulated amino acid and lipid metabolism, particularly pathways related to glycine, serine, alanine, and threonine. THC influenced metabolic pathways associated with hexose phosphorylation and vitamin E metabolism, suggesting effects on energy production and antioxidant responses. Combined CBD/THC treatment triggered broader metabolic alterations, affecting fatty acid, amino acid, and carbohydrate metabolism, indicating a more integrated regulation of cellular metabolic networks.

In summary, this study highlights the power of metabolomics to uncover distinct molecular signatures associated with full-spectrum cannabis oil and isolated cannabinoid treatments. These findings provide novel insights into the metabolic mechanisms underlying cannabis-derived therapies for neurodegenerative diseases and highlight the value of pharmacometabolomics in drug evaluation and mechanistic research.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1148 A — Metabolomics profiling reveals increased gut proteolytic microbial metabolites during spaceflight

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SUB THEME: *Other*

Spaceflight has been shown to play an important role into astronauts´ health, with adverse effects such as bone loss, muscle loss, and constipation. Understanding the effect of microgravity on human physiology and metabolism is crucial for preventing or mitigating adverse effects of spaceflight. The aim of this study was to use untargeted metabolomics to analyze the metabolic profile of astronauts on International Space Station missions to obtain insights into their metabolic state.

Ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS) was used to analyze 488 plasma samples from 54 astronauts at multiple points before, during, and after flight. The samples were analyzed with a qTOF MS and pre-processed using the R package XCMS. Features that significantly changed during or after spaceflight compared to pre-flight were selected by both multi- and univariate statistical analyses. Forty-six compounds were identified by interpretation of mass spectra and match with reference standards.

The metabolites affected by spaceflight include food-, host-, and microbiota-derived compounds. We observed increased plasma levels of several microbial proteolytic metabolites, commonly associated with prolonged transit time. Constipation is common during spaceflight and may be due to changes in diet and physical activity, or simply the effect of weightlessness on gastrointestinal motility. Prolonged transit time is known to result in increased gut bacterial proteolytic fermentation. Some of the resulting proteolytic metabolites have been associated with negative health outcomes in humans, and this requires further evaluation. These findings highlight the importance of bowel function during spaceflight, and the role of metabolomics in understanding human adaptation to space travel.

1149 A — Combining clinical chemistry with metabolomics for metabolic phenotyping at population levels.

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SUB THEME: *Other*

Untargeted metabolic phenotyping, also known as metabotyping, has been shown to be able to discriminate reliably between different physiological or clinical conditions. However, we believe that standard panels of routinely collected clinical chemistry data also have the potential to provide assay panels that complement metabotyping.

To test the above hypothesis, we compared multivariate analyses (MVA) from metabolomics from 977 individuals with matching clinical chemistry data to predict sex, age and BMI. Metabotyping of serum included GC-MS and LC-MS (ESI+ and ESI- modes) as previously reported (HUSERMET study: Dunn et al. (2015) Metabolomics 11, 9-26; https://doi.org/10.1007/s11306-014-0707-1). Clinical chemistry data were obtained from the same individuals at the time of blood collection and these 19 measurements included assessing liver and kidney function, blood pressure, serum glucose, cations, as well as lipids.

MVA included support vector machines, random forest and partial least squares. To predict sex, age and BMI, these models used as inputs: (i) the clinical chemistry data alone; (ii) the three metabolomics datasets; (iii) combinations of clinical chemistry with the metabolomics data. Model predictions were rigorously validated using 1,000 bootstrapping (with re-sampling) coupled with permutation tests.

We shall report that MVA on the clinical chemistry data obtained for these healthy participants could be used to predict: their sex, based on creatinine; their age, based on systolic blood pressure, total serum protein and serum glucose; as well as BMI using alanine transaminase and diastolic blood pressure, as well as waist-to-height ratio (perhaps not surprising). These predictions were between 71-84% accurate (depending on the MVA used) and compared favourably with metabolomics (71-91 depending on analytical method). Combining clinical chemistry and metabolomics data sets enhanced the predictions of these characteristics to 77-93% accuracy, suggesting that this augmentation of methods may be a useful approach in the search for clinical stratification and biomarker discovery.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1150 A — Impact of PFAS on lipid metabolism in MASLD is mediated through steroids

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SUB THEME: ***Other***

The liver plays an essential role in the metabolism of nutrients, synthesis of glucose and lipids as well as hormone metabolism. However, the hepatic metabolic functions change especially during the metabolic dysfunction-associated steatotic liver disease (MASLD). In MASLD, the regular homeostasis of liver metabolism can be disrupted by the interactions between the environmental, genetic, and epigenetic factors. We previously found that the exposure to per- and polyfluoroalkyl substances (PFAS) is associated differentially to lipid and bile acid metabolism in females as compared to males, suggesting that steroid hormones are playing a role in mediating the impact of PFAS exposure. Here we investigated if the steroid hormones are associated with the clinical classifications of the liver disease. In addition, we investigated whether the steroid hormones were mediating the impact of PFAS exposure on lipids and bile acids, and whether this impact is different between males and females. For these purposes, we used mediation analysis and a bivariate analysis. We found that PFAS exposure had sex-specific impact on lipids and bile acids, both directly or indirectly via steroids. The PFAS had a clearly stronger impact on steroid hormones in females than in males. The association of androgens to MASLD was clearly different between the sexes. The general mediation pattern of the indirect impact of PFAS to the bile acids or lipids via steroids was largely similar, with females having higher average causal mediated effects as compared to males. This suggests that the impact of PFAS exposure on hepatic metabolome is mediated via steroids in MASLD.

1151 A — Assessing the muscle metabolome in Malignant Hyperthermia

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CO-AUTHORS: ***Dominik Kopczynski, Harald Schoeny, Andrea Michalek-Sauberer, Sergej Milovic, Lukas Weigl, Carlos Ibarra Moreno, Janusz Pawliszyn, Coral Barbas, Barbara Bojko, Gunda Koellensperger***

SUB THEME: ***Other***

Introduction

Malignant Hyperthermia (MH) is a rare muscle disorder triggered by fluorinated anesthetic gases, leading to metabolic hyperactivation and severe complications without immediate intervention. A muscle biopsy is required for reliable MH diagnosis, but it involves consequential risks.[1] Therefore, less invasive alternatives are needed, making metabolomics a promising approach. In this study, we aim to identify key molecules characteristic of MH that could serve as a basis for a novel diagnosis method. We emphasize that MH metabolic research remains limited, and this work will provide valuable insights into the underlying pathophysiology of MH.

Methods

MH muscle samples underwent a two-phase extraction protocol to assess metabolites and lipids, incorporating isotopically labeled internal standards for normalization.[2] Extracts were analyzed using a dual-HPLC setup with orthogonal chromatographic methods (HILIC, RP), coupled with a high-resolution orbitrap tribrid mass spectrometer for MS1 and MS2 spectra acquisition in both polarities.[3] This approach covers a broad physicochemical range, enabling both non-targeted and targeted analysis with mzMine, spectral database matching, and Skyline. Machine learning based on support vector machines (SVM) was applied for data filtering and elucidation of MH characteristic metabolites.

Results

Preliminary results from the SVM highlight several notable metabolites and lipid species that distinguish the MH patient cohort from the control group. The corresponding cross-validation score exceeds 0.9, supporting the concept of a metabolome-based, less invasive diagnostic approach. Further validation with certified reference materials, focusing on level 1 annotation and absolute quantification, is ongoing.[4]

Conclusion

Using LC-MS-based metabolomics and machine learning, we identified key molecules in MH muscle samples distinguishing MH patients from non-MH individuals. Ongoing analysis will validate our findings, paving the way for improved diagnostics and a deeper understanding of MH-related metabolic alterations.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1152 A — Metabolome trajectory of exercise physiology- from validated workflows to sex differences in sports

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CO-AUTHORS: *Bruno Stelzer, Theresa Hofbauer, Florian Reisenbauer, Yasin El Abiead, Juergen Scharhag, Gunda Koellensperger*

SUB THEME: *Other*

Integrating metabolomics in sports science provides valuable insights into the biochemistry during physical activity. Sex-specific metabolic responses need to be addressed to close a significant gap in exercise research, where female participation remains underrepresented. However, due to their invasiveness, traditional blood sampling methods present challenges in sports settings.

To address this, we explored volumetrically accurate microsampling (VAMS) as a dried blood spot (DBS) technique for assessing metabolomic changes in response to cardiopulmonary exercise testing in more than 130 participants. The study employed a targeted quantitative approach using isotopically-labeled internal standards to measure over 100 metabolites in DBS, providing accurate and traceable results. An accuracy assessment using standard reference material and stability testing over 90 days further evaluated the suitability of DBS for sports metabolomics. Additionally, the influence of the hormonal changes during the menstruation cycle was subsequently studied by following 15 female athletes across two cycles collecting cardiopulmonary exercise data at three different phases: menstruation, ovulation and luteal phase.

Our findings confirm that DBS offers a valid approach to capturing metabolic changes during exercise, reporting a wide panel of metabolites including key metabolites of energy pathways, which correlate well with plasma-derived data but also less studied classes such as pyrimidines. Implementing a straightforward standardization concept established metabolic perturbations upon bout exercise as differences of absolute concentrations. While metabolic regulations upon exercise are similar in both sexes, differences in the correlation with fitness-related metadata such as peak oxygen consumption (V O2peak) and performance, indicate a higher complexity in women and a limitation of previous knowledge to men only.

Underscoring the transformative potential of the minimally invasive and practical DBS in sports science aligns with the needs of athletes while the quantification approach paves the way to expand this type of research towards other fields of personalized medical services.

1153 A — An integrated approach based on mass spectrometry, nuclear magnetic resonance and Raman spectroscopy to shade light on the systemic response following new synthetic opioid intake

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CO-AUTHORS: *Ilenia Bracaglia, Adele Bosi, Simona Cesaroni, Greta Petrella, Giorgia Corli, Sabrine Bilel, Claudia Fasolato, Fabiana Di Rosa, Daniel Oscar Cicero, Matteo Marti, Manuel Sergi*

SUB THEME: *Pharmacometabolomics*

Novel psychoactive substances (NPS) represent a broad class of new drugs that often allow passing drug-screening tests. They are characterized by a variety of structures, thus creating an ever-changing scenario with evolving analytical targets.

This study aimed at developing an indirect screening strategy for NPS monitoring, and specifically for new synthetic opioids, based on assessing changes in endogenous urinary metabolite levels resulting from the systemic response following their intake.

The experimental design involved in-vivo CD-1 mice models. Twenty animals (10 male, 10 female) received different treatments. On day 0 mice were treated with a vehicle and urine was collected at 0-12h and 12-24h. On day 1 brorphine was administrated to half of the animals of both genders, while the other half received etonitazene; urine samples were collected at the same time intervals . A second drug administration and subsequent urine collection occurred on day 8. Finally, following a one-week washout, urine was collected again on day 15.

The urine samples were analyzed with an untargeted metabolomics workflow by exploiting several analytical platforms, including LC-HRMS/MS, NMR, and Surface Enhanced Raman Scattering (SERS) spectroscopy, obtaining multiple datasets.

For each dataset, by combining multivariate and univariate analysis, it was possible to identify the most altered metabolites following acute drug administration. These included metabolites involved in lipid peroxidation, inflammatory processes, oxidative stress, nucleotide metabolism and others. To assess metabolome changes based on different factors such as collection time, gender, and drug treatment, ANOVA Simultaneous Component Analysis (ASCA) was applied after data fusion.

The proposed approach offered a new perspective to address the scarcity of studies on the urinary metabolic profile of opiate-treated mice. The combination of complementary techniques enabled a more comprehensive and reliable analysis, leveraging the strengths of each methodology.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1154 A — Characterization of Gut Microbial Transformation of Sertraline and Its Implications for Multidrug Resistance and Antidepressant Tachyphylaxis

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SUB THEME: ***Pharmacometabolomics***

Selective serotonin reuptake inhibitors (SSRIs) are widely prescribed antidepressants, yet their use is frequently associated with adverse effects and rapid tolerance development (antidepressant tachyphylaxis, ADT). To explore the metabolic role of gut microbiota in this phenomenon, four sertraline (STR)-resistant strains had been isolated in this study, including a uropathogenic Escherichia coli (E. coli G5100a). Transcriptomic and efflux pump inhibition assays revealed activation of RND- and ABC-type multidrug efflux systems in E. coli G5100a, conferring cross-resistance to several antibiotics. Metabolomic profiling using LC-MS and GNPS showed that G5100a metabolized approximately 25% of STR, yielding two key metabolites: sertraline ketone and N-acetylsertraline, identified via MS/MS and NMR. These metabolic conversions correlated with elevated expression of oxidative stress enzymes (SodA, KatE, KatG) and acetyl-CoA biosynthesis genes (pflB, pta). Additionally, similar oxidative and acetylated metabolites were observed for citalopram and fluoxetine, suggesting a conserved microbial detoxification pathway. BBB permeability predictions by DeePred-BBB indicated that these metabolites exhibited limited CNS permeability due to altered polarity and charge. Collectively, our study highlight a gut microbe-mediated metabolic pathway that transforms SSRIs into non-CNS-permeable forms, potentially contributing to ADT and multidrug resistance. This study underscores the significance of host-microbiota metabolic interactions in shaping SSRI efficacy and side effects.

1155 A — NMR-based Metabolomic Profiling of Serum Samples to Unveil IL-4/IL-13/JAK Inhibitor Resistance Mechanisms in Atopic Dermatitis Patients

PRESENTING AUTHOR: ***Linda Fantato, University Of Florence, Italy***

CO-AUTHORS: ***Alessia Vignoli, Chiara Boggio, Marta Armari, Federica Veronese, Elia Esposto, Barbara Azzimonti, Leonardo Tenori, Moreno Lelli, Paola Savoia***

SUB THEME: ***Pharmacometabolomics***

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by epidermal barrier dysfunction, immune dysregulation and Staphylococcus aureus overgrowth. Despite the efficacy of new biological therapies, such as IL-4/IL-13 inhibitors(1) and Janus Kinase (JAK) inhibitors(2), a subset of patients fails to respond or experiences adverse effects, highlighting the need for predictive biomarkers to guide personalized treatment strategies.

Metabolomics is a valuable tool to discover molecular alterations underlying this disease by examining small molecules and revealing changes in metabolic pathways and biomarkers.(3)

This study aims to deepen molecular insight on AD in mediating resistance to IL-4/IL-13 and JAK inhibitors in patients, analyzing metabolic differences in serum samples.

We enrolled 15 adult patients with severe AD (EASI ≥ 24), treated with either dupilumab (anti-IL-4 receptor α antagonist) or upadacitinib (selective JAK1 inhibitor). Serum samples were collected at baseline (T0), 8 weeks (T8), and 16 weeks (T16) of treatment. Nuclear Magnetic Resonance spectroscopy coupled with statistical analysis was employed to identify metabolomic differences.

Univariate analysis identified six key metabolites (alanine, citrate, glutamine, glycine, histidine, tyrosine) and 12 lipoprotein-related parameters that significantly varied among the three groups. These metabolic changes correlated with clinical response, suggesting a potential role for metabolic markers in predicting treatment outcomes.

The identification of predictive biomarkers is crucial for optimizing therapeutic strategies in AD, improving patient outcomes, and reducing healthcare costs. Our findings provide new insights into systemic therapies, paving the way for personalized medicine approaches in AD management.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1156 A — Prediction of renal function decline using plasma and urine metabolomics

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CO-AUTHORS: ***Miho Iida, Naoko Miyagawa, Minako Matsumoto, Ryota Toki, Aya Hirata, Shun Edagawa, Akiyoshi Hirayama, Masahiro Sugimoto, Tomoyoshi Soga, Toru Takebayashi***

SUB THEME: ***Precision health***

Background: Predicting kidney function decline is a significant public health issue, and biomarkers that can detect it early are needed. However, the relationship between kidney function and blood metabolome has been poorly studied in longitudinal studies, and few studies have combined blood and urine metabolomics. Therefore, this study aimed to identify biomarkers for predicting kidney function decline using plasma and urine metabolome.

Methods: The study included 1,671 men and women aged 60 years or older at wave 1 of the Tsuruoka Metabolome Cohort Study (TMCS) in Japan who participated in both wave 1 in 2012-2014 and wave 3 in 2018-2020 and excluded participants with a history of cardiovascular disease or cancer. Plasma and urine samples from all the participants were measured using CE-MS, a method established at Keio IAB lab and validated for large-scale measurements. To assess kidney function, eGFR was calculated using the CKD-EPI equation with Japanese coefficients of 0.908, incorporating both creatinine and cystatin C. A mixed regression model was used to examine the association between the annual decline in eGFR and metabolite concentrations (plasma and urine) at wave 1. Additionally, PLS analysis was employed to predict eGFR decline by combining multiple metabolites.

Results: After adjusting for confounding factors (alcohol intake, smoking, hypertension, diabetes) and performing multiple comparison adjustments (FDR method), multiple metabolites were associated with eGFR decline. Four metabolites were significant in plasma and three in urine, but none were significant in both plasma and urine. Excluding participants with eGFR < 60 in wave 1 did not alter the results. Furthermore, PLS regression analysis using these metabolites predicted eGFR decline.

Conclusion: Using plasma and urine metabolomics may enable the prediction of future kidney function decline.

1157 A — Characterization of Seminal Plasma Lipidome: A New Approach for Identifying Biomarkers of Male Infertility

PRESENTING AUTHOR: ***Luz Alonso Dasques, Centre of Metabolomics and Bioanalysis (CEMBIO), Spain***

CO-AUTHORS: ***Ana Gradillas, Safouane Hamdi, Coral Barbas, Víctor González-Ruiz***

SUB THEME: ***Pregnancy, maternal and neonatal health***

The global decline in birth rates has persisted for many years, influenced by sociocultural factors and fertility challenges. Over half of fertility problems in couples arise from the male partner, yet traditional semen analysis fails to determine underlying causes. Omics approaches offer a new strategy to extract a broader range of information from seminal fluid, providing insights into male subfertility and infertility. Since seminal plasma is an accessible sample, it is a promising matrix for biomarker discovery.

This study aims to characterize the lipidomic profile of seminal plasma in the context of human fertility issues. To optimize lipid extraction, four sample preparation protocols –two biphasic, a monophasic, and a double strategy– were compared using an LC-QTOF-MS (Agilent 6546) untargeted lipidomics approach. Results indicated that the Matyash method achieved the highest extraction efficiency, was convenient, and exhibited optimal performance across a broad range of lipid polarities.

A comprehensive database for seminal plasma lipidome was built. Initially, an in-house lipid LC-MS database1) was used for known-lipid exploration. The library was expanded with additional lipid species identified through Lipid Annotator and MS-DIAL software. After merging and manual curation, a total of 231 lipid species were characterized across 20 subclasses. Higher relative levels of sphingomyelins and phosphatidylethanolamines were observed in seminal plasma compared to blood plasma, highlighting their relevance in male reproductive function.

Finally, the seminal plasma lipid database was used to characterize human samples with various pathological conditions and their distinct lipidomic patterns. These findings contribute to the growing understanding of the lipid composition of seminal plasma and its potential role in reproductive health assessment, paving the way for improved diagnostic tools in male fertility evaluation.

Acknowledgements

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1) https://doi.org/10.1016/j.jlr.2024.100671

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1158 A — The influence of microbiome and extrinsic factors in early-life fecal metabolite development

PRESENTING AUTHOR: ***Milla F Brandao Gois, Department of Genetics, University Medical Center, Netherlands***

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SUB THEME: ***Pregnancy, maternal and neonatal health***

The interplay between the environment, host, and gut microbiome during the first year of life plays a crucial role in early-life development. Despite growing research in the field, the metabolic interactions between the gut microbiome and host health during this period remain incompletely elucidated. This study aims to bridge this gap by characterizing environment-host-microbiome metabolic networks during infancy. We longitudinally assessed the fecal metabolome and microbial profiles of 329 infants and 147 mothers from the Lifelines-NEXT cohort. A total of 1203 fecal samples were included, infant samples were collected at 5 timepoints from 0.5 to 12 months of age, while maternal samples were obtained at birth and 3 months postpartum. Extensive questionnaire data regarding birth-, mother- and infant- related factors was collected. Untargeted metabolomics was used to analyze fecal metabolites, and deep metagenomic sequencing determined the gut microbiome composition. A total of 760 metabolites were included in the analysis. We observed that feeding mode was the primary driver of fecal metabolite variation in early infancy ($P = 1\times10^{-4}$, $R^2 = 0.09$), while later timepoints showed a stronger influence of variables related to dietary diversification, including the introduction of grains ($P = 1\times10^{-4}$, $R^2 = 0.015$) and eggs ($P = 1.4\times10^{-3}$, $R^2 = 0.012$). Interestingly, delivery mode had a weaker impact, being significant only in the first weeks of life ($P = 0.02$, $R^2 = 0.017$). This contrasts with previous studies on gut microbial colonization, where delivery mode is a key driver of microbiome composition. Other birth and maternal factors, such as parity and gestational age, influenced both overall fecal metabolite composition and individual metabolite profiles. By integrating fecal metabolomics with gut microbiome data, this study provides novel insights into the dynamic cometabolism shaping early-life development and highlights the differences between taxonomic and functional perspectives on gut microbiome-host metabolism interactions.

1159 A — Metabolomic Profiling in Early Pregnancy: A Novel Approach for Predicting Preterm Birth.

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CO-AUTHORS: ***William Fernando Hidalgo Bucheli, Monica Patricia Cala Molina, Bladimiro Rincon Orozco***

SUB THEME: ***Pregnancy, maternal and neonatal health***

Preterm birth (PB) is defined as any birth occurring before 37 weeks of gestation. It is a leading cause of morbidity and mortality in children under five years of age worldwide, responsible for approximately one million child deaths in 2020 alone. Despite its significant impact, the exact underlying causes of PB remain unclear. Consequently, no reliable tool has been developed for early predictive diagnosis. Such a tool would be crucial in mitigating and preventing the adverse effects associated with this medical condition in neonates. This research aims to investigate the metabolic profile of pregnant women during their first trimester to identify differential chemical profiles in plasma with potential predictive value for PB. To achieve this objective, a retrospective case-control study was conducted with a 1:2 ratio, including 199 pregnant women—64 who experienced PB and 135 who had full-term births (TB). A non-targeted metabolomic analysis was performed on plasma using high-performance liquid chromatography coupled with mass spectrometry (RP-LC/MS-QTOF), employing a quadrupole detector and time-of-flight technology. Prior to analysis, protein precipitation was initiated using methanol followed by vortex mixing and centrifugation. The metabolites present in the extract's supernatant phase were subsequently measured. A total of 15 significant metabolic features ($p < 0.05$) were identified when comparing the PB and TB groups. Notably, the study observed the presence of lipids, specifically phospholipids derived from phosphatidylcholines, which are known to be involved in inflammatory and stress-related pathways. Additionally, Machine Learning models were employed to assess the predictive potential of these metabolites. Some algorithms such as Random Forest and Support Vector Machines (SVM), were trained to classify PB and TB cases, improving the robustness of predictive analysis. These findings suggest that integrating metabolomics with Machine Learning could enhance early PB detection, paving the way for a reliable predictive diagnostic tool for preterm birth.

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1160 A — Reversed-phase liquid chromatography/mass spectrometry approach for analysis of polar to mid-polar metabolites in spontaneous preterm birth human placenta samples

PRESENTING AUTHOR: ***Miroslav Lísa, University of Hradec Králové, Czech Republic***

SUB THEME: ***Pregnancy, maternal and neonatal health***

The metabolome of biological samples is an extremely complex mixture of compounds with a wide range of polarities, molecular weights, and concentrations. We aim to establish a straightforward and versatile reversed-phase liquid chromatography/mass spectrometry (RP-LC/MS) methodology for analyzing a wide range of polar and mid-polar metabolites utilizing a single instrument, column, and mobile phase. The RP-LC/MS platform utilizes a C18 column compatible with 100 % water mobile phase and a mobile phase consisting of 0.2 % formic acid, acetonitrile, and propan-2-ol, effectively separating polar and mid-polar metabolites through various mobile phase gradients. Our developed method outperforms the conventional hydrophilic interaction liquid chromatography metabolomic method, yielding a higher number of detected metabolites and better chromatographic performance. The RP-LC/MS platform demonstrates excellent intrabatch and interbatch retention time repeatability (<0.8 %). Furthermore, the determined concentrations of metabolites show strong agreement with certified and published concentrations of metabolites in the SRM 1950 plasma sample. The developed RP-LC/MS approach was used for the analysis of polar metabolites and endocannabinoids in a well-characterized cohort of spontaneous preterm birth (n=72) and healthy full-term (n=30) human placenta samples. A comprehensive metabolomic evaluation of the placenta revealed that spontaneous preterm birth was associated with significant changes in the levels of N-acylethanolamines, monoacylglycerols, and polar metabolites involved in intracellular energy metabolism and biochemical activity, including amino acids, purine metabolites, and small organic acids.

This work was supported by projects NW24-07-00129 and 22-13967S.

1161 A — Characterizing COPD phenotypes with a targeted signaling lipids metabolomics approach

PRESENTING AUTHOR: ***★ Lu Zhang, Leiden University, Netherlands***

SUB THEME: ***Respiratory disease***

Aims: This study aimed to elucidate clinically-relevant classifications of COPD using a targeted metabolomics approach focusing on signaling lipids.

Materials and methods: Using a targeted LC-MS/MS platform, 166 metabolites including free fatty acids, prostaglandins, isoprostanes, lysophospholipids, endocannabinoids, and bile acids were profiled in a cohort of 49 COPD patients. The study integrated metabolomic data with clinical parameters to identify key metabolites and related pathways for various COPD classification systems including Global Initiative for Chronic Obstructive Lung Disease (GOLD) grading stages, Koninklijk Nederlands Genootschap voor Fysiotherapie (KNGF, Royal Dutch Society for Physiotherapy) profiles, and Systemic (SYS) subtypes and explored the association of these classification systems.

Key findings: The GOLD stages showed correlations with 15 metabolites, including lysophospholipids, oxylipins, and bile acids. KNGF profiles were linked to 13 metabolites, predominantly lysophospholipids, while SYS subtypes were associated with 9 metabolites, mainly oxylipins. A specific cluster of oxylipins, including HETEs and HDoHEs, was notably correlated to prognostic factors of COPD.

Significance: This study identified distinct metabolic patterns associated with GOLD stages, KNGF profiles, and SYS subtypes. Additionally, the findings indicate that 14-HDoHE/DHA may serve as a potential biomarker for COPD exacerbation and suggest possible therapeutic targets for COPD, including pathways involving lipoxygenases, G-protein coupled receptors, and the Farnesoid X receptor.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1162 A — Comparative plasma metabolomics of SARS-CoV-2 infection in human and mouse: Toward mechanistic insights

PRESENTING AUTHOR: *Yeonseo Jang, Seoul National University, South Korea*

CO-AUTHORS: *Yufei Li, Hyeran Kim, Nam-Hyuk Cho, Joo-Youn Cho*

SUB THEME: *Respiratory disease*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes severe respiratory symptoms and is associated with systemic inflammatory responses. Previous studies have primarily focused on human coronavirus disease 2019 (COVID-19). However, the lack of studies on SARS-CoV-2 mouse models has led to a poor understanding of metabolic changes and disease mechanisms, highlighting the need to identify species-specific or shared metabolic changes. In this study, we analyzed plasma samples from COVID-19 patients and SARS-CoV-2 mice to find insights into metabolic disorders for subsequent mechanistic studies and evaluate metabolite differences between species.

Untargeted analysis was performed with Orbitrap Exploris 120 Mass Spectrometer on both human and mouse samples. A total of 74 metabolites were selected from COVID-19 patients based on p-value less than 0.05 in Student's t-test. Among them, 35 metabolites, particularly arginine biosynthesis metabolites, were considered to have significant correlations with COVID-19 severity. A total of 21 metabolites were selected from SARS-CoV-2 3-day post-infection mice based on p-value less than 0.05 in Wilcoxon rank-sum test.

Arginine biosynthesis was significantly changed in COVID-19 patients, and pantothenic acid and CoA biosynthesis were identified as major metabolic pathways in SARS-CoV-2 mice. Notably, arginine was identified as an important metabolite in both human and mouse studies.

In conclusion, arginine biosynthesis and pantothenic acid and CoA biosynthesis may play key roles in severe respiratory infectious diseases. This study provides insights into the metabolic mechanisms of SARS-CoV-2 and potential therapeutic targets for treating SARS-CoV-2.

1163 A — Cardiotoxic effect of Doxorubicin in 3D in vitro models of cardiac tissue: a multi-omics approach

PRESENTING AUTHOR: *Antoni Del Pino Rius, Centre For Omic Sciences – Eurecat, Spain*

CO-AUTHORS: *Iris Samarra Mestre, Ilaria Gisone, Jose Barrera, Lorena García, Salvador Fernández Arroyo, Gianluca Ciardelli, Nuria Canela, Federico Vozzi, Antoni Del Pino Rius*

SUB THEME: *Toxicology*

Recent advances in three-dimensional (3D) cardiac models have enabled more accurate assessments of drug-induced cardiotoxicity. We developed an in vitro model combining human stem cell-derived cardiomyocytes and coronary artery cells embedded in gelatin-metacryloyl (GelMA) hydrogel in poly(ester urethane) functionalized with poly(acrylic acid) and fibronectin (PUR-FN) scaffolds. This setup more realistically replicates the structure and properties of human heart tissues compared to traditional 2D cultures.

We applied a multi-omics approach (metabolomics and proteomics) to investigate the effects of the antitumor drug doxorubicin (DOX), known for its cardiotoxic profile.

Protein and metabolite extraction was performed after enzymatic digestion of the hydrogel using collagenase. Proteomic analysis used TMT-based mass spectrometry, while metabolomics assessed lipids and amino acid derivatives via liquid chromatography-mass spectrometry.

DOX-treated cells exhibited significant alterations in key metabolic pathways such as glycolysis, the Krebs cycle, fatty acid beta-oxidation, and tryptophan metabolism. Additional signs of oxidative stress, DNA damage, calcium dysregulation, and impaired protein synthesis were observed. Results confirm that DOX toxicity involves widespread mitochondrial dysfunction, oxidative stress, and accelerated cellular aging in cardiomyocytes leading to structural muscle damage and impaired contraction, highlighting the complexity of DOX's mechanism of action.

Our 3D cardiac model, combined with multi-omics analysis, offers a powerful platform for detailed cardiotoxicity assessment. It supports personalized risk evaluation and the development of safer therapeutic strategies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1164 A — Metabolomics study of nicotine exposure in THP-1 monocytes

PRESENTING AUTHOR: ***Silvio Uhlig, Nordic Institute Of Dental Materials, Norway***

CO-AUTHORS: ***Bergitte Olderbø Pearl, Jan Tore Samuelsen, Solveig Uvsløkk, Lada Ivanova, Camille Vanderstraeten, Lene Grutle Aiko, Oscar Daniel Rangel-Huerta***

SUB THEME: ***Toxicology***

Nicotine, a tobacco alkaloid, activates neuronal nicotinic acetylcholine receptors. Exposure to nicotine occurs mainly through conventional smoking and increasingly via e-cigarettes. In Scandinavia, the consumption of nicotine via snuff or nicotine pouches is also popular. The use of snuff has been associated with several adverse health effects such as inflammatory reactions of the oral mucosa and oral cavity cancer. In the present study, we performed a metabolomic analysis of nicotine-exposed THP-1 human monocytes. The monocytes were exposed to 5 mM nicotine for up to four hours. Both the cell extracts and cell culture medium were subjected to untargeted liquid chromatography high-resolution mass spectrometry. Nicotine-related metabolites were extracted from the dataset using the built-in biotransformation prediction option in the Compound Discoverer software and molecular network analyses, resulting in the tentative annotation of 17 nicotine biotransformation products. Cotinine and nornicotine were identified as two major cellular biotransformation products. The application of multi- and univariate statistical analyses resulted in the annotation of, up to a certain level of identification, 12 compounds in the cell extracts and 13 compounds in the medium that were altered by nicotine exposure. Of these, four were identified as methylthioadenosine, cytosine, uric acid, and L-glutamate. Methylthioadenosine levels were affected in both cells and the medium, whereas cytosine, uric acid, and L-glutamate levels were affected in the medium only. The effects of smoking on the pathways involving these metabolites have been previously demonstrated in humans. Most of the other discriminating compounds that were tentatively or not fully identified were amino acids or amino acid derivatives. In conclusion, our preliminary data suggest that some of the potentially adverse effects related to smoking may also be expected when nicotine is consumed via snuff or nicotine pouches, which are regarded as “healthier” options for nicotine consumption.

1165 A — Metabolic phenotypes of doxorubicin-induced cardiotoxicity among patients with breast cancer

PRESENTING AUTHOR: ***Ping-ching Hsu, University Of Arkansas For Medical Sciences, United States***

CO-AUTHORS: ***Amarnath Singh, Se-Ran Jun, Katherine Wallis, Reid D. Landes, Valentina Todorova, L. Joseph Su, Sam Makhoul, Ping-ching Hsu***

SUB THEME: ***Toxicology***

Background. Doxorubicin (DOX)-based chemotherapy has greatly increased the number of long-term cancer survivors, but it has also led to an increasing number of patients experiencing DOX-induced cardiotoxicity (DIC). Currently, there are no validated biomarkers that can predict the early development of DIC. Therefore, novel biomarkers of DIC are urgently needed to identify patients who are at an increased risk, allowing early detection of the cardiotoxicity before it causes permanent cardiac damage.

Methods. Breast cancer patients who received DOX-containing chemotherapy were stratified based on the degree of change in left ventricular ejection fraction (LVEF) into two groups: 20 patients who maintained normal cardiac function (normal, decline in LVEF < 10%) and 8 patients who developed cardiotoxicity (abnormal, decline in LVEF > 10%). Blood samples were collected before, during, and after DOX-containing chemotherapy, and plasma was used to perform the untargeted metabolomic profiling. Univariate analysis with baseline data and multivariate analysis with a pre-post study design were used to find dynamic changes in metabolite markers that might serve as early indicators of DIC. Stepwise logistic regression was further computed and validated via Random Forest modeling with the performance in predicting DIC visualized via a receiver operating characteristic curve.

Results. Significant separation of plasma metabolomics profiles was observed between normal and abnormal cardiotoxicity groups at baseline and after first cycle of DOX chemotherapy. The analysis identified 100 significant metabolites at baseline (T0) and 78 after the first cycle (T1), with 10 metabolites common to both timepoints: 3-phosphoglycerate, 2-hydroxyphenylacetate, inosine, taurine, suberate (C8-DC), sebacate (C10-DC), sphingadienine, oxindolylalanine. Machine learning models identified key metabolites (e.g., sebacate [C10-DC], 2-hydroxyhippurate, orotate, picolinate, and suberate [C8-DC]) as robust predictors of cardiotoxicity, achieving high sensitivity and specificity.

Conclusions. Metabolomic profiling shows potential for early detection of DIC in breast cancer patients, supporting personalized interventions to prevent irreversible cardiac damage.

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

1166 A	Cracking the Hemp Code: How Extended Steroid Profiling Unravels the Impact of Cannabis on Male Fertility

PRESENTING AUTHOR: ★ ***Mathieu Galmiche, University of Geneva, Switzerland***

CO-AUTHORS: ***Isabel Meister, Fanny Zufferey, Michel Rossier, Rita Rahban, Serge Nef, Julien Boccard, Serge Rudaz***

SUB THEME: ***Toxicology***

In Switzerland, a vast study revealed that 17% of young men displayed suboptimal sperm concentration. Drug use may be one of the environmental factors affecting male fertility. In particular, cannabis consumption causes human exposure to several phytocannabinoids. They can affect the function of cannabinoid receptors, which are particularly abundant in the Hypothalamic-Pituitary-Gonadal (HPG) axis. HPG dysregulation leads to disturbances of steroid metabolism and sex hormone balance, detrimental to human fertility.

To investigate potential endocrine-disruptive effects of cannabis, the circulating steroidome of 94 Swiss young men was measured. Their self-reported cannabis use was confirmed by the determination of THC-COOH in urine.

While most of steroidomics studies are generally restricted to 20-to-50 compounds due to numerous analytical difficulties (separation of isobaric compounds, poor ionization, etc.), the present work utilized a multi-targeted approach with an optimized LC separation on a Biphenyl phase and an enhanced Multiple Reaction Monitoring (MRM) detection with post-column addition of ammonium fluoride. This enabled the inclusion of 172 target compounds covering all steroid subclasses, out of which 74 were consistently detected, thus considerably surpassing the current state-of-the-art in steroidomics.

Multivariate analyses using PLS-based models confirmed higher androgen concentrations in cannabis consumers. This trend was more pronounced at higher THC-COOH levels. Androstenedione, testosterone and dihydrotestosterone were particularly increased, according to complementary univariate analyses ($p < 0.05$). However, there was no significant effect of cannabis consumption on C11-oxy androgens, which are specifically produced in the adrenal. This extended steroid profiling thus enabled the distinction of the androgenic steroidome relative to the different steroid-producing organs and suggested that only gonadal (testicular) androgen synthesis might be dysregulated by phytocannabinoids. Additionally, 11-beta-hydroxyprogesterone and 5-beta-dihydroprogesterone levels were markedly elevated in cannabis users. While their biological role is yet to be deciphered, these progestogens might be new markers of cannabis-related endocrine disruptions.



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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1185 A — Metabolomics Reveals the Impact of Harvest Time on the Chemical and Volatile Profiles of Jasmine Rice Leaf Tea

PRESENTING AUTHOR: ***Arashaporn Uthairangsi, Chulalongkorn university, Thailand***
CO-AUTHORS: ***Nureesun Mahamud, Supat Chaiyakul, Rossarin Tansawat***
SUB THEME: ***Crop quality research***

Jasmine rice (Oryza sativa L. cv. KDML105) is globally prized for its distinctive grain aroma, and its young leaves are gaining attention as functional ingredients in herbal teas and rice leaf juice. These applications not only enhance nutritional value but also promote product diversity and sustainability by utilizing agricultural byproducts. However, the chemical and aromatic profiles of rice leaf tea remain underexplored. This study aimed to evaluate the impact of harvest time on the chemical composition, volatile profile, and antioxidant activity of KDML105 rice leaf tea using untargeted metabolomics approach. Young leaves were collected at three harvest times: days 14, 20, and 28 (n = 10/group)—for chemical profiling by LC-QTOF-MS/MS and volatile profiling by GC-TOF-MS. Results showed that partial least squares discriminant analysis (PLS-DA) from both platforms clearly separated the samples by harvest time, emphasizing its influence on tea quality. LC-QTOF-MS/MS identified 175 metabolites, with eremanthin as a key antioxidant compound peaking at day 14 (VIP > 2.8; $p < 0.05$). GC-TOF-MS revealed 190 volatile compounds, with 2-acetyl-1-pyrroline (2-AP), known for its popcorn-like aroma and a key contributor to jasmine rice fragrance, being most abundant in tea harvested on day 20. Additionally, antioxidant assays, including DPPH and FRAP methods, were conducted to determine potential health benefits of the rice tea. Results showed that day 14 samples exhibited the highest antioxidant activity, with 84.78 ± 0.77% DPPH inhibition and 40.60 ± 1.07 μM Fe^{2} /g FRAP value. In conclusion, day 20 samples offered a balanced profile of fragrance and potential benefits, making them a valuable option for both sensory and functional applications. These findings underscore the importance of harvest time in shaping the health-promoting and aromatic qualities of rice leaf tea.

1186 A — Leveraging High-Throughput LC-MS Metabolomics to Unlock the Potential of Fruit Tree Crop Wild Relatives for Sustainable Agriculture

PRESENTING AUTHOR: ***Claudia Rouveyrol, INRAE France, France***
CO-AUTHORS: ***Adam Rochepeau, Sylvain Prigent, Morgane Roth, Nicola Busatto, Véronique Decroocq, Pierre Pétriacq***
SUB THEME: ***Crop quality research***

Crop Wild Relatives (CWRs) are wild plant species genetically related to domesticated crops, offering a rich yet under-explored source of biochemical and genetic diversity, particularly valuable for improving the resilience, sustainability, and nutritional quality of fruit crops. While current fruit production largely depends on a limited pool of elite cultivars, the exploration and characterization of diversity across wild and cultivated populations is essential to reintroduce beneficial traits into commercial varieties and to develop tools for biodiversity-based crop management.

The FruitDiv project (HE project 101133964) integrates metabolomics with global multi-omics approaches including genomics and phenomics to investigate core collections of Malus, Pyrus, and Prunus. By profiling metabolites associated with key agronomic traits such as fruit quality, stress tolerance, and pest and disease resistance, FruitDiv aims to elucidate genotype-to-phenotype relationships and develop pioneering technology for phenotypic trait prediction using computational modeling.

At the Bordeaux Metabolome Facility, a large-scale, untargeted LC-MS workflow was applied to over 1,500 leaf and fruit samples of Prunus. Samples underwent automated ethanolic extraction, including Quality Controls preparation under identical conditions, followed by UHPLC-LTQ Orbitrap (ThermoScientific) analysis in negative ion mode. Data processing and compound annotation were performed using MS-DIAL, and statistical analysis using MetaboAnalyst. Further computational modeling and parameter optimization are ongoing to build predictive models linking biomarkers of plant performance and fruit quality. Preliminary results in Prunus indicated clear metabolomic shifts between cultivated and wild relatives of apricot leaves and fruits, highlighting key redox and secondary pathways involved, as previously exemplified by genomic studies (Groppi et al., 2021).

Beyond advancing scientific knowledge on fruit crop adaptation, FruitDiv also supports breeders and conservation stakeholders in developing fruit production systems more resilient to environmental challenges.

High-throughput metabolomics thus provides key insights into the potential of CWRs to address challenges related to climate change, sustainable agriculture, and global food security.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1187 A — Longitudinal Metabolomic Signatures Reveal the Trajectories between Pre- and Peri-conceptional Traffic-Related Air Pollution Exposures and Preterm Birth Risk

PRESENTING AUTHOR: ***Mengyuan Ren, Emory University, United States***

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SUB THEME: ***Environmental exposures***

BACKGROUND: African Americans (AA) experience disproportionately high traffic-related air pollution (TRAP) exposures and bear the highest burden of preterm birth (PTB). Exposure to TRAP has been linked to an increased risk of PTB. However, the molecular mechanisms underlying TRAP-PTB associations remain unclear. We aimed to investigate metabolic signatures and pathways linking pre- and peri-conceptional TRAP exposure to PTB among AAs.

METHOD: We included 326 participants recruited between 2014-2019 with singleton pregnancies from the prospective Atlanta AA Maternal-Child Cohort. TRAP exposure, including fine particulate matter (PM2.5), carbon monoxide (CO), and nitrogen oxides (NOx), were estimated using spatiotemporally resolved model with 250×250m resolution for 1-year, 6, 3 and 1-months prior to biomonitoring. Prenatal longitudinal metabolic serum profiles in early (8-14 gestational weeks) and late-pregnancy (24-32 weeks) were characterized with untargeted high-resolution metabolomics. Generalized linear models were utilized to evaluate the associations between TRAP, metabolomic signatures and pathways, with all PTB (AllPTB) and spontaneous PTB (SpPTB) using a meet-in-the-middle approach.

RESULTS: We identified 16 and 22 metabolic signatures during early-pregnancy, and 16 and 28 during late-pregnancy associated with AllPTB and SpPTB, respectively. Intracellular signaling through prostacyclin receptor and prostacyclin, pyrimidine metabolism were the overlapping pathways linked with both TRAP exposure and PTB. We confirmed the identity of multiple metabolites with Level-1 evidence, including 5-hydroxy-L-tryptophan, leukotriene-B4, alanine, and endogenous phthalate metabolites. One-year exposure window prior to biomonitoring was the most sensitive time window with 3055, 145, and 100 metabolomic features significantly associated with PM2.5, CO, and NOx (FDR $q<0.05$), respectively.

CONCLUSIONS: Perturbations in amino acid metabolism and inflammatory pathways and metabolites centering around neurotransmission and glycoconjugate biosynthesis were associated with both longitudinal TRAP exposure and PTB in this AA cohort. These findings reveal novel mechanistic insights underlying the effects of prenatal TRAP exposure on PTB risk.

1188 A — Microdialysis for time-resolved in-vivo studies of pesticide effects on metabolome of pollinating insect E. tenax

PRESENTING AUTHOR: ***Simon Palmer, Lund University, Sweden***

CO-AUTHORS: ***Peter Spégel, Elisa Rigosi, David O'Carrol***

SUB THEME: ***Environmental exposures***

Pesticide use has been implicated in decline of populations of pollinating insects, threatening food production globally. Remarkably, it has been demonstated that pesticide use cause insect death even when exposure is far lower than lethal doses. This has sparked an interest in studying sublethal effects, i.e. adverse effects from pesticides caused by doses sometimes thousands of times lower than lethal.

Examples of such effects are impaired manouverability or disrupted energy metabolism. Intoxication of hoverflies with neonicotinoid pesticides even below lethal doses can show very detrimental effects over short timespans. While the animals are able to recover in a laboratory setting, this would likely be fatal in the wild.

Thus, it is of high interest to study how the metabolic profile of pollinating insects develop over time after exposure. Due to the small size of these animals, it is not possible to draw significant liquid volumes repeatedly for study. For this reason, we have developed a microdialysis method for time-resolved in-vivo measurements of hoverfly metabolism. Dialysates are studied using gas chromatography – mass spectrometry for untargeted metabolomics analysis.

Previous work investigated the feasability of microdialysis in insect brain compared to in the hemolymph, followed by studies of the relevant effects and what timescale they appear at when exposed to high pesticide doses. Future work will see insects exposed to field-relevant doses of various pesticides, alone and in combination.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1189 A — Yogurt fermented with extra strains modulates metabolism and immune tissues in gnotobiotic mice

PRESENTING AUTHOR: *Grégory Pimentel, Agroscope, Switzerland*

CO-AUTHORS: *Ueli Buetikofer, Thomas Roder, Cornelia Bär, Ueli von Ah, Sandro Christensen, Zahra Sattari, Cristina Kalbermatter, Rémy Bruggmann, Stephanie C. Ganal-Vonarburg, Guy Vergères*

SUB THEME: *Fermented foods*

Dietary diversity is increasingly recognized as a determinant of health. It is associated with better nutritional status in the elderly and protective effects against cardio-metabolic diseases. Likewise, reduced gut microbiota diversity has been linked to poorer health outcomes. Fermented dairy products are an important source of beneficial microbes and bioactive compounds, and expanding microbial diversity in fermentation may influence host metabolism and immunity.

We aimed to evaluate whether increasing microbial diversity in yogurt fermentation translates into greater metabolic diversity in the product and in the host. Specifically, we tested whether milk fermented with five lactic acid bacteria (LAB) strains (instead of the traditional two) modulates metabolic and immune-related profiles in mice.

Seventeen gnotobiotic mice were fed daily for 3 days with either milk, normal yogurt, or a test yogurt fermented with two standard LAB plus three additional strains. Blood was collected on day 1 (2 h and 4 h post-gavage) and day 3 (4 h post-gavage). On day 3, mesenteric lymph nodes (MLNs) and spleens were also collected. Untargeted UHPLC-MSMS metabolomics was performed on the products, serum, spleen, and MLN samples. Bulk transcriptomic analysis was performed on ileal tissue. Multivariate analyses (PLS-DA, OPLS-DA) were applied to the metabolomics data as well as pathway analysis. Differential gene expression and GSEA was performed on the RNA sequencing data.

Metabolomics analysis of the products confirmed increased metabolic diversity in the test yogurt. Distinct metabolic profiles in the blood and organs depending on the ingested product could be revealed: (O)PLS-DA analysis clearly differentiated samples from mice treated with milk, normal yogurt, or test yogurt. Pathway enrichment using Mummichog and compound identification are ongoing and will be presented. Preliminary transcriptomic data and integrated multi-omics insights are under analysis and will be presented.

1190 A — Metabolomics Highlights the Distinct Impacts of Different Partial Pulp Removal Methods on Cocoa Flavor Improvement

PRESENTING AUTHOR: *Aulia Gusning Ati, Osaka University, Japan*

CO-AUTHORS: *Indah Anita Sari, Hendy Firmanto, Abdul Malik, Eiichiro Fukusaki, Sastia Prama Putri*

SUB THEME: *Fermented foods*

Incorporating additional steps to partially remove cocoa pulp by 10–30% before fermentation has shown potential for improving cocoa flavor. Common methods for pulp removal include pre-drying, which involves spreading the beans in a single layer to reduce pulp through moisture loss, and mechanical de-pulping, which uses physical abrasion to remove the pulp rapidly with a machine. Although both approaches can achieve similar levels of pulp reduction, they may differ in their effects on flavor development. Therefore, this study compares the two approaches through a 25% pulp reduction and evaluates their impact on flavor through sensory evaluation. Alongside this, metabolite profiling of cocoa liquor was carried out using GC-MS after derivatization for non-volatile compounds and HS-SPME GC-MS for volatile compounds. Results show that pre-drying improved cocoa liquor flavor, while mechanical de-pulping failed to achieve positive sensory effects. Pre-drying enhanced fresh fruit and acidity attributes ($p<0.05$) while reducing bitterness and astringency, whereas mechanical de-pulping led to higher bitterness and astringency score attributes ($p<0.05$). Partial Least Square Regression (PLS-R) analysis correlated 85 non-volatile and 85 volatile metabolites to sensory attributes, identifying several compounds—organic acids (citric acid, malic acid, tartaric acid), sugars (fructose, trehalose), and esters (methyl phenylacetate, ethyl octanoate)—that were found at higher levels in pre-dried samples and contributed to fresh fruit and acidity attributes. In contrast, mechanical de-pulping resulted in higher levels of off-flavor compounds like butyric acid, butanoic acid, 1-phenylethyl alcohol, and valeraldehyde in the cocoa liquor, which aligned with the observed increase in bitterness and astringency. These findings highlight that the method used is crucial for flavor improvement and provide a basis for optimizing de-pulping strategies to enhance cocoa quality.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1191 A — Enhancement of the fruity flavor in coffee beverages by solid-state fermentation in green coffee: a multi-block approach

PRESENTING AUTHOR: *Mónica Cala, Universidad de Los Andes, Colombia*

CO-AUTHORS: *Santiago Moreno, Juan David Dereix, Andrea Ramirez, Sebastián Velásquez, Tatiana Sepúlveda, Ana María León-Inga, Julián Zapata, Mónica Cala*

SUB THEME: *Fermented foods*

This study aimed to develop specialty coffee profiles from regular coffee, with an emphasis on the desirable fruity flavor. Solid-state fermentation (SSF) of green coffee beans was evaluated, with yeast hydrated in water at a concentration of 10 g/L per batch of 100 kg of coffee. The yeast was incubated at room temperature for 15 minutes before being added to the samples. Additionally, selected conditions based on a screening experiment were applied to generate varying levels of fruity flavor after roasting. Green coffee beans were steamed, and the inoculum and additives were introduced before fermentation in closed containers for at least 12 hours. The coffee samples were then dried at 70 °C for 3 hours or until the moisture content reached 10-12%. Once dried, the samples were stored in polyethylene bags at -20 °C for further analysis.

The samples were analyzed in three blocks: physicochemical analysis; volatile compound analysis using headspace solid-phase microextraction (HS-SPME), gas chromatography coupled to an olfactometer (GC-O), and gas chromatography coupled with a time-of-flight mass spectrometer (GC-QTOF-MS); and non-volatile compound analysis through untargeted metabolomics using both liquid and gas chromatography with high-resolution mass spectrometry (RP-LC-QTOF-MS/GC-QTOF-MS). Fermentation of the green coffee beans increased fructose content from 0.01% to over 1%, while glucose levels increased by 80% in the same samples. Metabolomic analysis revealed changes in carbohydrates, cinnamic acids, glycerophospholipids, organic acids, and flavonoids as a result of fermentation. Despite a slight reduction in aroma and fragrance, high-fruity samples were preferred overall and exhibited a more complex profile. The bioprocess also resulted in three specialty coffee samples, with one showing the most significant improvement, a 10-point increase. Consequently, the process was demonstrated to be effective in modulating the fruity flavor attribute, and the multi-block approach provides a solid framework for studying the metabolic pathways occurring during fermentation.

1192 A — Characteristics and variability of the aroma composition of three Japanese strawberry cultivars

PRESENTING AUTHOR: *Fukuyo Tanaka, Research Center for Advanced Analysis, NARO, Japan*

CO-AUTHORS: *Hironori Juichi, Yuko Suga*

SUB THEME: *Foodomics and food security*

Strawberry aroma varies depending on the cultivar, and detailed information on the aroma composition is essential for developing cultivars that align with consumer preferences. Furthermore, both the aroma and flavor of the fruits are influenced by the cultivation environment.

We conducted a comparative analysis of strawberry samples grown under two cultivation conditions: (a) ground-soil and (b) raised-bed systems using a coconut shell-based medium. The analyzed cultivars—'Tochiotome,' 'Koiminori,' and 'Yotsuboshi'—are known to exhibit distinct aroma profiles.

A total of 167 peaks were aligned, and the integrated peak intensity data were used for analysis. Among these, 85 peaks differed significantly among the cultivars (FDR-$p<0.05$). Cluster analysis of the significant components revealed that the cultivar 'Tochiotome' grown in soil exhibited the most characteristic composition, rich in mesifuran, furaneol, 1-methoxy-2-propanol, and various ethyl esters. Terpenoid alcohols and lactones were rich in 'Koiminori,' whereas 'Yotsuboshi' had elevated levels of methyl anthranilate and its precursor, 2-aminobenzaldehyde.

1193 A — Wide-Targeted Analysis of Metabolites in Rice from Different Production Area

PRESENTING AUTHOR: *Hitomi Tsujihata, Shimadzu Corporation, Japan*

CO-AUTHORS: *Yutaka Umakoshi, Nanami Sakashita, Yuki Sakamoto*

SUB THEME: *Foodomics and food security*

In recent years, numerous cases of fraudulent labeling of food origins have been reported, which affects food safety and product value. To counter this, the development of more advanced analysis is required. Commonly used testing methods include DNA analysis, elemental analysis, stable isotope ratio analysis, and compositional analysis. Rice contains many nutrients and is important as an energy source. It contains a wide variety of metabolites, including sugars, amino acids, fatty acids, and vitamins. Comprehensive analysis of these metabolites may clarify relations between production areas and metabolite profiles. In this study, we performed comprehensive metabolomic analysis on eight samples of the same variety (Koshihikari) of rice collected from six prefectures in Japan. The polished rice was frozen-ground, and based on method of Bligh & Dyer, hydrophilic metabolites were extracted using mixed solution with methanol, dichloromethane, and water. After performing trimethylsilyl derivatives, a gas chromatograph mass spectrometer (GCMS-TQ8040 NX) and Smart Metabolite Database ver.2 was used to wide-targeted analysis of five hundred components in MRM mode (n=3). Data processing was performed with LabSolutions Insight. As a result, a total of sixty-five components such as sugars, amino acids, and fatty acids, were identified in all samples. Furthermore, multivariate analysis, principal component analysis (PCA) was performed with eMSTAT Solution using fifty-five components with a p-value of 0.05 or less. The score plot showed that the samples could be classified based on their cultivation locations. The loading plot showed that amino acids were more abundant in Chiba, monosaccharide in Kagawa, fatty acids in Niigata, and organic acid in Toyama, which was indicated differences in metabolites depending on the region. monosaccharides and polysaccharides were located at opposite positions, suggesting that the sugar profiles differ depending on samples. The result of others will be discussed in the poster presentation.

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★ AWARD WINNERS

PLANTS, FOOD, ENVIRONMENT AND MICROBES

1194 A — Metabolite signatures and marker compounds for the authentication of Coffea arabica and robusta

PRESENTING AUTHOR: ★ *Stefan Pieczonka, The University of Queensland, Australia*
CO-AUTHORS: *Anna Dzemajili, Silke Heinzmann, Michael Rychlik, Philippe Schmitt-Kopplin*
SUB THEME: *Foodomics and food security*

As food production scales and supply networks globalize, ensuring the quality and authenticity of food products has become increasingly complex. In coffee production and processing, mislabeling lower-value Robusta as premium Arabica coffee can yield a profit margin of up to 40%. Existing analytical efforts for authenticity verification rely on solvent extraction, focus on raw products, and are univariate in nature.

Conversely, analysis of a diverse set of 130 standardized brewed coffee samples using Fourier-transform ion cyclotron mass spectrometry (FT-ICR-MS) provided a comprehensive, ultra-high resolution perspective on their molecular complexity, annotating unambiguous molecular formulas to 11,500 mass signals. OPLS-DA statistics and mass difference networks revealed numerous hydroxycinnamic acid derivatives, whose compositions varied by coffee type: Arabica plants predominantly conjugated these metabolites with quinic acid and as glycosides, while tryptophan conjugates were exclusive to Robusta coffees.

Complementary LC-ToF-MS^2 analysis, targeted at these compounds, confirmed their core structures and conjugation patterns through SIRIUS/CANOPUS class annotations and structural similarities in GNPS feature-based molecular networks.

Both the FT-ICR-MS classification model and the LC-ToF-MS^2 screening of significant hydroxycinnamic acid conjugates achieved flawless differentiation (130 out of 130) between Arabica and Robusta samples. The mislabeling of four Robusta coffees as Arabica was further validated by targeted NMR analysis of solvent extracts from the beans.

Our study establishes an effective and reliable non-targeted method for coffee authentication, harnessing the water-soluble nature of hydroxycinnamic acid conjugates to enable accurate verification across raw and roasted beans as well as ready-made coffee products. This approach provides a robust solution for safeguarding coffee authenticity throughout the entire production and supply chain.

1195 A — LC-MS-Based Lipidomics and Proteomics for Meat Species and Breed Authentication

PRESENTING AUTHOR: *Renata Garbellini Duft, University of Aberdeen, United Kingdom*
CO-AUTHORS: *David A. Stead, Julian L. Griffin*
SUB THEME: *Foodomics and food security*

Ensuring food authenticity is crucial in the globalized food industry, especially after high-profile fraud scandals that raise concerns about meat integrity and cost the United Kingdom an average of £2 billion annually. Liquid chromatography-mass spectrometry (LC-MS) is a highly sensitive and selective tool to detect mislabelling, fraud, allergens, and cross-contamination by detecting small molecules. We aimed to apply a combination of LC-MS lipidomics, proteomics, and multivariate statistical analysis to discriminate between and within species, using lipid and protein profiles to verify meat provenance. Beef, pork, chicken and lamb samples were purchased from retailers and farm shops in Scotland. For lipidomics, samples were homogenised, extracted, and analysed by ion mobility quadrupole time-of-flight mass spectrometry coupled to ultra-high-performance LC (UHPLC-IM-QTOF-MS). For proteomics, samples were cooked, washed, homogenised, reduced, alkylated and digested with trypsin. The peptides were desalted, and then injected into a nano flow-LC-MS/MS system. Orthogonal partial least squares discriminant analysis (OPLS-DA) revealed clear segregation between and within species based on both lipids and proteins. Chicken samples contained high levels of phosphatidylethanolamine (PE (16:0/20:4)) and phosphatidylcholine (PC (18:1/18:2)) compared to other species. Pork samples showed higher levels of PC (16:0/18:2) and PE (18:0/20:3), while lamb contained different triglyceride chains, TG (12:0/14:0/14:0) and TG (15:0/16:1/18:2). Unique peptides from proteins included myozenin 1 and nebulin (beef); carbonic anhydrase 3 and myoglobin (pork); AHNAK nucleoprotein (lamb); and calpastatin and creatine kinase (chicken), which were verified by BLAST database. Two cattle breeds were discriminated based on TG (52:0), PC (O-36:4), and PE (18:0/22:5) lipid chains, as well as peptides from cytochrome c oxidase subunit 5B. The findings demonstrate the reliability of integrating LC-MS lipidomics and proteomics in distinguishing between meat species and breeds, thereby identifying specific lipid and peptide biomarkers for detecting food fraud and verifying the authenticity of processed food products.

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1196 A — Beyond Fats: How Food Lipidome Insights for better Health and Sustainability

PRESENTING AUTHOR: ***Rosário Domingues, University of Aveiro, Portugal***

CO-AUTHORS: ***Felisa Rey, Diana Lopes, Tiago Conde, Bruna Neves, Ana S. P. Moreira, Pedro Domingues***

SUB THEME: ***Foodomics and food security***

Food is a crucial source of lipids, essential for various bodily functions such as cell membrane structure, cellular signaling, and energy storage. Despite their importance, there are widespread misconceptions about food lipids. Many people believe all fats are unhealthy or directly cause weight gain. However, it is well-recognized that certain lipids, such as omega-3 fatty acids, are beneficial for heart and brain health. Additionally, low-fat diets, while often recommended for weight management, can increase the risk of non-communicable diseases (NCDs) like cardiovascular disease and type 2 diabetes by depriving the body of essential lipids.

The lack of understanding about food lipids largely stems from limited knowledge of the food lipidome. Nutritional labeling typically provides only total fat and certain fatty acids, offering an incomplete picture. However, advancements in mass spectrometry-based lipidomics have shown that the food lipidome is complex and diverse, consisting of thousands of lipid species from different classes, beyond just fatty acids.1 Results indicate that food lipidomes are specific to both the food source and the species from which they originate. This specificity can be used as an identity signature to improve food quality, traceability, authenticity and the detection of food fraud, as seen with olive oil and seafood (e.g., octopus).2 It also provides essential evidence for evaluating the nutritional value and health effects of different lipids. Studying food lipids also helps uncovereninng sustainable food systems by supporting alternative, resilient lipid sources (e.g., algae)3 and enabling the reuse of food waste (e.g., avocado by-products)4. This is relevant for addressing global challenges like food security, climate change and management of natural resources, or resources scarcity.Evidence-based lipidomics benefits consumers, industry, and policymakers, enabling informed food choices and regulations. Advancing research on food lipids is key to healthier diets and sustainable practices.

1197 A — Existing food processing classifications overlook the phytochemical composition of processed plant-based protein-rich foods

PRESENTING AUTHOR: ***Kati Hanhineva, University of Turku, Finland***

CO-AUTHORS: ***Jasmin Raita, Hany Ahmed, Kang Chen, Veera Houttu, Retu Haikonen, Anna Kårlund, Maaria Kortesniemi, Baoru Yang, Ville Koistinen, Kati Hanhineva***

SUB THEME: ***Foodomics and food security***

According to existing food processing classification systems, plant-based protein-rich (PBPR) foods are often considered “ultra-processed” - and therefore perceived as unhealthy – despite their ability to provide various bioactive compounds potentially beneficial for human health. On the other hand, foods made from plant-based ingredients are often automatically considered healthier choices, regardless of their nutritional and overall biochemical content. Here, we used a non-targeted liquid chromatography – mass spectrometry metabolomics approach to analyze the impact of processing on the biochemical composition of PBPR foods (Nature Food, in press). Results reveal that existing food classification systems may provide questionable categories for PBPR foods without considering their overall biochemical composition including phytochemicals. An analysis focusing specifically on biochemical compounds of soy-based products manufactured with various technologies showed no clear distinctions between processing groups in the principal component analysis based on the NOVA and Poti classification. However, the phytochemical profile obtained from the metabolomics analysis clearly distinguished the food products according to their processing technologies. In particular, the bioavailability of phytochemicals in fermented soy-based products is seemingly high, as exemplified with isoflavonoids. The products made from refined plant-based proteins contained only trace amounts of the potentially bioactive phytochemicals that can mediate beneficial health effects. Although the food processing classification systems are welcome in their attempt to guide consumers towards healthy choices, they should be improved to more accurately reflect the biochemical composition of PBPR foods.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1198 A — Ensuring Food Safety through Comprehensive Chemical Residue and Contaminant Testing in Meat, Poultry, Siluriformes, and Egg Products

PRESENTING AUTHOR: ***Candice Ulmer Holland, USDA-FSIS, United States***

SUB THEME: ***Foodomics and food security***

The USDA-FSIS Eastern Laboratory (EL) plays a critical role in ensuring the safety and regulatory compliance of meat, poultry, Siluriformes (catfish), and egg products. Utilizing a comprehensive suite of 26 analytical instruments, including LC-MS/MS, GC-MS/MS, and LC-HRMS/MS platforms, the EL employs metabolomics approaches to detect over 180 veterinary drugs, pesticides, and chemical contaminants. Each year, the EL processes approximately 4,500 samples from the field using 18 validated ISO 17025 analytical methods to support national food security initiatives.

One FSIS regulatory effort, in particular, aims to perform routine PFAS testing to address the growing concern of per- and polyfluoroalkyl substances (PFAS) contamination in food matrices. FSIS currently detects 16 PFAS compounds using a ZORBAX RR Eclipse Plus C18 delay column on an Agilent 1290 Infinity UHPLC coupled to a Sciex 6500+ instrument, achieving limits of detection as low as 0.5 ppb. However, harmonization with other federal stakeholders such as USDA-ARS and FDA methodologies is needed to ensure consistency in regulatory applications. As a result, active research efforts are focused on expanding the existing PFAS testing methodologies to allow for the detection of 32 PFAS compounds across priority food matrices, optimizing MRM transitions and source parameters for the Sciex 6500+ instrument, evaluating matrix effects and extraction efficiency in FSIS-regulated food products, modifying the LC gradient and column selection to improve peak resolution, and standardizing internal standards, including the assessment of isotope-labeled alternatives. This multi-agency collaboration will also ensure that FSIS methodologies align with FDA and EPA standards (C-010.02, EPA 533, and EPA 537). Preliminary results demonstrate enhanced cholic acid separation, improved matrix recovery, and optimized detection limits with a QuEChERS extraction.

This presentation will also highlight multi-year trends (2020-2025) in food contaminants, discuss method standardization challenges, and explore future directions for chemical residue testing in the U.S. food supply.

1199 A — Enhancing coverage and spatial resolution of the coral-microalgal metabolomes

PRESENTING AUTHOR: ***Wing Yan Chan, The University Of Melbourne, Australia***

CO-AUTHORS: ***Evan Heit, Mei-Chen Liu, Vinzenz Hofferek, David Rudd, Madeleine van Oppen, Malcolm McConville***

SUB THEME: ***Marine metabolomics***

Coral reefs represent some of the most diverse ecosystems on Earth, but the majority are at risk due to climate change. Corals are dependent on algal symbionts for nutrition and growth via metabolic exchange. This intimate and highly evolved coral-algal interaction is disrupted at elevated temperatures, leading to coral bleaching (algal expulsion) and often death. Our ability to design effective reef restoration and conservation strategies hinges on in-depth knowledge on coral symbiosis and the mechanism underpinning coral resilience. Metabolomics is a powerful approach to examine cellular mechanisms underpinning climate change resilience. However, traditional approaches typically 1) have limited coverage in coral/symbiont central carbon metabolism, 2) do not provide knowledge on metabolic fluxes, 3) require a large amount of biomass, and 4) utilize whole tissue homogenates, which prevent spatial dissection of coral or symbiont metabolism and the two-way transfer of metabolites between each partner in situ. Using a combination of gas chromatography- and liquid chromatography-mass spectrometry (GC-MS, LC-MS), > 300 metabolites associated with coral and symbiont central carbon metabolism and beyond were identified using minimal biomass. Changes in the levels of these metabolites and metabolic fluxes associated with the uptake and metabolism of different 13C-labeled precursors were also assessed under conditions of thermal stress. Finally, to more precisely map changes in the symbiont versus the coral host, mass spectrometry imaging (MALDI-MSI) was used to map specific metabolites/lipids at a single microalgal cell resolution (5 µm), separating metabolic signatures originating from different cellular layers (e.g., epidermis, gastrodermis) and co-occurring symbiont species. These novel findings highlight key aspects of the coral-symbiont central carbon metabolism, providing new insights into the metabolic strategies underpinning coral thermotolerance.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1200 A — Lipidomic Workflow for the In-Depth Exploration of Microchloropsis gaditana Lipidome

PRESENTING AUTHOR: ***Marie Valmori, Université Paris-Saclay, CEA, INRAE, Département, France***

CO-AUTHORS: ***Juliette Jouhet, David Touboul, François Fenaille, Benoit Colsch***

SUB THEME: ***Marine metabolomics***

The green microalgae Microchloropsis gaditana is well known for its high triacylglycerol content, making it a promising candidate for 3rd generation of biofuel product. Recent untargeted lipidomics studies have reported other lipid classes within this microalgae, including diacylglyceryltrimethylhomoserine (DGTS), mono- and di-galactosyldiacylglycerols (MG-DGDG), and sulfoquinovosyldiacylglycerols (SQDG). In this study, we propose to conduct in-depth lipidomic analysis of MS/MS spectra and molecular networking.

A total lipid extract from Microchloropsis gaditana was analyzed using reverse-phase liquid chromatography coupled to an Orbitrap Fusion Tribrid mass spectrometer (RPLC-Orbitrap) operated in electrospray ionization and data-dependent HCD MS/MS acquisition modes. Lipid annotation

was achieved with the MS-DIAL software using the Lipidblast spectral library and annotations were further manually validated. For deeper analysis, t-distributed Stochastic Neighbor Embedding (t-SNE) algorithm was employed using the MetGem software.

In a first instance using MS-DIAL, we identified up to 200 unique lipid species distributed in 14 distinct classes. Analysis of both annotated and not annotated features using the t-SNE algorithm facilitated annotation propagation thanks to feature aggregation into clusters. Among them, 25 additional features were putatively annotated through careful examination of their fragmentation patterns (diagnostic ions, neutral losses). New clusters revealed pigments-like chlorophyll a and astaxanthin, as well as redundantly annotated lipid species with different adducts. The lipidome coverage obtained under these conditions is the most extensive recorded to date for Microchloropsis using mass spectrometry-based lipidomics. However and despite those methodological improvements, many features (~520) still remain unannotated as isolated nodes owing to weak spectral similarities or non-informative enough MS/MS spectra (e.g. low signal intensity). As a result, current efforts are focused on implementing more sensitive and complementary MS/MS techniques, such as HCD MSn, CID, and EAD fragmentation workflows, to get the broadest lipidome coverage. The final goal is to create a comprehensive spectral library for Microchloropsis gaditana.

1201 A — Mapping mycosporine-like amino acids in flatfish organs using (un)targeted metabolomics

PRESENTING AUTHOR: ***Fabian Hammerle, University of Innsbruck, Austria***

CO-AUTHORS: ***Romina Schöpf, Anastasiia Fedorova, Johanna Lückenbach, Cornelia Karg, Simone Moser, Johanna Gostner, Ulf Karsten, Markus Ganzera***

SUB THEME: ***Marine metabolomics***

Marine organisms mitigate harmful UV radiation through various strategies, such as accumulating photoprotective compounds with high molar extinction coefficients in the UV range, like mycosporine-like amino acids (MAAs). While well documented in cyanobacteria and algae, their organ-specific distribution in fish and potential physiological benefits remain unclear.

Using advanced chromatographic and mass spectrometric techniques, this study investigates MAA accumulation in the eyes and internal organs of flounder, turbot, and plaice. Ten fish per species were dissected into eight tissue types, freeze-dried, and extracted with methanol. HPLC-UV analysis [1] confirmed that the highest MAA concentrations were in the eyes. UHPLC-HRMS/MS, combined with feature-based molecular networking [2], enabled the identification of additional low-abundance MAAs. Notably, several MAAs were detected for the first time in kidneys and intestines.

To explore the potential physiological roles of these marine natural products, porphyra-334 was tested in a 2D human corneal epithelial cell model under a certified sunlight simulator. It significantly reduced UV-induced ROS formation in a dose-dependent manner without impairing cell viability, supporting its photoprotective function.

Our findings suggest that MAAs may act as natural sunglasses for fish by accumulating in ocular tissues. Additionally, our results indicate broader physiological roles, underscoring the ecological and pharmacological significance of MAAs in marine organisms.

[1] Orfanoudaki M, et al. Chemotaxonomic study of Bostrychia spp. (Ceramiales, Rhodophyta) based on their mycosporine-like amino acid content. Molecules. 2020 Jul 17;25(14):3273.

[2] Zwerger MJ, et al. Application of feature-based molecular networking in the field of algal research with special focus on mycosporine-like amino acids. J Appl Phycol. 2016 Jan 24;35:1377-1392.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1202 A — Bacterial bodyguards: Characterising the virus-repressing effect of Wolbachia in Drosophila melanogaster using NMR-based metabolomics

PRESENTING AUTHOR: *Sarah Walsh, Griffith University, Australia*

CO-AUTHORS: *Tyson Thomson, Karyn Johnson, Jeremy Brownlie, Horst Joachim Schirra*

SUB THEME: *Microbial metabolomics*

Wolbachia, an intracellular bacterial symbiont, exhibits antiviral effects in insects and has been employed to limit the spread of arboviruses. However, the mechanisms underlying this interference are not consistently understood. Our study employs nuclear magnetic resonance (NMR)-based metabolomics to characterise the bi- and tripartite host-Wolbachia-virus interactions using the model insect Drosophila melanogaster, the protective Wolbachia strain wMel and the pathogenic Drosophila C virus (DCV).

The findings reveal that wMel-infected flies showed increased simple carbohydrate catabolism and elevated purine metabolite levels relative to uninfected Drosophila. DCV infection perturbed nucleotide synthesis and nucleotide abundance in Drosophila compared to uninfected Drosophila, driving metabolism to likely meet the viral replication demands imposed on the host. Notably, co-infected Drosophila exhibited a metabolic profile more similar to wMel-infected flies than DCV-infected flies, suggesting wMel generates a metabolic environment where there is competition for host metabolites between wMel and DCV inhibiting viral replication. The study also suggests that wMel competes with the host for oxygen, creating a hypoxic environment that generates reactive oxygen species (ROS). ROS are effector molecules well known to trigger specific immune pathways that have been previously proven to contribute to Wolbachia-mediated antiviral protection.

We therefore propose that Wolbachia-mediated antiviral protection should be viewed as a multimodal response resulting from wMel's influence on host metabolism, rather than a single mechanism. Results suggest that wMel drives metabolism in a direction that at least temporarily inhibits DCV replication and is metabolically similar to single wMel infections; in doing so, it concomitantly triggers immune pathways that contribute towards Wolbachia-mediated pathogen blocking. This perspective may guide future research and contribute to the continued success of Wolbachia-based vector control strategies against RNA arboviruses, potentially leading to novel approaches for defending against such pathogens and improving vector control strategies.

1203 A — Decoupling Meta-Metabolome and Taxonomic Dynamics in Aquatic Biofilms: Implications for Herbicide Sensitivity Under Global Change

PRESENTING AUTHOR: *Nicolas Creusot, INRAE, France*

CO-AUTHORS: *Arthur Medina, Olivier Lepais, Melissa Eon, Zoe Delporte, Jhodi Avizzara, Chloe Bonnineau, Sebastien Boutry, David Carayon, Sylvain Prigent, Remy Cordazzo, Guillaume Marti, Nicolas Mazzella, Aurelie Moreira, Soizic Morin, Erwan Guichoux, Nicolas Creusot*

SUB THEME: *Microbial metabolomics*

In the face of global changes, understanding how environmental microbiomes respond to multiple stressors is crucial for preserving ecosystem functions and services. Regarding chemical contamination in aquatic systems, a key challenge is to determine how fluctuations in microbial community structure and function influence their sensitivity to micropollutants. The combined use of metabolomics and metagenomics is particularly relevant, as it enables the assessment of microbial activity, taxonomic diversity, and gene composition. In this context, this study aimed to (i) analyze the synchronicity between the natural dynamics of microbial activity (meta-metabolome) and structural biodiversity (species and genes) over a one-year cycle, and (ii) assess how these variations affect the sensitivity of the meta-metabolome and photosynthesis to a model herbicide. To achieve this, biofilms were colonized monthly on glass slides in a pilot pond. After quenching and freeze-drying, samples were processed for both omics analyses. In parallel, each month, a portion of the collected biofilm was exposed for 4 hours to serial dilutions of terbuthylazine (TBA) to evaluate community sensitivity to this herbicide. The results revealed an asynchronous and uncorrelated shift between the meta-metabolome and microbial composition, suggesting distinct assembly mechanisms. Principal Component Analysis based on Aitchison distance highlighted significant taxonomic and meta-metabolome discrepancies across months, with temperature exerting a strong influence on microbial composition. DIABLO analysis confirmed this effect, whereas meta-metabolome structuring appeared to be driven by other factors (e.g., NO_3). These fluctuations likely influenced changes in TBA sensitivity, primarily affecting the meta-metabolome. Further investigations are underway to disentangle the role of internal (metabolism) vs external (water properties) factors in this shift, potentially linked to microbial and metabolite co-occurrence under specific climatic conditions. Overall, these findings provide insights into meta-metabolome and taxonomic assembly mechanisms and the functional impairment of microbiomes under chemical stress in the context of global change.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1204 A — Unlocking Actinomycete Metabolic Diversity: A Case Study Towards an Easy and Universal Workflow

PRESENTING AUTHOR: ***Michaela Plechatá, Institute Of Microbiology CAS Prague, Czechia***
CO-AUTHORS: ***Tommaso Stefani, Aninda Mazumdar, Jan Masák, Zdeněk Kameník***
SUB THEME: ***Microbial metabolomics***

Actinomycetes are well known for producing a wide array of specialized metabolites, but there is still a need for more systematic studies on how extraction protocols influence the detection of these compounds. In our study, we examined ten non-pathogenic actinomycete strains—including Streptomyces, Nocardia, Nonomuraea, Mycobacterium, Amycolatopsis, Saccharomonospora, Kribbella, Actinoplanes, and Micromonospora—isolated from diverse habitats such as water sediments, various soil types, Roman catacombs, and the root surface of an eggplant. While Streptomyces was included as a well-known reference, our focus was on less-studied genera to broaden the chemical diversity that we aimed to explore.

To evaluate how extraction methods affect metabolite coverage, we tested several solid-phase extraction protocols, using reverse-phase and mixed-mode ion-exchange sorbents. We obtained four extract types per strain and medium, enabling us to assess how different protocols capture metabolites with varying polarity and acid-base properties. Metabolomic profiling was performed using ultra-high performance liquid chromatography coupled with ion mobility and high-resolution timsTOF mass spectrometer.

Data analysis combined Bruker MetaboScape, GNPS molecular networking, and SIRIUS for compound annotation, with metabolite networks visualized in Cytoscape. Our results revealed considerable chemical diversity across samples, shaped by both the culture medium and the extraction protocol used. The outcome of our study is a recommendation on which culture media and extraction methods are generally preferred to maximize captured chemical diversity of actinomycetes when only a limited number can be used due to capacity constraints.

1205 A — Untargeted Metabolomics in Depressed Humans and Animals via Exposure to Soil Inoculated with Streptomyces rimosus

PRESENTING AUTHOR: ***Sowon Yang, Konkuk University, South Korea***
CO-AUTHORS: ***Jin Hee Kim, Risu Kim, Sin-Ae Park, Myung Sook Oh, Choong Hwan Lee***
SUB THEME: ***Microbial metabolomics***

The acceleration of urbanization and reduced exposure to nature have contributed to an increase in mental health issues such as anxiety and depression. Soil, a vital component of natural environments, derives its characteristic aroma from volatile organic compounds released by soil microorganisms, particularly Actinomycetes. Among Actinomycetes, the genus Streptomyces—the largest group—releases secondary metabolites such as 2-methylisoborneol and geosmin during its metabolic processes, which are the primary contributors to the characteristic earthy smell of soil. To characterize the metabolites released by a soil microorganism, volatilome profiling of Streptomyces rimosus was conducted using HS-SPME-GC-MS, and the metabolic effects of exposure to soil inoculated with S. rimosus were investigated through untargeted metabolomics in (1) patients with depression using GC-TOF-MS, and (2) a mouse model of depression induced by chronic restraint stress (CRS) using GC-TOF-MS and UHPLC-Orbitrap-MS/MS. (1) In patients with depression, increased levels of glycolate, acetate, carbamate, benzoate, succinate, carnitine, N-methyl-L-glutamate, pyroglutamate, threonine, proline, octanoate, nonanoate, caprate, and laurate in serum were observed after exposure. Pathway analysis revealed that metabolic pathways associated with energy metabolism, such as the citric acid cycle, glyoxylate and dicarboxylate metabolism, and fatty acid biosynthesis, were upregulated. These changes showed significant correlations with the phenotypes, suggesting an ameliorative effect on energy metabolism impaired by depression. (2) In the CRS mouse model, exposure led to changes in amino acid metabolic pathways, including alanine, aspartate and glutamate metabolism, and phenylalanine, tyrosine and tryptophan biosynthesis in the hippocampus and plasma. Glutamate and sphingosine in the hippocampus, as well as 5-hydroxytryptophan, methionine, valine, and acetylcarnitine in the plasma, exhibited significant correlations with the phenotypes, indicating improvements in depression-related metabolic alterations. These findings suggest that exposure to the volatilome of S. rimosus has therapeutic potential for alleviating depression and highlights its possible role in identifying biomarkers associated with depression amelioration.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1206 A — Metabolomics Analysis for Enhancing Probiotic Productivity and Unveiling Health Benefits of Probiotics

PRESENTING AUTHOR: *Hyeon Ji Jeon, Kyungpook National University, South Korea*
CO-AUTHORS: *Young Hoon Jung*
SUB THEME: *Microbial metabolomics*

Metabolomics analysis has been conducted to improve the productability of probiotics and reveal the health benefits of probiotics and postbiotics.

When Bifidobacterium animalis IDCC 4301 and Lacticaseibacillus rhamnosus IDCC 3201 were cultured to stationary phase and lyophilized, their viability and lactate production were improved compared to the mid-exponential phase of the bacteria. Intracellular and extracellular metabolites were analyzed by gas chromatography-mass spectrometry (GC-MS), and it was found that trehalose and arabinose, which are produced by the nutrient-depleted stationary phase of bacteria to protect themselves, could act as protective substances under the lyophilization stress.

The intracellular/extracellular metabolome of Lactiplantibacillus plantarum IDCC 3501 was analyzed upon addition of red ginseng dietary fiber (RGDF) compared to fructooligosaccharides (FOS), a commercial prebiotics. It was found by GC-MS that RGDF altered the central carbon metabolism of the bacteria, resulting in a higher level of prebiotic effects (e.g., increased viability and short-chain fatty acids (SCFAs) production, enhanced intestinal adhesion, and inhibition of pathogen adhesion) than utilizing FOS.

Limosilactobacillus reuteri IDCC 3701 could survive under alcohol stress and metabolize alcohol by possessing alcohol dehydrogenase (ADH). The hepatoprotective effect of L. reuteri IDCC 3701-derived postbiotics was confirmed by downregulating TGF-β1 mRNA, a marker of liver injury, in alcohol-treated HepG2 cells. Transcriptomics of HepG2 cells by RNAseq revealed that the postbiotics exerted alcohol detoxification effects by regulating the factor related to gene expression and immune response.

Fecal fermentation of irritable bowel syndrome constipation (IBS-C) with a mixture of eight probiotics increased SCFAs and improved the composition of microbiomes. Specifically, the proportion of E. coli decreased, and the beneficial bacteria Lactobacillus spp. increased. Metabolites involved in intestinal barriers and immune responses increased after fecal fermentation.

Consequently, metabolomics could help a high level of industrial application by revealing the mechanisms of probiotics and postbiotics.

1207 A — Mass spectrometry-based metabolomics reveal cyanobacteria metabolic profiles under environmental and stress conditions

PRESENTING AUTHOR: *Triantafyllos Kaloudis, NCSR Demokritos, Greece*
CO-AUTHORS: *Sevasti-Kiriaki Zervou, Sofia Iliakopoulou, Eleni Aidini, Angelika-Ioanna Gialleli, Theodoros Triantis, Anastasia Hiskia*
SUB THEME: *Microbial metabolomics*

Freshwater cyanobacteria exhibit remarkable biological and chemical diversity, producing a vast array of secondary metabolites—including potent toxins—with over 3,000 compounds reported in the literature to date. These cyano-metabolites may serve diverse ecological functions, exhibit toxicological effects, and hold promise for biotechnological applications. Several of these compounds are also known to be synthesized in response to environmental stressors. We apply an “aquatic metabolomics” approach, using non-targeted analytical workflows that integrate high-resolution mass spectrometry (HRMS) with advanced computational tools. Our workflow typically includes data-dependent acquisition (DDA), data preprocessing in MZmine, feature-based molecular networking (FBMN) and annotations in GNPS2, suspect screening with CyanoMetDB, compound- and class-level annotations with SIRIUS, the CMMC workflow to gain insights into co-occurring microbial communities and FBMNstats to determine significant metabolic changes. We demonstrate the application of these workflows in analysing metabolites from cyanobacterial bloom events, and lake water samples, including investigations into cyanobacterial stress responses and the transformation pathways of cyano-metabolites.

We demonstrate the application of these workflows in the analysis of metabolites from cyanobacterial bloom events and lake water samples, including investigations into cyanobacterial stress responses and transformation pathways of cyano-metabolites. We present three recent case studies illustrating the use of this approach:

A) Analysis of samples from a natural lake revealed the co-occurrence of cyanotoxins with pesticides and pharmaceuticals, suggesting contamination from agricultural runoff and wastewater discharges.

B) A study on Microcystis aeruginosa under UV-B stress identified mycosporine-like amino acids (UV-blockers) as significant metabolites, highlighting their potential as biomarkers and for biotechnological applications in consumer products.

C) Investigations into the environmental fate of cyano-metabolites upon exposure to reactive radicals such as hydroxyl radicals (•OH) revealed multiple hydroxylation and oxidative cleavage events, providing insights into their transformation pathways in natural waters and during advanced oxidation processes used in water treatment.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1209 A — Exploring the Mode of Action of Biofungicides: A Rapid Method Based on Metabolic Fingerprinting

PRESENTING AUTHOR: *Cedric Bertrand, Université De Perpignan, France*
CO-AUTHORS: *Robin Cahuzac, Christian Espinoza, Justine Bertrand-Michel, Floriant Bellvert, Fabien Jourdan, Cedric Bertrand*
SUB THEME: *Microbial metabolomics*

Given the increasing concerns regarding the environmental and health impacts of synthetic pesticides, Inulagreen—a plant-based extract derived from Dittrichia viscosa and developed by AkiNaO—represents a promising biocontrol alternative. However, its mode of action (MOA) remains poorly characterized, a common limitation among emerging biosolutions. A comprehensive understanding of MOA is critical for evaluating off-target effects, resistance development risks, and for optimizing application strategies.

In the absence of genomic data for the target phytopathogenic fungus, a multi-omics and multi-platform metabolomics strategy (MSXM, MetaToul) was employed. Targeted analyses were conducted on lipid classes using GC-MS, LC-MS, and GC-FID, as well as on energy cycle intermediates using IC-MS. Additionally, an untargeted metabolomics approach based on reversed-phase LC-HRMS was implemented to explore alterations in central metabolic pathways.

To infer the MOA of Inulagreen, the metabolic fingerprints of treated fungal samples were compared to those exposed to synthetic fungicides with well-characterized modes of action. Twelve treatment conditions were assessed, including Inulagreen, with a minimum of two reference fungicides per MOA category and five biological replicates per condition.

Hierarchical clustering analyses revealed distinct metabolic similarities between Inulagreen and specific classes of fungicides. Preliminary results indicate that Inulagreen may interfere with ergosterol biosynthesis—a key process in maintaining fungal membrane integrity—and potentially alters membrane permeability. Furthermore, metabolic resemblance to a β-tubulin-targeting fungicide suggests that Inulagreen may exert a multi-site mode of action.

These findings highlight the utility of metabolomics-based fingerprinting as a robust, rapid, and scalable approach to elucidate the MOA of biofungicides in the absence of genomic resources.

1210 A — Metabolomics Profiling of Salmonella Infection in Chicken Gut over a 35-day trial: The Role of Synbiotics

PRESENTING AUTHOR: *Topi Meuronen, Afekta Technologies Ltd., Finland*
CO-AUTHORS: *Soile Turunen, Urvish Trivedi, Victoria Drauch, Claudia Hess, Michael Hess, Kati Hanhineva, Olli Kärkkäinen*
SUB THEME: *Model organisms*

Food-borne infections caused by Salmonella are a major global health concern, with the serovar Infantis becoming increasingly significant. Salmonella (S.) Infantis infections on farms pose the risk to be transmitted to humans leading to gastrointestinal disease.

To address the growing concern of S. Infantis infections, we conducted a study using a synbiotic product (PoultryStar®) containing three probiotic strains and inulin on growing broiler chickens. The goal was to assess the product's potential to improve metabolic outcomes in broiler chickens challenged with S. Infantis. Metabolomics was employed to uncover disease mechanisms and the function of synbiotic product, potentially leading to a decrease of S. Infantis burden in broiler chickens and limiting the risk of human infections.

The trial involved five groups of Ross 308 broiler chickens including positive and negative control groups and three treatment groups which were infected with S. Infantis at the age of 7 days and received synbiotic product with different application routes: a) via water and feed, b) via water only, or c) via feed only. Animals were euthanized and samples from the caecum tissue and digesta were collected weekly until the age of 35 days. Non-targeted LC-MS QTOF metabolomics provided comprehensive metabolic profiling and insights into the effects of the synbiotic product on the host and microbial metabolism in caecum.

Results show that the S. Infantis infection caused metabolic alterations in the host tissue with the peak of changes occurring 21 days after infection. When applicated via water and feed, the synbiotic product was able to prevent some of the metabolic alterations caused by S. Infantis (e.g. increased abundances of amino acids, dipeptides and polyamines). Overall, the present study provides longitudinal insight into the metabolic alterations caused by S. Infantis infection in chickens, and how synbiotic products can diminish these metabolic changes associated with Salmonella infection.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1211 A — ECOLIP DH5: a highly curated lipidome database from Escherichia coli DH5α strain generated by deep lipidomics profiling

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SUB THEME: ***Model organisms***

Lipidome databases comprehensively characterize the lipidome of a biological matrix, facilitating global lipidomic analyses. However, high-quality lipidome characterizations of bacterial model organisms remain scarce, limiting the translation from global lipidomics to a deeper systems-level understanding of membrane lipid biochemistry.

To address this methodological gap, we used the Escherichia coli DH5α, a strain widely used in molecular cloning and genetic engineering. We analyzed highly 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.

We combined software assisted MS/MS annotation with literature-driven in silico prediction of lipid molecular species and comprehensive in silico assessment of coelutions, all followed by MS and MS/MS inspection according to reference criteria [2]. 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 (diagnostic MS/MS ions and neutral losses, retention time behavior, and semiquantitative adduct profile) as previously performed for NIST SRM 1950 human plasma reference material [3].

Our approach culminated in ECOLIP DH5, a highly curated database with >350 lipid molecular species, representing a >50% increase in coverage compared to the E. coli reference lipidome characterization [4]. Our database provides the most curated lipidome characterization of conserved bacterial lipid metabolism up to date including >100 phosphatidylethanolamine, >75 phosphatidylglycerol and >50 cardiolipin molecular species within a 19-minute runtime. ECOLIP DH5 is readily available for the microbial metabolomics community, streamlining lipidomics data processing and curation. It is easily scalable to other E. coli strains and holds applications in multiple fields including lipid biochemistry, microbial genetics, synthetic biology, lipidome engineering and infectious disease research.

References:

[1].https://doi.org/10.3390/ijms241311150
[2].https://doi.org/10.1038/s41467-021-24984-y
[3].https://doi.org/10.1016/j.jlr.2024.100671
[4].https://doi.org/10.1016/j.celrep.2019.04.018

1212 A — Computational metabolomics reveals overlooked chemodiversity of alkaloid scaffolds in the Piper genus

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SUB THEME: ***Natural products***

Plant specialized metabolites play key roles in physiological processes and ecological interactions. Identifying novel metabolites, as well as discovering known compounds in new species, is crucial for answering broader biological questions. The Piper genus (Piperaceae family) is renowned for its special alkaloids biochemistry, with more than 300 alkaloids (“piperamides”) characterized in the past four decades. In a recent study, we investigated the alkaloid diversity of Piper fimbriulatum, a myrmecophytic plant native to Central America, using a metabolomics workflow that combines untargeted LC-MS analysis with a range of state-of-the-art computational tools. Specifically, we leveraged open MS/MS spectral libraries and metabolomics data repositories for metabolite annotation and guided isolation efforts toward structurally-novel compounds. As a result, we identified 22 alkaloids belonging to 5 different classes, and isolated one novel seco-benzylisoquinoline alkaloid carrying a linear quaternary amine moiety which we named fimbriulatumine. Notably, several of these alkaloids had never been reported in Piper plants, despite the extensive phytochemical investigation of the genus. We later supported this discovery by sampling 12 additional Piper species and confirmed the presence of these alkaloids across the genus. Finally, we contextualized our findings within a broader biological framework by systematically mining literature reports of the identified alkaloid scaffolds across angiosperms. Specifically, we searched the Wikidata knowledge base using the SPARQL query language and mapped the results onto the angiosperm tree of life. By doing so, we highlighted the remarkable alkaloid diversity of the Piper genus and provided a framework for generating hypotheses on the biosynthetic evolution of these specialized metabolites. Overall, our findings expand the known Piperaceae alkaloid diversity and demonstrate the value of revisiting well-studied plant families using state-of-the-art computational metabolomics, which can uncover previously overlooked chemodiversity and advance our understanding of plant specialized metabolism.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1213 A — UPLC-QTOF/MS-Based Identification of Phenolic Compounds in Fruit and Leaf of Highbush Blueberry (Vaccinium corymbosum L.)

PRESENTING AUTHOR: *Inwook Choi, Korea Food Research Institute, South Korea*
CO-AUTHORS: *Jun Seung Kim, Guijae Yoo, Sang Yoon Choi, Inwook Choi*
SUB THEME: *Natural products*

Vaccinium corymbosum L. (highbush blueberry) is valued for its health benefits, largely due to its rich phytochemical content. While the fruit is known for antioxidant, anti-aging, and glycemic-regulating effects, the leaves exhibit antioxidant, anti-inflammatory, and vision-protective activities. These functional differences may stem from distinct metabolite profiles in each part. In this study, ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS) was used to analyze and compare metabolites in the fruit and leaf. Characteristic phenolic compounds specific to each part were successfully identified, highlighting their biochemical diversity.

1214 A — Caffeoylglucaric from Inula japonica Leaves and Their Anti-Skin Aging Effects in TNF-α-induced Normal Human Fibroblast Damage

PRESENTING AUTHOR: *So-Ri Son, Kyung Hee University, South Korea*
CO-AUTHORS: *Sullim Lee, Dae Sik Jang*
SUB THEME: *Natural products*

We investigated the bioactive compounds from Inula japonica leaves and their anti-skin-aging effects. LC-MS-based molecular networking (MN) and compound isolation identified caffeoylglucaric acids as major constituents of the 30% ethanolic extract (IJE). Targeted isolation based on MN led to the discovery of three previously undescribed caffeoylglucaric acids (compounds 1, 2, and 4), characterized through comprehensive spectroscopic data analysis.

IJE and its compounds significantly inhibited ROS and MMP-1 production in TNF-α-induced NHDF cells. Notably, compound 4 suppressed ROS, MMP-1, IL-6, and IL-8 levels while enhancing procollagen type I α1 production. Mechanistic studies showed that compound 4 inhibited the phosphorylation of ERK, JNK, NF-κB, and c-Jun, suggesting modulation of inflammatory cytokines and prevention of collagen degradation via MAPK-NF-κB/AP-1 pathways. Additionally, compound 4 downregulated iNOS and COX-2, and upregulated HO-1 expression, indicating its potential in modulating inflammatory responses associated with skin aging.

1215 A — Characterization and Discrimination of Acanthopanax Species Based on UPLC-QTOF-MS Combined Molecular Networking with Multivariate Statistical Analysis

PRESENTING AUTHOR: *Bo-Ram Choi, Rural Development Administration, South Korea*
CO-AUTHORS: *Jin-Kyu Jang, Dahye Yoon, Woo-Cheol Shin, Dae Young Lee*
SUB THEME: *Natural products*

The genus Acanthopanax is widely distributed across Korea, Japan, China, and the far-eastern region of Russia. In Korea, over 18 species of Acanthopanax are known to be present, and they have traditionally been used as medicinal materials in Korean herbal medicine. Various parts of Acanthopanax spp. have been traditionally used for therapeutic purposes. In particular, the fruits of Acanthopanax spp., traditionally used to treat symptoms related to 'wind disorders', indicating paralysis and stroke-like conditions.

However, most studies have focused on the stems, leaves and roots, while research on the fruits remains relatively limited. In this study, we aimed to distinguish the species and maturity stages of Acanthopanax fruits. Using UPLC-QTOF/MS, an analytical method was optimized, allowing comprehensive analysis of Acanthopanax fruits in less than 30 minutes and a total of 39 components derived from Acanthopanax were profiled. This in-house library was employed to identify species and maturity related metabolites through multivariate statistical analysis. In the loading line plot of orthogonal partial least squares discriminant analysis (OPLS-DA), hederagenin-3-glucuronic acid was identified as a metabolite showing significant variation in mature fruits of A. senticosus and A. sessiliflorus. Also, chiisanoside was identified as a marker component for A. sessiliflorus. In addition, molecular networking using the GNPS platform visualized the structural relationships among metabolites observed in Acanthopanax fruits and revealed distinct clusters associated with species and maturity differentiation.

Overall, the results of this study indicate that UPLC-QTOF/MS based metabolomic profiling represents a reliable and effective tool for distinguishing Acanthopanax spp. fruits by species and maturity stage.

Bo-Ram Choi and Jin-Kyu Jang equally contributed to this manuscript.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1216 A — Integrating mass spectrometry based molecular networks and comparative metabolomics for origin tracing of Panax ginseng

PRESENTING AUTHOR: *Woo Cheol Shin, Kyungpook National University, South Korea*
CO-AUTHORS: *Jin-Kyu Jang, Dahye Yoon, Bo-Ram Choi, Dae Young Lee*
SUB THEME: *Natural products*

Panax ginseng C. A. Meyer is widely cultivated in East Asian countries, including Korea, China, and Japan. Owing to its diverse pharmacological effects, ginseng has garnered significant attention as a health-promoting functional food, with metabolites such as ginsenosides known to contribute to these effects. Primary metabolites are directly involved in plant growth, development, and reproduction, whereas secondary metabolites, although not essential for these primary processes, play important ecological roles. In plants, the composition of secondary metabolites can vary depending on environmental factors such as cultivation region and climate. As a result, ginseng with identical genetic backgrounds may exhibit distinct metabolic profiles according to the cultivation environment.

This study aimed to analyze and compare the metabolic profiles of P. ginseng cultivated in South Korea, China, and Japan, with the goal of establishing a comprehensive metabolite database to support future research. To this end, a mass spectrometry-based analytical strategy was employed to capture a broad spectrum of metabolites. Ginsenoside profiles were characterized using UPLC-QTOF/MS under optimized conditions for high-resolution peak separation, and region-specific metabolites were identified through multivariate statistical analyses, including PCA and PLS-DA. Additionally, primary metabolite profiles and contents were analyzed using GC-TOF/MS following derivatization, enabling simultaneous detection of sugars, amino acids, and organic acids.

Furthermore, molecular networking was applied to visualize the chemical diversity and structural relationships among ginsenosides, facilitating the interpretation of metabolic variation across origins. The resulting database of primary and secondary metabolites offers valuable reference data for origin authentication, quality control, and pharmacological research on P. ginseng. This integrative approach provides insight into the influence of environmental factors on metabolic composition and establishes a foundation for future standardization and functional evaluation of ginseng products.

1217 A — Olive mill solid waste induces beneficial mushroom-specialized metabolite diversity revealed by a computational metabolomics strategies.

PRESENTING AUTHOR: *Soliman Khatib, MIGAL-Galilee Research Institute, Tel Hai College, Israel*
CO-AUTHORS: *Idan Pereman, Elizabeth Kostanda, Mitja Zdouc, Nirit Ezov, Ron Schweitzer, Justin J. J. van der Hooft*
SUB THEME: *Natural products*

Mushrooms contain a diverse pallet of specialized metabolites that can be bioactive in both a beneficial and harmful manner. For example, Hericium erinaceus and Pleurotus eryngii are edible and medicinal mushrooms grown commercially around the world. In nature, H. erinaceus grows on old or dead tree trunks, and P. eryngii grows on Apiaceae plant roots, whereas indoors, they grow on substrates mainly consisting of dry wood chips, straw, and cereals. To make their farming more sustainable, supplements such as olive mill solid waste (OMSW) have been added to support mushroom development. However, so far, the impact of substrate additives on the edible mushroom metabolic content has not been assessed. Here, we examined the effect of different proportions of OMSW added to the substrate on the metabolic profiles of the fruiting body (FB) and mycelium of H. erinaceus and P. eryngii mushrooms. We used computational metabolomics strategies including GNPS molecular networking, MS2Query, and FERMO, to organize, annotate, and prioritize metabolite features from the untargeted Q-Exactive Plus HR-LC-MS/MS metabolomics data. Following chromatography-based fractionation, the metabolite annotation of four metabolite features was further validated or fine-tuned using 1H-NMR, i.e., to resolve structural isomers. The computational metabolomics strategies employed showed several annotated metabolite features to be affected by the OSMW. In general, the methanolic extracts of H. erinaceus FB and mycelium were more highly enriched with specialized metabolites than those of P. eryngii. Interestingly, OMSW increased several hericenone analogues in the H. erinaceus FB, to which beneficial properties are assigned, such as anti-inflammatory, anticancer and neuroprotective properties, as well as several erinacerin metabolites from the mycelium. In addition, OMSW decreased the toxic enniatin metabolite abundance. The study highlights the importance of including computational metabolomic strategies to investigate new sustainable growth options for edible mushrooms and other natural foods.

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★ AWARD WINNERS

PLANTS, FOOD, ENVIRONMENT AND MICROBES

1218 A — Unlocking the nutraceutical potential of chestnut shells – Untargeted versus targeted metabolomic approaches combined with in vitro digestion and intestinal permeability model

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CO-AUTHORS: *Julián Lozano-Castellón, Anallely López-Yerena, Ana Margarida Silva, María de la Luz Cádiz-Gurrea, Antonio Segura-Carretero, Rosa M. Lamuela-Raventós, Anna Vallverdú-Queralt, Cristina Delerue-Matos, Francisca Rodrigues*

SUB THEME: *Natural products*

In recent decades, the nutraceutical market has experienced significant growth, with food by-products being explored as sources of antioxidants for potential nutraceutical applications [1]. Chestnut (Castanea sativa) shells (CS), an abundant yet undervalued by-product, are recognized for their health-promoting properties attributed to phytochemicals [1,2]. Nonetheless, the European legislation on nutraceuticals' validation remains vague, reinforcing the need to evaluate the in-vitro and in-vivo bioactivity and metabolic profiling after gastrointestinal digestion and intestinal absorption to guarantee their efficacy and safety.

This study investigates the nutraceutical potential of CS extract prepared by supercritical fluid extraction, focusing on the impact of gastrointestinal digestion and intestinal permeability on the bioaccessibility, bioavailability, and bioactivity of CS extract using targeted and untargeted metabolomic techniques assessed by LC-ESI-LTQ-Orbitrap-MS. The intestinal permeability was evaluated on a Caco-2/HT29-MTX co-culture model. The bioactivity in acetylcholinesterase, α-amylase, and antioxidant enzymes was explored.

The results confirmed a reduction in phenolic concentrations after digestion, with bioaccessibility reaching 30%. The CS extract demonstrated antioxidant/antiradical, hypoglycemic, and neuroprotective effects upon digestion. The metabolomic analyses revealed the biotransformation of complex phenolic acids, flavonoids, and tannins present in the undigested extract (45.8 µg/mg DW of total phenolic concentration) into hydroxybenzoic, phenylpropanoic, and phenylacetic acids after digestion (35.5 µg/mg DW). The untargeted metabolomic profile of permeation samples indicated that 60% of the metabolites identified were lipids and 30% phenolic compounds, unveiling 29 metabolic pathways. Phenolic acids, flavonoids, and coumarins permeated the intestinal barrier, alongside phase I (hydrogenation) and II (methylation) metabolites. The permeation rates increased during 4 h as follows: ellagic acid < o-coumaric acid < p-coumaric acid < ferulaldehyde ≤ hydroxyferulic acid ≤ dihydroferulic acid < ferulic acid < trans-caffeic acid < trans-cinnamic acid < dihydrocaffeic acid. These findings validate CS extract as a novel nutraceutical ingredient with proven efficacy after in vitro digestion and intestinal permeation.

1219 A — A metabolomics exploration of lotus seeds using matrix-assisted laser desorption/ionization mass spectrometry imaging

PRESENTING AUTHOR: *Chunping Tang, Shanghai Institute of Materia Medica, CAS, China*

CO-AUTHORS: *Ying Chen, Jia Liu*

SUB THEME: *Natural products*

Lotus (Nelumbo nucifera Gaertn.) is a quintessential medicinal and edible plant, exhib-iting marked differences in therapeutic effects among its various parts. The lotus seed constitutes a key component of this plant. Notably, the entire seed and the plumula nelumbinis display distinct medicinal properties. To investigate the "homologous plants with different effects" phenomenon in traditional Chinese medicine, this study established a Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imag-ing (MALDI-MSI) method. Conductive double-sided tape was employed for sample preparation, and the method was applied to analyze entire lotus seeds. Complete lon-gitudinal sections of the seeds were obtained, followed by MALDI-MSI analysis to identify and visualize the spatial distribution of metabolites within the seeds. The re-sults unveiled the diversity of metabolites in lotus seeds and their differential distri-bution across tissues, with pronounced distinctions in the plumula nelumbinis. These findings enhance our understanding of the nutritional and medicinal value of lotus seeds, offering novel insights into their therapeutic potential.

1220 A — Computational Metabolomics Approach to Characterize the Metabolome of Three Medicinal Helichrysum Species

PRESENTING AUTHOR: ★ *Motseoa Lephatsi, University Of Johannesburg, South Africa*

CO-AUTHORS: *Fidele Tugizimana, Mpho Choene, Ntakadzeni Madala, Abidemi Kappo*

SUB THEME: *Natural products*

Helichrysum species, with 35% native to South Africa, are widely recognized for their diverse medicinal properties. However, their chemical composition remains largely unexplored, limiting the potential for drug discovery. This study employs a computational metabolomics approach to comprehensively characterize the metabolic landscape of three underexplored species—H. italicum, H. petiolare, and H. splendidum. Metabolites were extracted using hexane, ethyl acetate, and methanol to enhance metabolome coverage and analyzed via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Data processing through molecular networking (MN) strategies provided an in-depth annotation of the metabolomes. Results demonstrate that multi-solvent extraction offers a more complete representation of Helichrysum chemistry, revealing a metabolome rich in phenylpropanoids, lipids, and lipid-like molecules. Comparative analysis identified distinct differences in flavonoid glucoside and triterpenoid profiles among the three species, suggesting species-specific biochemical pathways. These findings contribute to a deeper understanding of Helichrysum phytochemistry, facilitating the discovery of bioactive compounds with pharmaceutical potential. By expanding the knowledge of medicinal plant metabolomes, this research aligns with the United Nations Sustainable Development Goal #3 (Good Health and Well-being), supporting efforts to harness natural products for therapeutic applications.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1221 A — A Paired Omics Data Resource for Plant Natural Product Discovery

PRESENTING AUTHOR: ***Felicia Wolters, Wageningen University And Research, Netherlands***

CO-AUTHORS: ***Klaas Bouwmeester, Justin J. J. van der Hooft, Eric Schranz, Tina Woldu, Marnix H. Medema***

SUB THEME: ***Natural products***

The diversity of plant specialized metabolites provides a promising yet underexplored resource for drug development and agrochemicals. Glycosylation, hydroxylation and methylation of plant natural products determine bioactivity and structural diversity of many compound classes. Yet, annotation of genes encoding modifying enzymes poses a challenge due to rapid evolution and promiscuity of acceptor domains.

Availability of public genomic data has accelerated data-driven approaches for biosynthetic pathway annotation. However, automated inference of biosynthetic pathway modules needs to account for plant genome complexity, including polyploidy and sub-genome dominance.

To overcome this challenge, integration of multiple omics data types has been suggested as a promising approach, as it provides multiple layers of evidence for pathway reconstruction, particularly when datatypes originate from the same source and/or are derived across taxonomically related species.

Here, we present a paired transcriptomics and metabolomics dataset that we generated for ten plant species of the Brassicaceae family – spanning two tissues, four conditions and three time points.

To facilitate public data integration, the experimental design was standardized using curated metadata ontologies, in accordance with FAIR Data guidelines. LCMS/MS data pre-processing pipelines were developed to facilitate modular integration of software, including MZmine, mass spectral networking, and SIRIUS, amongst others.

Preliminary analysis revealed modification patterns of all annotated terpenoids and alkaloids across species, with 53 and 67 molecular families, respectively, containing glycosylations, hydroxylations, and/or methylations. Per species, abundance and intensity of 45 and up to 366 mass features were significantly altered by plant hormone treatments, including jasmonic acid, salicylic acid, and abscisic acid. In total, 187 features responded over a time-course of 3, 8, and 12 hours after treatment.

Obtained insights on biosynthetic pathway genes, metabolite features, biotransformations, and their evolutionary relationships, are expected to accelerate natural product discovery. They will also provide new resources for synthetic biology and targeted plant breeding.

1222 A — Meta-Metabolomic Insights into Environmental Drivers of Periphyton Metabolic Variation Across 75 French Rivers

PRESENTING AUTHOR: ***Pierre Petriacq, University of Bordeaux, France***

CO-AUTHORS: ***Jhodi Avizara, Amelie Malherbe, Melissa Eon, Chloe Bonnineau, Sebastien Boutry, David Carayon, Pierre Petriacq, Guillaume Marty, Sylvain Prigent, Nicolas Creusot***

SUB THEME: ***Non-targeted analysis in biomonitoring and environmental monitoring***

In ecotoxicology, microbial periphyton communities are critical for assessing freshwater ecosystem responses to chemical stressors. However, field-based interpretations remain limited due to insufficient understanding of how environmental conditions shape these communities. This study leverages meta-metabolomics to unravel how spatial heterogeneity in water chemistry, climate, and micropollutant exposure influences periphyton metabolic activity across 75 French rivers with diverse ecological and physicochemical conditions. We measured in situ photosynthetic yield, water parameters (nutrients, pH, temperature, micropollutants), and analyzed untargeted LC-HRMS metabolomics alongside biomass traits. Prelimary chemometrics showed discrepancies in the meta-metabolome fingerprints between the investigated sites. Hence, HCA highlighted 3 main clusters composed of sites from Nouvelle Aquitaine collected in Spring and Summer vs sites from the area collected in Automn vs the sites surrounding Lyon collected in Automn and Winter. Annotation based on Natural Product Classifier did not show marked differences at the pathway level whereas discrepancies were noted at the superclass level. For instance the superclasses of the fatty amides, fatty esters, glycerophospholipids, macrolides, carotenoids, the triterpenoids and the sequiterpenoids showed more marked differences between the sites. Contradictory, superclasses with similar intensity between the site might be constitutive of a core meta-metabolome which contribute to overall homoestasis of the whole community. Further multibloc approaches are ongoing to identify key environmental drivers of metabolome variation. By defining the “normal operating range” of the periphyton metabolome under natural conditions, this work advances biomarker discovery for chemical stress and strengthens frameworks for ecological biomonitoring.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1223 A — Impact of pre-calving feeding strategy on colostrum metabolome: Insights into metabolic status and nitrogen metabolism efficiency in Danish Holstein cows

PRESENTING AUTHOR: ***Paraskevi Tsermoula, University Of Copenhagen, Denmark***
CO-AUTHORS: ***Niels Bastian Kristensen, Bekzod Khakimov***
SUB THEME: ***Non-targeted analysis in biomonitoring and environmental monitoring***

Cow colostrum is the mammary gland secretion collected during the first milking postpartum and provides passive immunity and nutrients to the newborn calves1. Colostrum synthesis and accumulation of lacteal secretion in the mammary gland occur during the last month of pregnancy, while colostrum composition is affected by various factors including the cow's health, diet, parity and breed2,3. Metabolomic analysis of cow colostrum provides a great opportunity to identify potential markers for monitoring the physiological status of cows during the last month of pregnancy.

This study aims to evaluate the effects of four feeding strategies on the metabolic status of periparturient cows. The feedings were as follows: a) grass-clover silage and barley straw, b) magnesium chloride-acidified corn silage supplemented with canola cake, c) ammonium chloride-acidified corn silage supplemented with canola cake, and d) a rotation of one week of grass-diluted, magnesium chloride-acidified corn silage followed by two weeks of ammonium chloride-acidified corn silage. Eighteen-nine cows were randomly allocated to the feedings three weeks before their expected calving date. Colostrum samples were analyzed using proton nuclear magnetic resonance spectroscopy, which allowed the identification of 52 metabolites and quantification of 44.

Calving season influenced the concentration of 14 metabolites, suggesting that photoperiod affects colostrum metabolome. Independent of seasonal variation, acidified corn silage feedings showed decreased levels of tryptophan, acetate, and cytidine in colostrum, while the non-acidified grass-based diet resulted in greater concentration of fucose. Although colostrum synthesis and excretion are mainly regulated physiologically, our findings for the first time suggest that feeding strategies can induce shifts in four colostrum metabolites, reflecting the energy and nitrogen metabolism of cows before parturition.

(1)McGrath et al. (2016). Dairy Science and Technology, 96(2), 133-158.

(2)Mellenberger et al (2009). Journal of Mammary Gland Biology and Neoplasia, 14(3), 261-268.

(3)Westhoff et al. (2024). Journal of Dairy Science, 107(7), 4109-4128.

1224 A — Metabolomics in the barn: a decade of sheep research building the path from biomarker discovery to application

PRESENTING AUTHOR: ***Seyed Ali Goldansaz, Agri-Food and Biosciences Institute, United Kingdom***
CO-AUTHORS: ***Hamza Jawad, Olufemi Osonowo, Ghader Manafiazar, Francis Lively, Steven Morrison, Masoud Shirali***
SUB THEME: ***Other***

Over the past decade, we have collaborated with sheep producers in Canada, New Zealand and United Kingdom to understand key industry challenges and develop a research-driven strategy to improve profitability. Metabolomics has been central and complimentary to conventional approaches, helping us investigate key production, reproduction, and health traits in sheep. Our goals include reducing production costs through early detection of subclinical diseases and identifying efficient animals, while increasing revenue via early pregnancy detection, improve feed efficiency and carcass quality, and reduce methane production and environmental impact of production.

This work began with the creation of the Livestock Metabolome Database (LMDB), a multi-species catalogue of over 1,200 metabolites, which revealed both key references and critical research gaps—particularly the lack of biomarker studies in sheep.

Building on this foundation, we identified and validated serum biomarker panels for feed efficiency (FE), methane production, meat quality, pregnancy, litter size, parasitic infection, and mastitis using NMR and MS-based platforms. In ewes, metabolite signatures predicted early pregnancy and litter size by day 50 of gestation, with AU-ROC values up to 0.93. For FE, candidate biomarkers such as phosphatidylcholines and sphingolipids differentiated high-efficiency lambs with AU-ROCs> 0.75, offering insight into lipid metabolism and energy partitioning. Moreover, studies investigating resilience to parasitic infection identified metabolite panels capable of detecting subclinical Haemonchus contortus infection up to two weeks before faecal egg detection, with AU-ROCs ranging 0.69-0.87.

Looking forward, we aim to validate these biomarkers across commercial flocks globally. We are exploring traits related to environmental impact and the role of the microbiome in host performance, integrating multi-omics with machine learning and artificial intelligence. In an ideal world, we would like to translate biomarkers into diagnostic kits for centralized labs or on-farm use along with relevant Apps, enabling producers to make informed, real-time decisions on breeding, nutrition, and health.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1225 A — Metabolic adaptations to abiotic conditions and plant community descriptors in Arabidopsis thaliana

PRESENTING AUTHOR: *Thomas Dussarrat, Bielefeld University, Germany*
CO-AUTHORS: *Marcus Persicke, Victoria Goedde, Carine Chauveau, Chrystel Gibelin, Léa Frachon, Karsten Niehaus, Fabrice Roux, Caroline Müller*
SUB THEME: *Other*

Plant-plant interactions are a key component of ecosystem dynamics. Yet very little is known at whether plant community descriptors can lead to adaptive molecular changes, particularly on a realistic ecological scale. However, a previous study revealed adaptive loci in Arabidopsis thaliana associated with the diversity and composition of its neighbouring plants. Hence, plant metabolism must also adapt to biotic parameters (i.e. plant community descriptors). We explored the primary and specialized metabolisms of 150 A. thaliana genotypes grown under controlled conditions from seeds collected in 75 French natural sites for which both abiotic environment and plant communities have been previously characterised. We uncovered a set of chemical indices (e.g. richness of flavonoids) and chemical compounds that predicted environmental parameters with high accuracy. Besides, phytochemical diversity was affected by plant α-diversity and plant relative height (used as a competition index) as well as by the presence of specific plant species. The influence of abiotic parameters on the chemical traits predicting biotic parameters was then assessed. These results provide (i) a better understanding of adaptive metabolic changes to abiotic conditions and (ii) pioneering insights into the chemical traits governing plant-plant interactions and how A. thaliana evolved in response to plant community descriptors. Besides, as the full genome of these 150 accessions is available, links between adaptive loci and predictive metabolites will be investigated and therefore represent, for instance, a reservoir of innovative strategies for intercropping practices.

1226 A — Robustness and Reliability of Metabolomics Approaches in Addressing Biological Questions: A Multi-Year Comparative Study Using Wheat's Response to Deoxynivalenol

PRESENTING AUTHOR: *Christoph Bueschl, BOKU University, Austria*
CO-AUTHORS: *Maria Doppler, Bernhard Seidl, Bernhard Kluger, Marc Lemmens, Alexandra Simader, Rainer Schuhmacher*
SUB THEME: *Other*

Untargeted metabolomics is crucial for elucidating complex biochemical mechanisms, like plant stress responses. While within-experiment replication provides statistical certainty, derived values such as log2FoldChanges remain single-point estimates. Independent experimental replication strengthens findings by enabling confidence interval calculation for these estimators and better assessment of technical variation, enhancing result reliability. However, the typical extent of such repeated experiments often remains unexplored.

To investigate this, five repetitions of a complete metabolomics experiment ranging from biological treatment to data interpretation utilizing the same experimental protocol to investigate the response of flowering wheat plants to deoxynivalenol(DON)-induced stress, which is a key-virulence factor of Fusarium graminearum, were realized by Master- and PhD-students within a university lecture between 2015 and 2024.

Despite inherent challenges - resulting in a variable number of detected metabolites across years (ranging from 111 to 215 polyphenolic compounds, with 89 consistently detected in three or more repetitions) - a core biological outcome remained consistent: Each year, a clear differentiation between DON-treated and control groups was achieved, as expected. Furthermore, another even more remarkably consistent trend of increased abundance (average log2FoldChange ≥ 2) for numerous hydroxycinnamic-acids, which are known compounds involved in cell-wall reinforcement, in response to DON treatment was observed. Analysis of the log2FoldChange variability revealed that while metabolites with high average log2FoldChanges were significantly different in most datasets, their specific values varied considerably (e.g., standard deviation of 2.0 for a mean log2FoldChange of 2.2), whereas non-significant metabolites (log2FoldChange approximately 0.0) showed higher consistency across experiments. Notably, no correlation was observed between the mean log2FoldChange and its standard deviation across the datasets.

This multi-year investigation highlights the value of integrating repeated experiments in respect to biological interpretation, confirms the robustness of wheat's biological response to DON stress amidst expected technical variability, while simultaneously underscoring the inherent difficulties in longitudinal metabolomics comparisons.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1227 A — Untargeted HS-SPME-GC-MS-Based Metabolomic Profiling of Volatile Compounds in Different Parts of Allium monanthum

PRESENTING AUTHOR: *Gajin Kim, Kyungsung University, South Korea*
CO-AUTHORS: *Jihyeon Yu, Yoonjeong Kim, Jinseo Lee, Keono Kim, Jeehye Sung, Younghwa Kim*
SUB THEME: *Other*

Allium monanthum, commonly known as Korean wild chive, is a popular vegetable in Korea due to its distinctive aroma and flavor. This study applied headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS) to profile volatile metabolites in the root, bulb, leaf, and whole plant. Eleven different volatile metabolites were reported, including sulfur compounds, aldehydes, alcohols, esters, hydrocarbons, and siloxanes. Interestingly, sulfur compounds and aldehydes were abundant in all parts of the plant. The root contained the most aldehydes (11), while the whole plant had the largest number of sulfur compounds (12). The bulb and leaf also showed distinct profiles, with sulfur-containing volatiles being the major components. Multivariate statistical analyses revealed clear differences in the volatile compound profiles among plant parts. Principal component analysis (PCA) clearly separated the groups along PC1 (49.2%) and PC2 (28.8%). The partial least squares-discriminant analysis results showed a strong group separation (PC1: 37.5%, PC2: 30.9%), with high cross-validation results (R^2 = 0.997, Q^2 = 0.993). The multivariate metabolite analysis highlighted hexanal, hydrogen sulfide, and cycloheptasiloxane as important factors contributing to discriminating differences (VIP > 1). Orthogonal partial least squares-discriminant analysis was conducted for all pairwise comparisons among plant parts. All models showed stable model performance (R^2Y = 0.977–0.997, Q^2 = 0.960–0.995), supported by permutation tests. Hierarchical clustering supported these results, clustering the bulb and whole-plant samples together, and distinguishing them clearly from the root group. The results provide a foundation for utilizing the characteristic aroma and flavor of A. monanthum in the development of food products and functional ingredients.

1228 A — LC-MS-based metabolomics reveals organic versus conventional farming and cultivated variety-dependent alterations in pea seed metabolome

PRESENTING AUTHOR: *Sarvar Kakhkhorov, University of Copenhagen, Denmark*
CO-AUTHORS: *Søren Balling Engelsen, Bekzod Khakimov*
SUB THEME: *Other*

Farming practices and genetic factors are the two predominant determinants of the metabolome in staple crops, ultimately influencing their nutritional value. This study shows the application of LC-MS-based untargeted metabolomics coupled with mass spectral computational tools and molecular networking for annotation, and advanced chemometrics to characterize pea seed metabolome. The study includes ten pea cultivars grown across three geographically distant farms in Denmark, including one site practicing organic farming.

High-resolution mass spectrometry data were obtained from 80% methanolic-water extracts of pea seed flour using data-dependent acquisition in positive electrospray ionization mode. In total, 1230 features were extracted and 121 metabolites were annotated using spectral libraries implemented in Metaboscape software. Computational annotation was performed on SIRIUS platform using CSI:FingerID resulting in 178 hits with a COSMIC confidence score >0.4. ANOVA-simultaneous component analysis (ASCA) assessed the primary sources of metabolome variation. ASCA analysis revealed a predominant location effect, primarily distinguishing the organic farming site from the two conventional farming sites. This effect was most pronounced in metabolites associated with amino acid and isoflavonoid biosynthesis pathways, which were found at higher levels in peas grown under organic farming conditions. Overall, 41 individual metabolites exhibited consistent differences across the three farming locations, independent of the cultivated variety effect. Cultivar-specific differences were mainly pronounced on lipid oxidation products and polyunsaturated fatty acids levels. In total, 106 metabolites were significantly influenced by cultivar type. Lastly, SIRIUS-annotated 1-N-β-glucopyranosyl-2-oxindole-3-acetyl-N-aspartate, a phytohormone of auxin class, was significantly influenced by cultivar type and propagation of its annotation in molecular networking facilitated the putative annotation of three previously non-reported analogues of chloroindole-3-acetyl aspartate in pea.

This study shows farming practice/site - and cultivated variety-specific metabolite variations in pea seed. Computationally enhanced metabolomics has proven to be efficient to uncover previously unknown metabolites shedding a new light in alterations in biosynthetic pathways.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1230 A — Maternal per-fluoroalkyl substances predict brain structural and functional outcomes in children

PRESENTING AUTHOR: ***Alex Dickens, University Of Turku, Finland***

CO-AUTHORS: ***Aaron Barron, Jetro Tuulari, Tuulia Hyötylainen, Susanna Kortesluoma, Harri Merisarri, Elmo Pulli, Eero Silver, Venla Kumpulainen, Anni Copeland, Ekaterina Saukko, John D. Lewis, Linnea Karlsson, Matej Oresic, Hasse Karlsson***

SUB THEME: ***PFAS***

Per- and poly-fluoroalkyl substances (PFAS) are ubiquitous persistent organic pollutants that are implicated in various adverse health outcomes in humans. While PFAS are associated with autism spectrum disorder and behavioural outcomes, it is unclear whether they affect human brain development. The aim of this study which is set within the FinnBrain Birth Cohort, sought to explore the association between multiple maternal PFAS and multimodal brain structural, and functional, parameters in 5-year-old children.

The maternal PFAS were measured using UHPLC high resolution mass spectrometry and quantified with authentic standards. The MRI imaging were carried out in Turku University Hospital and consisted of volumetric, diffusion and bold sequences to get the whole range of MRI parameters.

Maternal PFAS including PFOA branched and linear and PFNA strongly predicted multimodal structural outcomes, dominated by corpus callosal integrity and occipital cortex volume, as well as hypothalamic microstructure, and brain functional connectivity. The associations were not mediated by birth outcomes or maternal physiological factors, and were not influenced by sex.

Collectively, our findings provide strong evidence that prenatal PFAS exposure affects human brain development. Interestingly the strongest area impacted was the corpus callosum which is one of the most lipid rich areas of the CNS and critical to brain development.

1231 A — Systematic characterization of wheat fungal infection with Zymoseptoria tritici: a metabolomics study

PRESENTING AUTHOR: ***Tobias Demetrowitsch, Kiel University, Germany***

CO-AUTHORS: ***Heike Seybold, Karin Schwarz, Eva Stukenbrock***

SUB THEME: ***Plant defense, pathogens and interactions***

Introduction:

Yield losses due to fungal diseases pose a major threat to global food production. One of the most devastating pathogens in wheat is Zymoseptoria tritici. Despite the importance of this fungus, the underlying mechanisms of the interactions between plant and pathogen have hardly been investigated. In our previous study, we show that the fungus (isolate IPO 323) suppresses the production of immune-related metabolites in one susceptible cultivar (doi: 10.1038/s41467-020-15633-x).

Aim:

The aim of recent study was to test whether the immune system reacts in the same way to an infection with Z. tririci in other susceptible and resistant cultivars as it does in the cultivars Chinese Spring and Obelisk. Further, we wanted to test the hypothesis that susceptible varieties produce flavonoids significantly fewer.

Method:

The wheat cultivars Ariana, Riband, Obelisk and Chinese Spring were treated with the isolated fungus. Therefore, plants were brushed with a fungus solution, controls only with water. Samples were taken at 1, 3-, 7-, 10- and 14-day(s) post infection. Samples were frozen and extracted with the SIMPLEX protocol. Extracts were analyzed by a non-targeted metabolomics approach utilizing a DI-FT-ICR-mass spectrometer. For the statical evaluation, linear mixed models were used in R.

Results:

The results show that the fungus also blocks immune metabolite synthesis in other susceptible varieties. We were able to show that, for example, the benzoxazionoids, hydroxycinnamic acids, flavonoids and other secondary plant compounds were measurable significantly later on the days post infection in the susceptible cultivars.

Conclusion:

We were able to show different infection mechanism, which Z. tritici uses to infect different susceptible wheat cultivars. Moreover, we could observe dramatically concentration changes between the different cultivars by this fungus infection.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1232 A — Biotransformation of HT-2 and T-2 toxins using plant apparatus and isolation of the resulting glucosides

PRESENTING AUTHOR: ***Adam Behner, UCT Prague, Czech Republic***
CO-AUTHORS: ***Martina Travnickova, Zbynek Dzuman, Jana Chrpova, Milena Stranska***
SUB THEME: ***Plant defense, pathogens and interactions***

Mycotoxins are contaminants found in a wide range of foods and feeds that can pose a serious health risk to humans and livestock due to their toxic effects. The mycotoxins HT-2 toxin (HT2) and T-2 toxin (T2), produced by Fusarium micromycetes, are found in food and feed in both free and conjugated forms with carbohydrates. These conjugated forms pose a potential 'hidden' health risk due to the hydrolysis and subsequent release of free mycotoxins within the mammalian gastrointestinal tract. However, the inaccessibility of analytical standards for conjugated forms of HT2 and T2 prevents routine analysis, which can lead to an underestimation of dietary intake. This study exploits the natural ability of plants (Triticum aestivum) to biotransform free mycotoxins (HT2 and T2) into their conjugated forms through their detoxification mechanisms after direct inoculation by these toxins in the flowering ears of spikelets.s. The potential of this plant apparatus for use for the routine production of HT2/T2-glycosides was investigated. The Kabot and Pexeso cultivars were used for direct inoculation with HT2/T2 solutions. In addition to the most abundant metabolite, HT2-3-glucoside (HT2-3-Glc), the presence of HT2 oligoglucosides, specifically HT2-diglucoside (HT2-diGlc), was also observed. HT2-diGlc was preparatively isolated and its structure was characterised in more detail using specific enzyme hydrolytic reactions in conjunction with UHPLC-HRMS/MS analysis of reaction products. Furthermore, other biotransformation products, including HT2-3-malonyl-glucoside, 3-acetyl-T2, feruloyl-T2, and HT2-3-diglucoside were also tentatively identified using UHPLC-HRMS/MS. These results represent the first step toward obtaining pure HT2 glucoside fractions that could be used as analytical standards for routine analyses and toxicological assessment.

1233 A — Rhizosphere metabolite profiling and metabolic analysis of the plant-protective effects of beneficial rhizobacteria on wheat plants responding to a fungal pathogen: Prospects for Sustainable Agriculture

PRESENTING AUTHOR: ***Manamele Dannies Mashabela, University Of Johannesburg, South Africa***
CO-AUTHORS: ***Msizi Mhlongo, Fidele Tugizimana, Lizelle Piater, Ian Dubery***
SUB THEME: ***Plant defense, pathogens and interactions***

The rhizosphere is a highly complex environment crucial for plant-microbe interactions, with plant root exudates and microbial metabolites playing key roles in these interactions. However, the biochemical makeup of the rhizosphere and the effects of plant growth-promoting rhizobacteria (PGPR) on plant metabolism remain poorly understood. Recent studies using untargeted metabolomics have begun to elucidate these dynamics, revealing significant metabolic changes in both the rhizosphere and above-ground tissues of bio-primed plants. For example, PGPR-treated wheat showed a differential accumulation of metabolites like phenylpropanoids, organic acids, lipids, and benzenoids, which are linked to plant-microbe communication and stress response. Furthermore, PGPR bio-priming induced systemic metabolic reprogramming in leaves, enhancing the plant's ability to adapt to biotic and abiotic stresses and contributing to sustainable agriculture practices. In the case of Puccinia striiformis f. sp. tritici (Pst), a devastating wheat pathogen, metabolomic profiling has revealed how wheat cultivars respond differently to infection. Resistant varieties like Koonap showed increased levels of phenolic compounds such as rutin and luteolin after infection, while susceptible cultivars like Gariep exhibited downregulation of these compounds, highlighting potential biomarkers for resistance. The metabolomics approach provided insight into the complex, non-linear metabolic reprogramming in response to pathogen attack, offering a better understanding of plant defence mechanisms. Overall, the application of advanced metabolomics tools, such as UHPLC-HDMS and LC-MS, has revealed the intricate metabolic changes in both PGPR-treated and Pst-infected wheat. These findings underscore the potential for metabolome engineering to improve crop resilience, contribute to the achievement of UN-SDGs, and advance sustainable agricultural practices.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1234 A — Bioguided Molecular Networking for the discovery of antimycobacterial compounds from French Guiana flora

PRESENTING AUTHOR: ***Célia Breaud, Aix-Marseille University, IMBE, France***

CO-AUTHORS: ***Clémentine Saunier, Stéphane Canaan, Sok-Siya Bun-Llopet, Elnur Garayev***

SUB THEME: ***Plant metabolomics***

Tuberculosis, caused by the bacterial organism Mycobacterium tuberculosis, continues to be a significant public health threat despite the availability of treatments and vaccines. In French Guiana, the high incidence of tuberculosis and the emergence of drug-resistant strains highlight the urgent need for alternative therapeutic options. The rich biodiversity of the region has been recognized as a potential source for novel therapeutic agents.

This study investigated the chemical composition of seven plant species from French Guiana using UHPLC-HRMS/MS to identify novel metabolites with potential anti-tuberculosis properties. The studied species are Curatella americana, Davilla nitida, Dipteryx punctata, Indigofera suffruticosa, Quassia amara, Tetradenia riparia and Zingiber zerumbet.

Concurrently, bioassays against Mycobacterium tuberculosis H37Ra strain were conducted. Preliminary findings, supported by existing literature, indicated promising bioactivity in the selected species (Pavan et al., 2009).

A combination of advanced metabolomics tools, including UHPLC-HRMS/MS, NMR, and MS-based Molecular Network analysis was employed, as it enables structural dereplication and facilitates the analysis of large datasets. Using MS/MS spectral databases, and by integrating the antimycobacterial screening results on the Molecular Network, clusters containing metabolites with potential activity against tuberculosis were identified. The combined use of computational metabolomics with bioassay-guided purification allowed the identification of previously unreported bioactive compounds.

Exploration of the annotated molecular network revealed unexpected antituberculosis activity of a chlorophyll derivative, pheophorbide A (MIC₅₀=10.6 µM). Moreover, Tetradenia riparia was selected for in-depth analysis, based on bioassay results and the presence of unidentified clusters. Bioassay-guided fractionation led to the isolation of several unknown metabolites, which are currently being identified through NMR and MS/MS data.

References

Pavan, F. R., Sato, D. N., Higuchi, C. T., Santos, A. C. B., Vilegas, W., Leite, C. Q. E. (2009). In vitro anti-Mycobacterium tuberculosis activity of some Brazilian « Cerrado » plants. Revista Brasileira de Farmacognosia, 19, 204-206.

1235 A — Decoding seasonal shifts in the plant metabolome in urban Atlantic Forest remnants in São Paulo, Brazil

PRESENTING AUTHOR: ***Fernanda Anselmo-Moreira, Environmental Research Institute (IPA-SP), Brazil***

CO-AUTHORS: ***Bruno Ruiz Brandão da Costa, Jarmo-Charles J. Kalinski, Eduardo Luís Martins Catharino, Agnès Borbon, Adalgiza Fornaro, Silvia Ribeiro de Souza, Daniel Petras, Cláudia Maria Furlan***

SUB THEME: ***Plant metabolomics***

Anthropogenic activities are reshaping natural ecosystems. Urban forests are exposed to intense abiotic stresses, such as drought and air pollution, which can trigger oxidative stress and drive to significant metabolic adjustments. However, the seasonal metabolomic responses of tropical species in polluted regions like the Metropolitan Area of São Paulo (MASP) remain poorly understood. The MASP includes Atlantic Forest remnants facing different pollution levels. For example, the Instituto de Biociências Forest Reserve (Matão-IAG) experiences higher pollution exposure, while the Morro Grande Forest Reserve (RMG) is relatively less impacted. This study evaluated the seasonal metabolomic responses of four Atlantic Forest species – Alchornea sidifolia, Casearia sylvestris, Guarea macrophylla, and Machaerium nyctitans – naturally occurring in these two urban forests. Leaf extracts from six biological replicates per species were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS, positive mode), followed by Feature-Based Molecular Networking (FBMN) in the Global Natural Products Social Molecular Networking (GNPS) platform, yielding 1,026 annotated features. Principal Coordinate Analysis did not reveal significant clustering by area (pseudo-F = 0.910, p = 0.391) or season (pseudo-F = 1.691, p = 0.163). Consistently, t-test analysis did not identify any significant features when comparing the two study areas, despite their contrasting pollution levels. However, 32 features showed significant seasonal differences: three features, identified as polypropylene glycols (PPGs), were more abundant in the rainy season, while the remaining significant features, primarily fatty acids and their derivatives, were more abundant in the dry season. We annotated compounds in 12 chemical families with statistically significant seasonal differences (Mann-Whitney test), including (poly)amines, fatty amides, PPGs, and triterpenoids (rainy season), as well as carotenoids, pheophytins, saccharides, glycerolipids, octadecanoids, and vitamins (dry season). These findings indicate that, despite varying pollution levels, seasonality is the primary driver of chemical variation in these urban forest plants. (FAPESP grant numbers: 2020/07141-2, 2022/07326-8, 2022/13213-1).

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1236 A — Application of untargeted metabolomics for the safety assessment of genetically modified maize (Zea mays L.)

PRESENTING AUTHOR: *Bo Kyeong Kim, Incheon National University, South Korea*

CO-AUTHORS: *Young Jin Park, Hyoun-Min Park, Sung-Dug Oh, Jae Kwang Kim*

SUB THEME: *Plant metabolomics*

Evaluation of unintended metabolic changes resulting from gene insertion is an integral part of the safety assessment of genetically modified (GM) crops. Untargeted metabolomics could serve as a valuable tool in the safety assessment of GM crops due to its capacity to detect broad metabolic changes. Two hybrid lines of GM maize, GM4 and GM6, were generated by introducing herbicide resistance gene into the Hi IIA genotype, followed by hybridization with the B73 genotype to enhance yield stability. This study aims to assess the applicability of untargeted metabolomics for GM crop safety evaluation by comparing the metabolic profiles of the GM lines (GM4 and GM6) with those of their parental lines (Hi IIA and B73) and four commercial cultivars (CM group).

Metabolite profiling was performed using UPLC-qTOFMS, detecting 18,387 peaks in positive ion mode and 12,978 peaks in negative ion mode. Among these, 134 metabolites were identified by matching with NIST20 Tandem Library. The identified metabolites included 39 fatty acids, 27 lipids, 15 amino acids, 9 organoheterocyclic compounds, 8 organic acids, 8 amines, 7 phenylpropanoids, 6 sugars, and 15 others. Principal component analysis revealed that the metabolite compositions of the GM lines were similar to those of their parental lines but distinctly different from those of the CM group. Orthogonal partial least squares discriminant analysis also revealed clear separation between the GM lines and their parental lines, and the CM group. The reliability of the model was confirmed through internal validation, permutation testing, and cross-validation ANOVA. Among the identified metabolites, fatty acids and phenylpropanoids contributed most significantly to the discrimination between the GM lines and their parental lines, and the CM group.

In conclusion, this study suggests that untargeted metabolomics could be considered a reliable and comprehensive approach for detecting potential unintended effects, such as metabolic changes, in GM crops.

1237 A — Towards plant pan-metabolome; metabolic diversity of 3,000 plant species

PRESENTING AUTHOR: *Markéta Macho, Max Planck Institute Of Molecular Plant Physiology, Czechia*

CO-AUTHORS: *Regina Wendenburg, Aleksandra Ivanova, Stefka Bogdanova, Mihail Angelov, Pietro Niccolini, Helena Romero, Riccardo Marconi, Ludvík Bortl, Petr Hanzelka, Klára Lorencová, Romana Rybková, Eva Smržová, Tomáš Procházka, Jana Navrátilová, Eduardo de la Peña Alonso, Lubomír Adamec, Vlastimil Rybka, Tsanko Gechev, Alisdair Fernie, Saleh Alseekh*

SUB THEME: *Plant metabolomics*

Plants produce a wide range of diverse metabolites that play many different roles in plant growth, development, and both biotic and abiotic stress. Understanding the variability of plant metabolome and the functionality of its components is an important basis for the study of plant compound biosynthesis, stress responses, immunity, defence, nutritional and medicinal properties, etc. This knowledge can be used for breeding crops with sustainable yield in stressful conditions, finding novel plant-based medicinal compounds, assessing the impact of climate change on biodiversity and ecosystems, or help protect endangered species.

To advance our understanding of plant metabolic diversity, our aim was to collect, extract, and analyse secondary metabolites from a wide range of species across the plant kingdom. In collaboration with several European botanical gardens, we collected over 4, 000 samples of primarily leaf tissue from over 3, 000 plant species representing 11 major classes of the phylum Embryophyta, including over 200 families in 60 orders. The sample pool is comprised of mosses, ferns, conifers, monocots, eudicots, etc., and includes, among others, rare or endemite species as well as species used by humans for food and medicinal properties.

Samples were harvested and immediately frozen in liquid nitrogen, extracted using an optimized methanol–chloroform protocol, and subsequently subjected to large-scale targeted and untargeted UPLC-MS/MS metabolic profiling. This study is in its scope one of the largest performed to date, and will bring high quality spectral data of a wide variety of plant species. The obtained data will not only compile the existing information into one dataset, but also offer broader overview of phytochemical diversity in a diverse array of plants. This will allow us to study the phylogenetic patterns of specialized metabolite biosynthesis, the relationship of ecology and function, identify unique compounds with potential biological activities, and help in biodiversity preservation.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1238 A — Metabolomic profiling of seed exudates from Glycine max treated at different temperatures

PRESENTING AUTHOR: *Cheol Woo Min, Pusan National University, South Korea*

CO-AUTHORS: *Na Won Park, Ji Soo Kim, Ravi Gupta, Jae Kwang Kim, Woo Duck Seo, Sun Tae Kim*

SUB THEME: *Plant metabolomics*

Soaking of soybean (Glycine max) seeds is a prerequisite for the production of soy-based foods, and the extent of water absorption under different imbibition conditions has been shown to directly influence the quality of the final products. During seed imbibition, exudates containing a diverse array of metabolites associated with germination, dormancy, antimicrobial activity, and defense responses are released. In this study, to investigate the temperature-dependent release of soybean seed exudates (SSEs), we conducted a metabolomic analysis of exudates obtained from soybean seeds soaked at 25°C and 55°C. The most pronounced metabolic changes were observed in the SSE collected at 55°C, which revealed accumulation of essential and non-essential amino acids, fatty acids, lipophilic compounds, and various isoflavones. Interestingly, SSE obtained at 25°C showed significantly higher levels of malonylated isoflavones, particularly malonyl-daidzin and malonyl-genistin, with 1.8-fold and 1.3-fold increases, respectively, as compared to SSE at 55°C. Besides, SSE extracted at 25°C exhibited a synergistic effect on bone morphogenetic protein-2 (BMP-2)-mediated osteoblastic differentiation. Taken together, our findings suggest that SSEs derived from both 25°C and 55°C soaking conditions possess distinct and potentially beneficial properties, highlighting their value for pharmaceutical and industrial applications.

1239 A — Multi-platformed widely-targeted metabolomics unveiled that indole-glucosinolate degradation metabolism contributes to cotyledon size regulation

PRESENTING AUTHOR: *Hiromitsu Tabeta, RIKEN, Japan*

CO-AUTHORS: *June-Sik Kim, Hiroyuki Koga, Atsuko Hirota, Yushiro Fuji, Tetsuya Mori, Muneo Sato, Makoto Hayashi, Keichi Mochida, Hirokazu Tsukaya, Ali Ferjani, Masami Hirai*

SUB THEME: *Plant metabolomics*

Reduced cell number in Arabidopsis leaves often enhances cell expansion, which is known as Compensation. This phenomenon suggests that a leaf can perceive its own size, and that decreased cell number as “input” is somehow translated into compensated cell enlargement (CCE) as “output”. This suggests the existence of a molecular network regulating leaf size, and indicates that understanding the molecular mechanism underlying compensation is fundamental towards unveiling the relationship between cell number and size in an organ-wide context. However, the detailed systems have not been uncovered.

Recently, we discovered that CCE in fugu5, a loss-of-function mutant of vacuolar H+-pyrophosphatase, is triggered by a transient excess of indole-3-acetic acid (IAA). Importantly, the IAA level was significantly increased in fugu5 via metabolic regulation at 8 days after sowing (DAS). Provided that the reduced cell number in fugu5 cotyledons has been ascribed to gluconeogenesis disorder during seed storage lipid mobilization (upon imbibition ~ 4 DAS), this relatively early metabolic alteration is likely to affect later IAA homeostasis at 8 DAS and accomplish compensation. Here, multi-platform widely-targeted metabolomics combined with bioinformatics has been adopted to test the above hypothesis.

Integrated metabolite data were analyzed through correlation networks and 3D module clustering to identify bioactive compounds associated with increased IAA levels. Our analysis indicates that indole-glucosinolate (iGSL) content was significantly altered at 8 DAS. RNA-seq and reverse genetics focusing on iGSL metabolism indicated that this process would be driven by the degradation of iGSL by the beta-glucosidases PYK10 and TGG2. Loss of these enzymes totally cancelled the CCE observed in fugu5 with high IAA accumulation, demonstrating that its cotyledon size can be regulated by iGSL degradation metabolism. Furthermore, single-nuclear RNA-seq revealed that auxin biosynthesis in epidermal cells drives cell enlargement in mesophyll through trans-tissue metabolic communication.

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★ AWARD WINNERS

PLANTS, FOOD, ENVIRONMENT AND MICROBES

1240 A — Independent Domestication of Cucumber (Cucumis sativus L.) Fruit Revealed by Cucurbitacin and Volatile Compound Profiling

PRESENTING AUTHOR: ***Eun Jin Lee, Seoul National University, South Korea***
CO-AUTHORS: ***Kyeonglim Min***
SUB THEME: ***Plant metabolomics***

Cucumber (Cucumis sativus L.) fruit possesses characteristic taste and aroma, which are crucial organoleptic traits influencing consumer preference and marketability. To enhance fruit quality and develop preferred cultivars, we profiled cucurbitacins responsible for bitterness and volatile compounds contributing to aroma using 69 cucumber genotypes with diverse fruit shapes, colors, and origins. Among six types of cucurbitacins (CuA–E, I), only CuC was detected in six inbred lines, while most genotypes lacked detectable levels, indicating that bitterness has been largely lost during domestication. Based on the profiles of cucurbitacins and volatile compounds, the 69 genotypes were classified into four distinct clusters. Clusters 2 and 3 exhibited the highest and lowest volatile contents, respectively, while Clusters 1 and 4 were distinguished by minor volatiles rather than major ones, suggesting unique background flavors. Additionally, Clusters 1 and 4 displayed differences in fruit phenotypes such as length and color, implying a relationship between fruit morphology and background flavor. However, clustering did not correspond with cultivar types or geographic origins, suggesting independent domestication processes for bitterness and aroma in different regions. Furthermore, analysis of 15 F1 hybrids derived from selected inbred lines revealed a reduction in total volatile compound (TVC) content, primarily due to a decrease in total alcohols, leading to an increased aldehyde-to-TVC ratio. These findings provide valuable insights into cucumber organoleptic quality improvement and offer useful information for selecting breeding materials to develop preferred fruit. This work is supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET; 821009-03-1-HD020, RS-2024-00398300) and National Research Foundation (RS-2023-NR076334), Republic of Korea.

1241 A — Application of Plant Metabolomics and Tissue Culture Technology to Investigate the Effect of Different Cultivation Condition on the Accumulation of Secondary Metabolite in Clinacanthus nutans

PRESENTING AUTHOR: ***Yun-Han Wang, Taipei Medical University, Taiwan***
CO-AUTHORS: ***Ching-Kuo Lee***
SUB THEME: ***Plant metabolomics***

Clinacanthus nutans (C. nutans) from the Acanthaceae family, The traditionally employed in the treatment of herpes simplex virus (HSV) infections, cancer, and various dermatological conditions. The previous study has demonstrated its anti-inflammatory, antipyretic, and anti-edematous activities, with clinical evidence further supporting its therapeutic potential against HSV and varicella-zoster virus (VZV) infections.

This study is to apply plant metabolomics and tissue culture techniques to investigate the effects of different cultivation durations on the accumulation of secondary metabolites in C. nutans, with a comprehensive analysis of its bioactive constituents and quality stability.

The results indicated Leaf explants cultured for 1, 2, and 4 weeks successfully developed into callus, Subsequent UPLC-MS/MS analysis coupled with multivariate statistical techniques identified 16 significantly altered metabolites. Several compounds were found to possess notable antioxidant, antiviral, and anticancer activities. According to the findings, this study proposes an efficient and comprehensive in vitro propagation approach for the effective acquisition of bioactive chemical constituents from Clinacanthus nutans.

1242 A — Untargeted Metabolomics Reveals Antioxidant and Anticancer Properties of Leaves and Rhizomes from Three Kaempferia Species via Network Pharmacology and Multivariate Analysis

PRESENTING AUTHOR: ***★ Nurhanis Hasmani, Universiti Sains Malaysia, Malaysia***
CO-AUTHORS: ***Lim Vuanghao***
SUB THEME: ***Plant metabolomics***

Kaempferia galanga, Kaempferia pandurata, and Kaempferia parviflora, belonging to the Zingiberaceae family, have historical significance in Asian medicine. Their bioactive metabolites exhibit potential anticancer and antioxidant properties, making them promising candidates for breast cancer research. This study integrates metabolomics and network pharmacology to elucidate key metabolites and molecular pathways underlying their therapeutic effects. Metabolomic profiling of polar and non-polar extracts was performed using UHPLC-QTOF-MS, revealing distinct phytochemical compositions between leaves and rhizomes. Leaves exhibited higher total phenolic content (22.26–51.96 mg GAE/g), while rhizomes had greater flavonoid content (10.15–32.51 mg QE/g). Antioxidant activity was assessed using DPPH, ABTS, and FRAP assays, with significant differences across species. Cytotoxicity evaluation on MCF-7 breast adenocarcinoma cells identified leaf extract of Kaempferia galanga as the most potent, with an IC_{50} of 62.50 µg/mL. Multivariate statistical analysis, particularly OPLS-DA ($R^2Y > 0.987$, $Q^2 > 0.983$, $p < 0.05$), identified 814 metabolites significantly associated with anticancer activity ($p(corr) \geq |0.5|$, $VIP > 1$). Key metabolites were mapped to metabolic pathways using MetaboAnalyst and KEGG, highlighting perturbations in flavone and flavonol biosynthesis; phenylpropanoid biosynthesis; and tropane, piperidine, and pyridine alkaloid biosynthesis pathways, all crucial in cancer progression. Network pharmacology analysis using Cytoscape further identified core target genes involved in breast cancer pathways, including CCNB1, CDK1, EGFR, PIK3CA, PIK3R1, STAT3, and SRC. This study underscores the power of integrating metabolomics, network pharmacology, and multivariate data analysis in identifying bioactive compounds and their mechanisms in cancer research. The findings provide a molecular basis for Kaempferia species as potential therapeutic agents and highlight the importance of metabolic pathway alterations in breast cancer treatment.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1243 A — Hordatines and Associated Precursors Dominate Metabolite Profiles of Barley (Hordeum vulgare L.) Seedlings: A Metabolomics Study of Five Cultivars

PRESENTING AUTHOR: ***Claude Yasmine Hamany Djande, University Of Johannesburg, South Africa***

CO-AUTHORS: ***Ian Dubery***

SUB THEME: ***Plant metabolomics***

In the process of enhancing crop potential, metabolomics offers a unique opportunity to biochemically describe plant metabolism and to elucidate metabolite profiles that govern specific phenotypic characteristics. In this study we report an untargeted metabolomic profiling of shoots and roots of barley seedlings performed to reveal the chemical makeup therein at an early growth stage. The study was conducted on five cultivars of barley: 'Overture', 'Cristalia', 'Deveron', 'LE7' and 'Genie'. Seedlings were grown for 16 days post germination under identical controlled conditions, and methanolic extracts were analysed on an ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC–HRMS) system. In addition, an unsupervised pattern identification technique, principal component analysis (PCA), was performed to process the generated multidimensional data. Following annotation of specific metabolites, several classes were revealed, among which phenolic acids represented the largest group in extracts from both shoot and root tissues. Interestingly, hordatines, barley-specific metabolites, were not found in the root tissue. In addition, metabolomic profiling revealed metabolites potentially associated with the plants' natural protection system against potential pathogens. The study sheds light on the chemical composition of barley at a young developmental stage and the information gathered could be useful in plant research and biomarker-based breeding programs.

1245 A — Seasonal Changes in Pollen Composition: A ^{1}H-NMR Study of Key Compounds in Bee Pollen

PRESENTING AUTHOR: ***Sofia Garcia, University Of Life Sciences Prague (czu), Czechia***

CO-AUTHORS: ***Saetbyeol Lee, Kateřina Kubiková, Jaroslav Havlik***

SUB THEME: ***Plant metabolomics***

Pollen is an important indicator of ecological diversity and bee colony health, reflecting plant species visited and environmental conditions. While many studies have explored seasonal variations in pollen composition, the metabolic variability throughout the flowering season, assessed using 1H NMR spectroscopy, remains insufficiently explored.

To address this gap, we collected bee pollen (bee bread from hind leg) from two hives in an apiary between March and September 2024, total 10 time points, categorized into 3 seasons: spring, summer and autumn. A 50 mg portion of each pollen was extracted with methanol-d_4 and deuterium oxide, then analyzed using a 1H NMR Bruker Avance III spectrometer (500.18 MHz) at 298 K with the 'noesypr1d' pulse sequence. Phase and baseline corrections were applied using TopSpin 3.6.5, and a total of 27 compounds were identified and quantified using Chenomx NMR Suite 9.0.

We compared the abundance of the compound across the seasons, applying the Kruskal–Wallis test with Dunn's procedure and Benjamini–Hochberg correction, we found that proline was the most abundant compound, showing an increasing trend from spring to autumn, with higher concentrations in autumn (q-values < 0.05). Among the 15 amino acids detected, 10 exhibited a significant decrease from spring to autumn (q-values < 0.05), while the remaining 5 showed an increase (q-values < 0.05). S-Methyl-L-cysteine sulfoxide, a plant secondary metabolite mainly found in rapeseed flowers, was significantly higher in spring compared to summer and autumn (q-values < 0.05). Among the sugars, fructose and glucose were higher in spring compared to summer (q-values < 0.05).

These results suggest that pollen metabolites fluctuate with the flowering season, with variations in amino acids and sugars reflecting changes in nutrient availability, emphasizing the significance of seasonal dynamics for bee colony health.

This research is supported by the Ministry of Agriculture of the Czech Republic (QK21010088).

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1247 A — Untangling metabolite structures and biosynthetic pathways in Populus nigra

PRESENTING AUTHOR: *Emma Bonné, VIB-UGent, Belgium*

CO-AUTHORS: *Héloïse Bastiaanse, Celien Van de Velde, Meng Peng, Anran Liu, Steven Vandersyppe, Veronique Jorge, Catherine Bastien, Geert Goeminne, Kris Morreel, Ruben Vanholme, Wout Boerjan*

SUB THEME: *Plant metabolomics*

Populus species are fast-growing trees with wide-ranging potential applications in both pharmaceutical and bio-refinery industries, and serve as established model organisms in tree research. Despite substantial knowledge, a more comprehensive understanding across multiple biological levels is essential to fully exploit their potential. In this study, we integrate genomics and metabolomics to investigate metabolic diversity and biosynthetic pathways in a population of 780 Populus nigra genotypes collected across 23 European river systems.

Leaf metabolomes were profiled using untargeted UPLC-QToF-MS analysis, followed by data processing with Waters Progenesis software and evaluation of several normalization strategies. To reduce data complexity and enrich for parent ion m/z features, we optimized a RAMClustR workflow for peak grouping, clustering related adducts, fragments, and artefacts into metabolite-level features.

From the refined dataset, we constructed a candidate substrate–product pair (CSPP) network based on characteristic mass and retention time differences associated with known biochemical conversions (e.g., hydroxylation, methylation, hexosylation). This network facilitates the visualization of putative biosynthetic pathways and supports compound annotation through integration with spectral library matching. GWAS was performed using metabolite intensities as traits. The resulting associations guide the prioritization of compounds and candidate genes for annotation, thereby contributing to the expansion of the metabolite library and advancing our understanding of the underlying biosynthetic networks.

As a case study, we focused on salicinoids, a class of specialized metabolites of which the biosynthesis is largely unknown. We annotated 92 salicinoids and found genome-wide associations with 35 enzyme-encoding genes. Novel genes potentially involved in salicinoid biosynthesis were identified and will be validated through reverse genetics. These results represent a major step toward resolving the salicinoid biosynthetic pathway. Future work will expand this framework to other compound classes and structural motifs.

This project is funded by the ERC Advanced Grant POPMET.

1248 A — Environmental Metabolic Footprinting Adapted to Track Dissipation of Complex Molecules based on Untargeted Metabolomics

PRESENTING AUTHOR: *Christian Espinoza, SAS. AKINAO, France*

CO-AUTHORS: *Marie-Virginie Salvia, Cédric Bertrand*

SUB THEME: *Xenobiotics and nutrient metabolism*

The EMF, based on Untargeted Metabolomics, was designed to study the global meta-metabolome through kinetic study, including the endo-metabolome (natural metametabolome of the environmental matrix, associated with micro-organisms) and the xeno-metabolome (product and by-products). In fact, the untargeted approach enables detection of even unknown compounds, offering a powerful tool to study chemically complex biocontrol products, especially those that are not well characterized and cannot be tracked by radiolabelling. This justification has led us to adapt the EMF approach to OECD 309 guideline “Aerobic Mineralisation in Surface Water” for estimating the dissipation time of a complex dye substance.

Three different modalities were used, one untreated (control) and two treated with two different concentrations of dye (100 µg/L and 5 mg/L). Samples were tracked for 30 days using nine sampling points (T0, T1, T2, T3, T7, T15, and T30 days). Analyses were performed using UHPLC-HRMS with ESI in positive and negative modes. The dye degradation was followed by Extraction Ion Chromatogram (EIC) while the by-products by untargeted approach. The main complexity of this approach lies in the separation of the endo-metabolome and xeno-metabolome by bio-infomatic processing. Here, only the xeno-metabolome was assessed. Discriminating features, potential by-products, were selected at each sampling point by comparison of treated and untreated groups using a combination of t-test (0.05) and fold-change (1.2).

The dye was fully degraded at T7 for low concentration (100 µg/L) treatment. However, for high concentration (5 mg/L) treatment, dye persisted even after 15 days (T15 and T30 are currently being processed). Moreover, the potential by-products detected followed the same trend as the dye, with few or no features for the low concentration (T7), but a significant number of features for the high concentration (T7). Therefore, the EMF approach provides an alternative to radiolabelling for tracking by-products of complex molecules through kinetic studies.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

1249 A	**A Streamlined Workflow for Rapid and Accurate Identification of Novel Bioavailable Forms of Microbial Metabolites by UHPLC-HRMS with Smart Searchable Databases**

PRESENTING AUTHOR: ***Danyue Daisy Zhao, The Hong Kong Polytechnic University, Hong Kong***

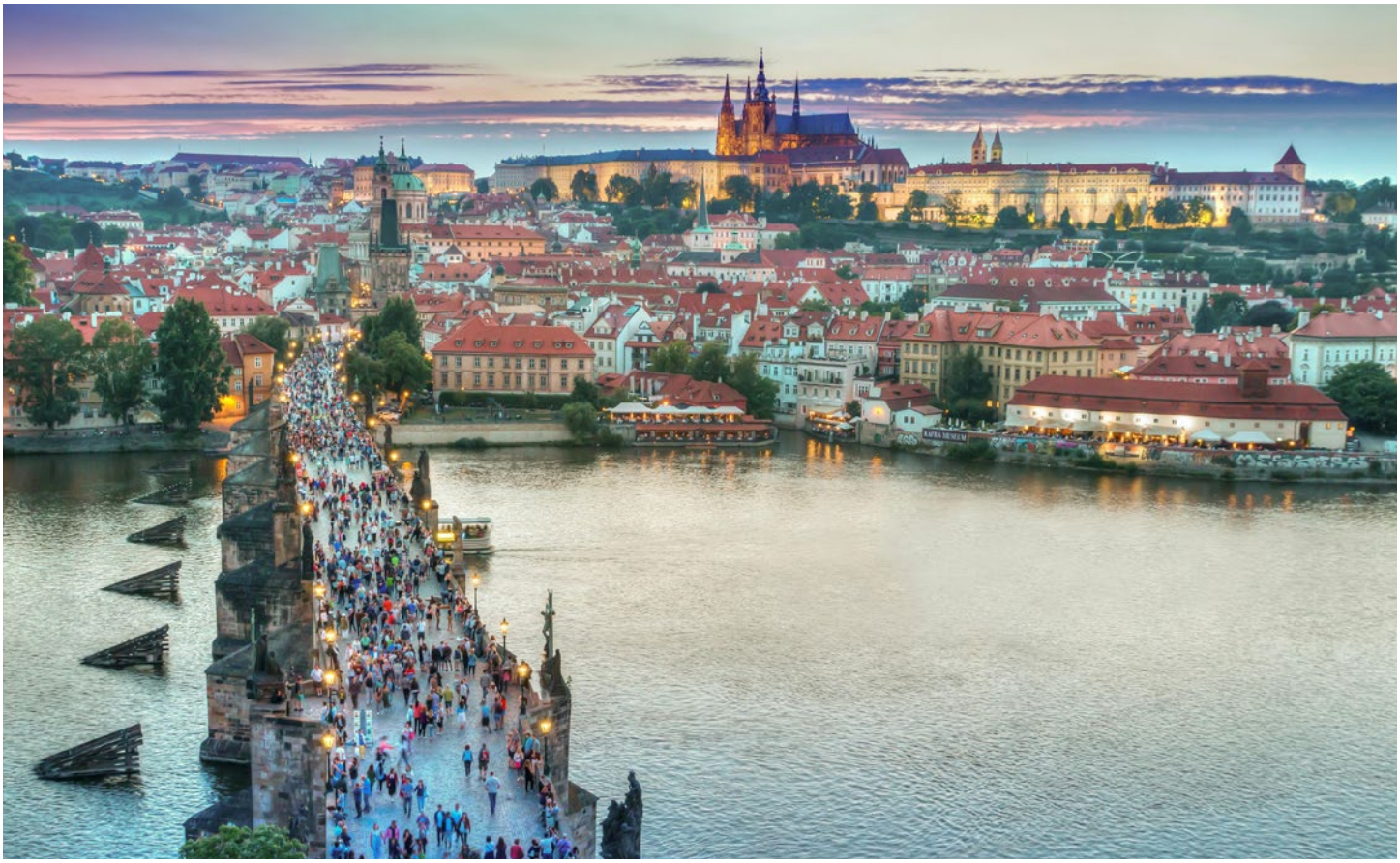
CO-AUTHORS: ***Jianing Liu, Fanghui Deng, Weipeng Li, Man-kin Wong***

SUB THEME: ***Xenobiotics and nutrient metabolism***

Microbial metabolites, such as urolithins produced from dietary ellagitannins and ellagic acid, have gained substantial attention for their remarkably higher bioavailability than precursors and multifarious health benefits. However, identifying the existing forms of these metabolites in vivo are challenging due to their complex biotransformation, limited reference databases and interfering bio-matrices.

In this study, an untargeted metabolomics workflow was developed to detect urolithin-related metabolites in mouse samples using UHPLC-Orbitrap-IQ-X-MSn. Raw data obtained from profiling mouse samples were preprocessed using MSnbase and XCMS packages in R for extracting relevant features, aligning retention times, and identifying MS2 fragments of urolithin metabolites. Mass defect filtering was applied to uncover metabolites that could be masked by matrix effect. A comprehensive database targeting urolithin-related metabolites (e.g., urolithin aglycones and their conjugates) was developed, including their precursor MS1 and product MS2 fragment ions for quantification and confirmation. This database is fully searchable, enabling quick matching of mass spectra data with known urolithin fragment patterns. Furthermore, its integration within R avoids switching between multiple software platforms, streamlining the data analysis process while saving resources. With this in-house database, we identified >60 urolithin-related metabolites, including multiple new phase II metabolites in mouse samples.

In summary, the proposed workflow addresses challenges in identifying bioavailable forms of microbial metabolites, exemplified by urolithin metabolites. By expanding the database, this workflow can be generalized to characterize a broad range of metabolites with distinct fragment profiles. Our approach paves the way for automated metabolite identification and provides a valuable tool for researchers to elucidate the active forms of microbial metabolites in vivo and their potential health benefits.



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TECHNOLOGY ADVANCEMENTS

1260 A — MetaFlowTrain: a highly parallelized and modular fluidic system for studying exometabolite-mediated inter-organismal interactions

PRESENTING AUTHOR: ***Silvina Perin, Max Planck Institute, Germany***

CO-AUTHORS: ***Guillaume Chesneau, Johannes Herpell, Sarah Marie Wolf, Silvina Perin, Stéphane Hacquard***

SUB THEME: ***At-home sample collection***

Ecosystems are colonized by diverse multi-kingdom microbial communities whose interactions are poorly understood, partly due to a lack of adequate experimental systems. To address this problem, we have developed a new system called MetaFlowTrain. It is a modular, inexpensive, semi-high-throughput, easy-to-assemble fluidic system in which multiple media can be flushed at adjustable flow rates into microchambers that house a variety of microorganisms, from bacteria to small eukaryotes. These microchambers can be used alone or connected in series to create trains of microchambers in which metabolites, but not organisms, move directionally between the microchambers to modulate the growth of the organisms. This fluidic system allows complete compartmentalization of organisms in ecosystems in a modular fashion, making it an effective tool for thoroughly deciphering exometabolite-mediated communication. We tested MetaFlowTrain using a representative synthetic community of the Arabidopsis thaliana root microbiota, consisting of 11 bacterial isolates and 4 fungal isolates belonging to 12 different families. We coupled this system with quantitative community profiling, targeted/non-targeted metabolomics and plant phenotyping. In the results, we demonstrate that a wide variety of bacteria and fungi can grow in the system under natural media conditions. Furthermore, we uncover the effects of soil conditioning on synthetic community structure and plant growth, and reveal microbial antagonism mediated by the temporal production of exometabolites. In conclusion, our study highlights MetaFlowTrain not only as a versatile system to investigate plant-microbe-microbe metabolic interactions, but also the potential of the system to discover metabolites that function as signaling molecules, drugs or antimicrobials in diverse biological systems.

1261 A — Metabolomics-guided biomarker selection for the development of a smart bioprocessing platform integrating real-time monitoring and advanced process control for autologous cell therapy

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SUB THEME: ***Bioprocessing***

Autologous immunotherapies have achieved impressive treatment results in patients with cancers. However, their broader clinical adoption and patient's accessibility of autologous immunotherapies remains hindered by the lack of efficient, consistent, cost-effective, and scalable manufacturing processes. To address this bottleneck, an international EU-funded consortium is developing a first-in-class, smart bioprocessing platform for personalised autologous cell therapies, integrating in-line process analytical technologies and advanced process control systems for the production of tumor-infiltrating lymphocytes (TILs). The aim of this project is to analyze metabolic profiles from spent media collected at key time points of the TIL expansion process and to search for correlations between these dynamic metabolic signatures and clinically relevant outcomes, such as T cell expansion rates and functional reactivity, to uncover metabolic biomarkers that serve as sensitive indicators of process performance and cellular quality. Using untargeted and targeted metabolomics, we have identified ~ 50 metabolites with a significant effect on cell expansion. Focusing on three key metabolites, alongside agitation speed and dissolved oxygen as critical process parameters (CPPs), we conducted design of experiment (DoE) studies and identified key metabolic determinants of T cell expansion and reactivity. We are investigating whether the real-time monitoring of the selected parameters can be performed with Raman and 2D-fluorescence probes that have been implemented within the bioreactor. Based on these results, subsequent work will seek to implement real-time monitoring and model predictive control strategies leveraging chemometric and mechanistic models to enhance process consistency and performance through the maintenance of an optimal culture environment for the production of TIL-based therapies.

TECHNOLOGY ADVANCEMENTS

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TECHNOLOGY ADVANCEMENTS

1262 A — Enhancing Urinary Volatilomics: A Dual Targeted-Untargeted Approach Using SPME Arrow for Biomarker Discovery and Environmental Exposure Assessment

PRESENTING AUTHOR: ***Raquel Cumeras, IISPV, Spain***
CO-AUTHORS: ***Maria Llambrich, Noelia Ramirez, Jesús Brezmes***
SUB THEME: ***Breath analysis/Volatolomics***

The analysis of urinary volatile organic compounds (VOCs) holds great potential for non-invasive diagnostics. However, the complexity of urinary matrices and the low concentrations of VOCs pose significant challenges for reliable detection, including the need of a semi-targeted urinary volatilomics approach. We focused on Solid-phase microextraction (SPME) Arrow urinary VOCs extraction combined with GC-HRMS, optimizing conditions for both targeted and untargeted analysis (Llambrich et al., Anal Chim Acta. 2024;1329:343261). A Design of Experiments (DOE) was applied to optimize extraction parameters based on both targeted (11 spiked standards) and untargeted outcomes (number of identified VOCs). Initial extraction conditions were selected based on the consensus of the reported conditions for SPME untargeted urinary analysis based on a bibliographic review we performed (Llambrich et al. Biological Procedures Online 2022;24(1):20). Among five fiber coatings tested, CAR/PDMS was selected for its superior extraction efficiency of both targeted and novel VOCs. Additional optimization steps included adjusting urine volume, salt concentration, and pH to enhance VOCs migration into the headspace. The final optimized method involved a 60 min extraction at 53°C, using 2mL urine, 0.25% w/v NaCl at pH 2. The method exhibited high repeatability and reproducibility (87% VOCs had RSD<20%), the method significantly enhanced result consistency. Compared to conventional SPME fibers, the SPME Arrow demonstrated greater extraction efficiency, detecting 44 VOCs versus 34 with standard SPME. Finally, the enhanced method was successfully applied to urine samples from children exposed and non-exposed to tobacco smoke, identifying specific VOCs such p-cymene and p-isopropenyl toluene related to tobacco exposure. By integrating both targeted and untargeted approaches, this method comprehensively captures the complexity of urinary volatilomics, ensuring the precise identification of known compounds and the discovery of novel biomarkers. This semi-targeted strategy provides a more complete metabolic profile, offering valuable applications in non-invasive diagnostics and environmental health studies.

1263 A — Creating Matrix-Matched Replicate Breath Samples: A New Approach to Quality Control in Breathomics and Beyond

PRESENTING AUTHOR: ***Carlos Gil, Markes International Gmbh, Germany***
CO-AUTHORS: ***Matthew Turner, James Swift, Laura Miles, Laura McGregor***
SUB THEME: ***Breath analysis/Volatolomics***

The development of robust quality control (QC) strategies remains a challenge in breathomics due to the inherent variability in breath samples and the inability to pool aliquots from multiple samples in the same way as blood or urine. While synthetic standards provide reliable solutions for instrument quality control and semi-quantitative analysis, they fail to reflect the complexity of the breath matrix, limiting their utility in monitoring analytical variance, evaluating data quality, and performing batch correction. This study presents a novel approach to generating replicate pooled composite breath samples to directly address these limitations.

Breath samples were collected using a structured protocol, including the ingestion of a peppermint-oil capsule, followed by serial sampling at multiple time points. The samples were processed using a system that redistributes breath onto multiple sorbent tubes to create composite pooled samples. Stability was assessed over five weeks at various storage temperatures (-80°C, -20°C, 4°C, 20°C) using TD-GC-MS analysis, with internal standard normalisation to evaluate analyte stability.

The pooled breath samples demonstrated reproducibility with %RSD (4.5–11%) for key volatile organic compounds such as eucalyptol, limonene, menthol, and isoprene. Control chart analysis confirmed that the samples remained stable for at least 21 days under all tested storage conditions, further confirming the utility of the pooled samples as true biological replicates for use in QC protocols. This method offers a reliable approach for creating matrix-matched replicates, addressing the challenge of breath sample variability. The ability to generate large numbers of replicate samples containing the breath matrix is applicable to longitudinal studies, cross-instrument validation, and proficiency testing schemes. Beyond breathomics, the approach shows great promise for advancing studies with other non-invasive matrices employed in volatolomics, for example as skin samples, providing a reliable means to stabilise samples onto sorbent tubes for transport and extended storage.

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1264 A — Pain Induces Rapid Characteristic Metabolic Signatures Detectable in Exhaled Breath

PRESENTING AUTHOR: ★ *Mélina Richard, University of Basel, Switzerland*

CO-AUTHORS: *Kapil Dev Singh, Dilan Sezer, Sarah Buergler, Luana Palermo, Yannick Schulz, Zhifeng Tang, Xin Luo, Urs Frey, Philippe Cattin, Xue Li, Jens Gaab, Pablo Sinues*

SUB THEME: *Breath analysis/Volatolomics*

Pain remains a primary reason for medical visits, yet its objective assessment poses a significant challenge, particularly in vulnerable populations such as children, elderly individuals, patients with disabilities, and unconscious patients. Current molecular studies on pain largely rely on blood and urine analyses, but real-time, non-invasive alternatives are needed. Here, we employ breath metabolomics combined with pathway analysis to investigate rapid metabolic shifts induced by a widely used experimental pain paradigm, namely the cold pressor test (CPT).

A discovery cohort (n=19) and an independent validation cohort (n=21) underwent CPT, during which 1920 breath samples were acquired and analyzed using secondary electrospray ionization-high-resolution mass spectrometry (SESI-HRMS). Within 15 minutes of CPT, 416 mass spectral features overlapping in both cohorts exhibited significant alterations across both cohorts (False Discovery Rate (FDR) < 0.01, Log2FoldChange > 1.5), revealing consistent metabolic responses. Pathway enrichment analysis highlighted aminoacyl-tRNA biosynthesis, cysteine/methionine metabolism, butanoate metabolism, and arginine/proline metabolism, with arginine and glutamate upregulation suggesting their involvement in nitric oxide production and nociceptive signaling. A neural network classifier distinguished pre- and post-CPT breath profiles with an AUC of 0.856 and 78% accuracy, demonstrating the feasibility of breath-based pain detection.

Comparisons with chronic pain studies revealed consistencies in amino acid and neurotransmitter-related pathways, supporting the universal relevance of these metabolic shifts. This study provides novel insights into the molecular mechanisms of acute pain and positions breath metabolomics as a promising, observer-independent tool for dynamic pain monitoring. Future research should explore whether these metabolic signatures can guide personalized pain management strategies.

1265 A — Metabolic Reprogramming and Extracellular Matrix Remodeling in Malignant Peripheral Nerve Sheath Tumor: The Hidden Role of Amino Acid Pathways

PRESENTING AUTHOR: *Denise Drago, IRCCS San Raffaele Scientific Institute, Italy*

CO-AUTHORS: *Radmila Pavlovic, Annapaola Andolfo, Martina La Spina, Andrea Rasola*

SUB THEME: *Flux analysis*

Malignant peripheral nerve sheath tumor (MPNST) is aggressive and poorly characterized cancer that can arise sporadically or in individuals with the genetic disorder Neurofibromatosis type 1 (NF1). Currently, no effective treatments are available for MPNST. Surgical intervention is often unsuccessful due to the tumor's deep localization, large size, and highly invasive and metastatic nature. As a result, the prognosis for MPNST patients remains dismal, underscoring the urgent need for a deeper understanding of the tumor's biology to inform the development of targeted therapies. This study seeks to address the challenges of MPNST progression by examining its malignant characteristics from a novel perspective. The tumor's ability to invade surrounding tissues is a critical prerequisite for its metastatic spread, which is the primary driver of lethality in MPNST. To become invasive, MPNST cells must actively remodel the surrounding extracellular matrix (ECM) - a complex process we hypothesize to be tightly linked to significant metabolic reprogramming. In this context, our investigation focuses on the unexplored interplay between metabolic adaptations and ECM remodeling in MPNST. Specifically, we evaluate the role of amino acid metabolic pathways in the biosynthesis of ECM components by performing metabolic flux analysis using 13C15N-labeled through liquid chromatography mass spectrometry (LC-MS/MS). Our findings consistently show that MPNST cells are avid consumers of glutamine, which they absorb from the extracellular environment and convert into proline, a key amino acid in ECM protein synthesis. By dissecting these metabolic processes, we aim to elucidate the mechanistic connections between ECM molecule production and the invasive potential of MPNST cells. Ultimately, our goal is to identify metabolic vulnerabilities in MPNST that can be therapeutically targeted to inhibit tumor growth and prevent metastatic dissemination.

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TECHNOLOGY ADVANCEMENTS

1266 A — In vivo RUBISCO activity in Synechocystis is regulated by RuBP availability

PRESENTING AUTHOR: ***Luisa Wittemeier, Max Planck Institute of Molecular Plant Physiology, Germany***
CO-AUTHORS: ***Stephanie Arrivault, Niels Neumann, Boris Maček, Nils Schmidt, Martin Hagemann, Joachim Kopka***
SUB THEME: ***Flux analysis***

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) is the main CO_2-fixing enzyme on earth and entry point of carbon into the Calvin-Benson-Bassham (CBB) cycle. Fueled by photosynthesis, C-assimilation by RUBISCO must be tightly controlled. RUBISCO regulation upon transition from light to darkness is not fully understood in the cyanobacterium Synechocystis sp. PCC 6803 (Synechocystis). Synechocystis does not have a RUBISCO activase that regulates RUBISCO activity in land plants by removing intrinsic sugar phosphate inhibitors from its active site. Instead, the regulatory CP12 protein of Synechocystis inactivates glyceraldehyde 3-phosphate dehydrogenase (GAPDH2) and phosphoribulokinase (PRK) during darkness. This mechanism indicates metabolic regulation of RUBISCO. We investigated C-assimilation in vivo at transition to darkness by dynamic $^{13}CO_2$ labeling experiments. We monitored RUBISCO activity by ^{13}C-incorporation into 1-C position of 3PGA using GC-MS. Other than the wild type, the Δcp12 mutant continued to assimilate $^{13}CO_2$ into 3PGA in darkness. RUBISCO abundances and specific activities were not altered in Δcp12 and upon light to dark transition. Complementation of Δcp12 by native CP12 (Δcp12::cp12) and CP12 with mutated conserved cysteines in its GAPDH2- and PRK-binding domains (Δcp12::cp12ΔCys) showed that both native binding domains are required to fully inactivate the CBB cycle in the night. RuBP levels were highly elevated in Δcp12 upon transition to darkness. Complementation with mutated and native CP12 variants gradually reduced RuBP to wild type levels and revealed highly significant correlation between RuBP concentration and the time-shifted ^{13}C-uptake into 3PGA. We propose that RUBISCO activity in Synechocystis at day-night transition is regulated through depletion and blocked regeneration of RuBP. ^{13}C-positional analyses of aspartate with GC-MS suggest regeneration of RuBP in Δcp12 via dysregulated gluconeogenesis and the oxidative pentose phosphate path. We demonstrate that RUBISCO activity of Synechocystis is present throughout diurnal growth and depends on the availability of its substrate.

1267 A — GCMS-based 13C-positional isotopologue analysis for the measurement of photosynthetic carbon assimilation fluxes

PRESENTING AUTHOR: ***Joachim Kopka, Max Planck Institute Of Molecular Plant Physiology, Germany***
CO-AUTHORS: ***Yogeswari Rajarathinam, Luisa Wittemeier***
SUB THEME: ***Flux analysis***

GC-MS technology induces abundant in source fragmentations where fragments can represent metabolite substructures. This phenomenon enables measurements of stable isotope enrichment at substructures of metabolites and can resolve even single C-atoms. Current developments of 13C-positional analyses extend in vitro studies of enzyme activities towards in vivo measurements of carbon assimilation. We provide an alternative to 13C-MNR technology that is the current technological solution for positional 13C analyses. With careful validation, GC-MS technology allows positional labeling analyses even of low to medium abundant compounds within complex metabolite fractions.

We apply this technology to photoautotrophic organisms that fix carbon by ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) and phosphoenolpyruvate carboxylase (PEPC). RUBISCO assimilates CO2 into 1-C position of 3-phosphoglycerate (3PGA). The Calvin-Benson-Basham cycle redistributes fixed carbon atoms into 2,3-C2 of the same molecule. PEPC uses phosphoenolpyruvate (PEP) derived from 3PGA and assimilates HCO3- into 4-C of oxaloacetate and its transaminase product aspartate. Positional isotopologue analysis of aspartate, the main product of oxaloacetate in the model cyanobacterium Synechocystis sp. PCC 6803 (Synechocystis), allows differentiation between PEPC, RUBISCO, and CBB cycle activities within one molecule. In source fragmentation of GC-EI-TOFMS at nominal mass resolution and of GC-APCI-TOFMS at high mass resolution in combination with chemical derivatization by N,O-bis(trimethylsilyl)trifluoroacetamide or N-methyl-N-(tert.-butyldimethylsilyl)trifluoroacetamide enabled the determination of fractional 13C enrichment (E13C) at 1-C 3PGA [1] and at each carbon position of aspartate [2].

These methods were validated by 13C-isotopomer mixtures of positional labeled aspartic acid and by in vivo metabolization of positionally labelled glucose using E. coli mutants. Accurate quantification of 3PGA and aspartate concentrations and positional fractional 13C enrichments from dynamic labelling experiments provided molar Ci assimilation rates during the day and night phases of photoautotrophic Synechocystis cultures. The validated method offers applications to characterize the photosynthetic Ci fixation fluxes in photosynthetic organisms.

[1] (https://academic.oup.com/plphys/article/197/2/kiaf020/7965674)

[2] (https://doi.org/10.1101/2024.05.07.592938)

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TECHNOLOGY ADVANCEMENTS

1268 A — Advances in GC–MS-Based ^{13}C-MFA Allow for Improved Characterization of the Central Carbon Metabolism

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CO-AUTHORS: *Melanie Hogg, Peter Radermacher, Ulrich Wachter, Vittoria Passarelli, Josef Vogt*
SUB THEME: *Flux analysis*

Isotope-based metabolic flux analysis (MFA) is a method for calculating production and consumption rates within biological networks such the central carbon metabolism encompassing glycolysis, the tricarboxylic acid cycle, and the pentose phosphate pathway (PPP). Even though the PPP plays a pivotal role in the cellular regulation of immune function, little is known about the metabolic interplay, especially regarding the non-oxidative PPP.

We developed a novel Bayesian MFA approach that was based on RStan, a library which uses user-defined models and data to return posterior estimates of prior defined parameters. The method focuses on the central carbon metabolism and relies on a) ^{13}C enrichments of intracellular metabolites of parallel tracer experiments with [U-$^{13}C_6$]Glucose, [1,2-$^{13}C_2$]Glucose, [4,5,6-$^{13}C_3$] Glucose, and [U-$^{13}C_5$]Glutamine, and b) the GC–MS-based measurement of extracellular production rates (e.g., $^{13}CO_2$ production of each tracer, lactate secretion).

Since we placed a special focus on the precise detection of hexose and pentose phosphates, the new methodology uses a two-step derivatization protocol: ethoxylamination followed by trimethylsilyloxime derivatization, which enables improved baseline separation and provides partial positional labeling of sugar phosphate isomers. The inclusion of multiple sugar phosphate fragments significantly enhanced flux resolution compared to traditional LC–MS methods. Furthermore, each of the parallel tracers contributed to the elucidation of different sections of the central carbon metabolism network, e.g., utilization of [4,5,6-$^{13}C_3$] Glucose allowed for distinguishing non-oxidative PPP and gluconeogenetic fluxes pertaining to upper glucose metabolism. Compared to traditional approaches relying on frequentist statistics, the Bayesian implementation provides full uncertainty distributions for fluxes, along with joint confidence regions and flux correlations. This enables straightforward assessment of flux relationships within complex biochemical networks. Overall, the novel approach yielded precise flux distributions and confidence intervals of immune cells analyzed ex vivo, enabling the determination of cellular metabolic responses under both physiological and pathophysiological conditions.

1269 A — Dual-HPLC-MS: A parallelised Method for Metabolite Analysis in Cardiovascular Disease Studies

PRESENTING AUTHOR: *Christian Faist, University of Münster, Germany*
CO-AUTHORS: *Edda Tacke, Thorsten Eierhoff, Heiko Hayen*
SUB THEME: *High-throughput metabolomics*

Today, many risk factors leading to cardiovascular diseases, e. g. aortic dissections, are known, but the influence of the gut microbiome has not yet been sufficiently explored. However, metabolites synthesized in the intestine by microorganisms from e. g. carnitine or dietary fiber, such as trimethylamine-N-oxide (TMAO) or short chain fatty acids (SCFA) have already been demonstrated an indirect influence on the endothelium. Due to the diverse physicochemical properties of these analytes, they cannot be analysed with one chromatographic and mass spectrometric method. Therefore, we developed a dual-high-performance liquid chromatography (HPLC) method in a time-saving procedure using only one mass spectrometer (MS). To investigate human serum samples in larger scale, this dual-HPLC-MS-based method was applied, using a HPLC system consisting of two individual flow paths, that can be addressed separately and simultaneously. The determination of carnitine und TMAO takes place by hydrophilic interaction liquid chromatography (HILIC)-MS in positive electrospray ionisation mode. After derivatisation, the SCFA can be separated on a reversed-phase column and detected in negative ionisation mode by a high-resolution Orbitrap-MS. This method was used to quantify these metabolites in human serum samples using stable-isotope dilution analysis. By integrating both chromatographic methods using a six-port valve and taking into account the equilibration times of the columns after each run, the measurement time at the MS can be used more effectively and hence saves valuable and cost-intensive detector time. The application of the dual-HPLC-MS method has already been successfully used for human serum samples, where differences between the levels of some metabolites could be observed. In addition, variations in the ratio of SCFA in relation to certain pathologies have been demonstrated, further emphasising the relevance of SCFA in relation to cardiovascular diseases.

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TECHNOLOGY ADVANCEMENTS

1270 A — Spatial metabolomics and lipidomics by on-tissue MS2 acquisition using mzmine

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CO-AUTHORS: *Robin Schmid, Carina Wolf, Arne Behrens, Arne Fütterer, Katharina Kronenberg, Ansgar Korf, Henning Richter, Astrid Jeibmann, Corinna Brungs, Uwe Karst*

SUB THEME: *Imaging MS*

While compound annotations in liquid chromatography (LC) based mass spectrometry (MS) experiments typically rely on accurate mass, retention times, and fragmentation spectra (MS2), MS imaging experiments often lack crucial MS2 information. Manual on-tissue MS2 acquisition can give confidential annotations, however, it is labour-intensive and typically limited to a set of targeted compounds. Here, we present the spatial-omics toolbox in mzmine to enable integrative analysis of LC-MS2 and MS imaging data and tools for dataset-dependent on-tissue MS2 acquisition and evaluation.

Sheep brain tissue was analysed using LC-ion mobility spectrometry (IMS)-MS and MALDI-IMS-MS. LC data were acquired in triplicate. Additionally, four adjacent rat brain tissue sections were analysed by MALDI-TIMS-MS. The spatial ion mobility-scheduled exhaustive fragmentation (SIMSEF) tool in mzmine was used to optimise an MS2 acquisition schedule for high-coverage, automated, on-tissue MS2 data collection by remote controlling the timsTOF fleX instrument, eliminating manual intervention.

The LC-IMS-MS2 and IMS-MS imaging datasets of sheep brain tissue were analysed in mzmine using the spatial metabolomics workflow. This associates the MS images with their respective LC-IMS-MS2 features, allowing robust MS2-based compound annotations for MS imaging data. The mobility-resolved images reveal isobaric overlaps with distinct spatial distributions, preventing misinterpretation. MS2 spectra from LC acquisitions allow confident compound annotation.

However, since the observed ion species of MALDI and LC datasets may differ, an automated on-tissue MS2 acquisition approach was developed. The SIMSEF algorithm analyses MS1 imaging data and identifies pixels for high-quality MS2 spectra for every detected compound, considering spatial distribution and ion mobility separation. Using this approach, a single rat brain sample yielded 29,000 MS2 spectra for 1,500 precursors, while achieving annotation rates of 13-15% across four replicates, comparable to LC-MS2 experiments.

mzmine then enables downstream analysis by spectral library matching, rule-based lipid annotation, and molecular networking within a unified open-source framework.

1271 A — MALDI mass spectrometry imaging of mouse tissue fine structure at 5-10 µm spatial resolution for accurate lipid and small-molecule profiling

PRESENTING AUTHOR: *Sabina Holm Skov, University Of Southern Denmark, Denmark*

CO-AUTHORS: *Henrik Max Jensen, Ole N. Jensen*

SUB THEME: *Imaging MS*

Mass spectrometry imaging (MSI) analysis enables visual localization of molecular components with high spatial resolution in sample specimens including biological tissues. Preserving morphology in some tissues is challenging due to fine structure and morphology, complicating MSI analysis.

The gut-liver axis encompasses bidirectional interactions between gut and liver, is important for well-being and implicated in liver disease progression. Intestinal tissue is essential for studying molecule absorption and conjugation but poses challenges due to its fine structure and inhomogeneous morphology. We use rapid fixation to preserve mouse colon morphology during MALDI MSI using microGRID and post-ionization (MALDI-2) technology assessing molecular integrity, signal intensity, diffusion effects, and fixation-induced mass shifts. We present findings from high spatial resolution (5-10 µm) MSI of colon and liver in a high-fat vs. lean diet mouse study. Different MALDI matrices in positive/negative mode enabled mass profiling of small molecules and lipids. Ion density maps of fixed and embedded colon tissue revealed spatial localization of molecules to the tubular structures of concentric layers in mouse intestinal tissue, providing insights into molecular mechanisms of the gut. MSI analysis of liver, performed without fixation and embedding, enabled visualization of compounds distributed throughout the homogenous liver tissue and identification of portal veins and bile ducts. Unsupervised segmentation distinguished high-fat and lean diet groups, and key clustering features were identified.

Computational processing of MSI datasets is ongoing, focusing on supervised and unsupervised segmentation and feature identification. We aim to classify relevant biomolecular features based on chemical structure and evaluate the biological role of selected compound classes. Data from liver and gut tissues will be correlated to address questions related to disease progression in mice on a high-fat diet. We aim to propose compounds that may serve as potential biomarkers of disease progression following a high-fat diet, facilitating further studies on obesity-related disease progression.

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TECHNOLOGY ADVANCEMENTS

1272 A — Enhanced Nano-LC-MS for Analyzing Dansylated Oral Cancer Tissue Metabolome Dissolved in Solvents with High Elution Strength

PRESENTING AUTHOR: *Ya-Ju Hsieh, Chang Gung University, Taiwan*
CO-AUTHORS: *Cheng-Yu Hung, Kun-Yi Chien*
SUB THEME: *New developments in instrumentation*

Background: Tissue metabolomics analysis, alongside genomics and proteomics, offers crucial insights into the regulatory mechanisms of tumorigenesis. To enhance metabolite detection sensitivity, chemical isotope labeling (CIL) techniques, such as dansylation, have been developed to improve metabolite separation and ionization in mass spectrometry (MS). However, the dissolution of hydrophobic derivatized metabolites in solvents with high acetonitrile content limits the use of liquid chromatography (LC) systems with small-volume reversed-phase (RP) columns. In this study, we developed a novel nano-LC-MS system with an online dilution design to address this issue, enabling sensitive analysis of oral cancer tissue metabolomes.

Results: Our nano-LC system features a unique flow path with online sample dilution before an RP trap column and backflushing of the trap column before entering the analytical column. Compared to other nano-LC systems, both with and without online dilution designs, our system demonstrates the superiority of the T-connector-based dilution method. Using only 1/20th of the sample required for popular micro-LC systems, our nano-LC detects a larger number of peak pairs with similar recovery rates for both hydrophilic and hydrophobic metabolites, ensuring unbiased results. Thirty-two matched pairs of oral squamous cell carcinoma (OSCC) tissue samples and adjacent noncancerous tissues (ANTs) underwent high-throughput CIL-metabolome analysis using our nano-LC system. Compared to our previous micro-LC methods, the nano-LC-MS system exhibits enhanced detection sensitivity, significantly reducing sample requirements.

Significance: Our findings highlight the efficacy of our platform for metabolomic analysis with limited sample amounts. The nano-LC system's ability to analyze samples dissolved in strong eluents suggests potential applications for handling other hydrophobic compounds using RPLC or other separation methods facing similar solvent incompatibility issues. This approach holds promise for identifying novel metabolite biomarkers for oral cancers, advancing our understanding of tumorigenesis, and enhancing clinical applications.

1273 A — Plasmonic nanomaterials in advancement of surface-assisted laser desorption/ionization mass spectrometry for applications in lipidomics and metabolomics trace analysis

PRESENTING AUTHOR: *Gulyaim Sagandykova, Core Facility Metabolomics, University of Tübingen, Germany*
CO-AUTHORS: *Radik Mametov, Christoph Trautwein*
SUB THEME: *New developments in instrumentation*

Surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) offers significant advantages in the analysis of low molecular weight compounds, which is particularly valuable in lipidomics, metabolomics and MALDI-2 Imaging. Plasmonic nanomaterials can offer advancement in sensitivity due to excitation of surface plasmon resonances, however rare efforts were dedicated to their application in development of SALDI-MS techniques. In this work, we present SALDI-MS performances of plasmonic nanomaterials beyond traditionally studied plasmonic metal nanoparticles such as anisotropic gold nanostars and silver nanostructures fabricated by chemical vapor deposition. The results showed high-intense SALDI-MS signals without background interferences for standards of lipids at nanomolar detection level (S/N in the range from 16 to 57) in case of gold nanostars (doi: 10.1016/j.ijms.2022.116872) and picomolar level for silver nanostructures (doi: 10.3390/ma15134660). Furthermore, silver nanostructures were applied for metabolic profiling of eight bacterial strains (doi: 10.1038/s41598-024-56107-0). The profiles were extracted with utilization of Bligh & Dyer method and both methanol and chloroform phases were analyzed. The data was processed using principal component analysis, hierarchical cluster analysis and random forest algorithm. Chloroform phase showed differentiation between all strains, while profiles for methanol phase failed to achieve specificity in profiles. Future perspectives of plasmonic nanomaterials in SALDI-MS includes further development of nanosubstrates for metabolic profiling in the low mass range as well as to extend their application to trapped ion mobility spectrometry (tims) time-of-flight (timsTOF) MS and MALDI imaging methods for applications in quantitative and spatial metabolomics allowing for sensitive, selective analysis of metabolites' profiles and distribution across various samples.

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TECHNOLOGY ADVANCEMENTS

1274 A — Data-independent analysis for Deep Metabolome Profiling of Plant Extracts

PRESENTING AUTHOR: *Adriano Rutz, ETH Zürich, Switzerland*
CO-AUTHORS: *Vincen Wu, Nicola Zamboni*
SUB THEME: *New developments in instrumentation*

Comprehensive metabolite annotation in complex plant extracts remains a significant challenge in untargeted metabolomics. MS2-based annotation often falls short, particularly due to the limited information and coverage of classical fragmentation techniques, or insufficient specificity and sensitivity in data-independent acquisition (DIA) modes.

We present a novel Q-TOF instrument that was optimized to attain higher sensitivity in all scan modes without sacrificing speed. The novel QTOF system employed has a >10x boost in sensitivity through a new source, ion optics, detector, and electronics compared to the SCIEX ZenoTOF 7600 system. The combination of sensitivity and speed pave the road to the acquisition of DIA data by scanning SWATH - at speed of 3000 Da/s and Q1 isolation windows that are equivalent to unit m/z resolution.

To explore the potential of this DIA setup, we analyzed a complex plant extracts using by a fast 4-min LC-DIA-MS method. The system could annotate over 45 (+100% in comparison to DDA) plant-derived metabolites with strong evidence and over 500 (+500%) with good evidence within a single injection. This was achieved without prior parameters optimization or extended acquisition time. Benchmarking focused on workflow-level performance metrics such as total unique annotations and spectral quality, rather than traditional limits of detection or signal-to-noise ratios.

This work illustrates that optimized instrumentation and procesing strategies can significantly enhance the depth of plant metabolome coverage, enabling high-throughput applications such as chemotaxonomy, phenotyping, and stress-response studies. Ongoing hardware and software refinements are expected to further improve the capabilities of DIA in plant metabolomics.

1275 A — In-depth analysis of hepatocellular carcinoma using a novel enhanced dynamic range MS mode reveals key metabolic pathways

PRESENTING AUTHOR: *Demi Rijlaarsdam, Utrecht University, Netherlands*
CO-AUTHORS: *Michał Kaczmarek, Christian Klaas, Jung-Chin Chang, Bernd Helms, Esther Zaal, Celia Berkers*
SUB THEME: *New developments in instrumentation*

Metabolic reprogramming is crucial for progression of tumors, including Hepatocellular Carcinoma (HCC). Hepatic stellate cells (HSCs) are important players in the liver tumor microenvironment, supporting HCC potentially by driving metabolic rewiring. A major challenge in metabolite detection is the broad concentration range, where high-abundant metabolites mask the presence of low-abundant metabolites, limiting the detection of potential key metabolites involved in cancer progression. Here, we apply a new high resolution mass spectrometry (HRMS) enhanced Dynamic Range (eDR) mode to identify novel metabolic therapeutic targets in HCC using a co-culture model of HCC and HSC cells.

Co-culture with HSCs resulted in increased proliferation rates of HCC compared to cells in mono-culture, suggesting the induction of a metabolic switch that is driven by soluble extracellular components. To investigate this metabolic switch, we next analyzed intracellular and extracellular metabolites in mono- and co-cultured HCC using a modified Thermo Scientific™ Orbitrap™ Hybrid mass spectrometer with a newly developed eDR mode. Using eDR, we find a 1.9-fold and 2.0-fold increase in the detection of intracellular and extracellular features, respectively. Moreover, significantly increased signal-to-noise ratios were found for 72% of the compounds in our semi-targeted analysis, including key metabolites involved in cancer growth, such as nucleotides, amino acid derivatives, NAD+/NADH and acetyl-CoA, which are often below detection limits in full scan mode of complex matrices. Mechanistically, co-culture resulted in a rewiring of glucose metabolism, characterized by enhanced 13C-glucose flux into glycolysis, de novo nucleotide synthesis, and the TCA cycle. Finally, we identified glucose transporter 1 as a target in HCC, where its inhibition reversed both the metabolic switch and the increased proliferation rates. Our results highlight the increase in sensitivity of the eDR mode, which enables the detection of low-abundant metabolites and in-depth analysis of HCC, allowing for the identification of novel metabolic targets for therapy.

1276 A — A Global Multi-Omics Workflow: Integrating Metabolomics, Lipidomics, and Proteomics to Compare Healthy Proximal and Distal Colonic Epithelium in Mice

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CO-AUTHORS: *Maryam Hemmati, Susanne I. Wudy, Franziska Hackbarth, Verena K. Mittermeier-Klessinger, Olivia I. Coleman, Dirk Haller, Christina Ludwig, Corinna Dawid*
SUB THEME: *New multi-omics workflows*

A comprehensive multi-omics workflow integrating metabolomics, lipidomics, and proteomics was developed to characterize the molecular interplay within proximal (PC) and distal (DC) colonic epithelium in healthy mice. This approach provides a foundational dataset for understanding regional tissue differences and investigating colon-related diseases such as colorectal cancer and inflammatory bowel disease. By optimizing the methyl tert-butyl ether extraction method, we successfully detected >350 reference-matched metabolites, >1850 reference-matched lipids, and >4500 proteins from a single tissue biopsy using targeted and untargeted mass spectrometry techniques. Each omics dataset was analyzed independently and subsequently integrated to reveal complex regulatory networks within the colon. Our findings highlight regional differences in mucin formation at both the substrate and enzyme levels, as well as alterations in lipid metabolism. Overall, this integrated multi-omics approach enhances our understanding of functional and regional distinctions between PC and DC tissues, offering deeper insights than individual omics techniques alone.

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TECHNOLOGY ADVANCEMENTS

1277 A — Integration of untargeted NMR-based metabolic profiling and gene expression analysis to identify potential biomarkers associated with Triton WR-1339-induced hyperlipidemia

PRESENTING AUTHOR: ***Mohammad Alwahsh, Al-Zaytoonah University of Jordan, Jordan***

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SUB THEME: ***NMR metabolomics***

Background: Hyperlipidemia is considered one of the main risk factors for cardiovascular diseases. Triton WR-1339 is one of the most commonly used compounds for the induction of hyperlipidemia in animal models, yet limited research has explored its impact on the metabolic level. This is the first metabolomics study employing nuclear magnetic resonance (NMR) spectroscopy to explore the plasma metabolomic profile of Triton WR-1339-induced hyperlipidemia rats.

Objectives: This study aims to explore the plasma metabolic profiles of Triton WR-1339-induced hyperlipidemic rats as well as to validate those changes on the molecular level via integrating metabolomics and gene expression analysis.

Methods: Male Wistar rats received an intraperitoneal administration of Triton WR-1339 to induce acute hyperlipidemia. Plasma lipid levels were evaluated to confirm hyperlipidemia induction.The plasma metabolic profiles were analyzed using 1H NMR spectrometer equipped with a cryogenic probe. Changes in gene expression levels of associated transporters were explored in liver,cardiac and kidney tissues using RT-PCR.Results: Lipid profile assay revealed significant elevation of triglycerides levels in hyperlipidemia rats.Both partial least squares-discriminant analysis (PLS-DA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) indicated a clear separation confirming the metabolic difference between control and hyperlipidemia group. Metabolomics results revealed a set of significantly altered metabolites associated with hyperlipidemia mainly related to disturbance in lipid metabolism, amino acid metabolism and energy metabolism. PCR analysis showed a significant change in the expression of several genes correlating with the observed metabolic changes.Conclusions:By identifying a set of potential biomarkers, this study provides a more comprehensive insights of the underlying metabolic disturbance induced by Triton WR-1339. Combining gene expression results with metabolomics have demonstrated differential expression of associated transporters that could be correlated with the alterations in the levels of potential biomarkers. These findings denote the importance of this integrative approach for the understanding of metabolic and molecular mechanism of complex diseases.

1278 A — Fast 2D NMR methods as an alternative to 1D ^{1}H NMR profiling: a case study on white wine

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CO-AUTHORS: ***Jérémy Marchand, Marine P.M. Letertre, Jean-Nicolas Dumez, Søren B. Engelsen, Patrick Giraudeau***

SUB THEME: ***NMR metabolomics***

Wine analysis by Nuclear Magnetic Resonance (NMR) presents challenges due to high concentrations of water and ethanol, which mask other metabolites and pose dynamic range issues. Therefore, multi-solvent suppression of water and ethanol is crucial to maintain NMR advantages like simple sample preparation and its ability to show a metabolomic fingerprint within a certain concentration range. While multi-solvent suppression 1D ^{1}H NMR methods have been used previously [1], the complex matrix leads to signal overlap limiting metabolite identification and quantification. Extension to 2D NMR methods can improve signal dispersion and provide unique structural information, though often suffering from long acquisition times. This problem has been investigated in NMR metabolomics in the past decade [2].

We compared three NMR methods on their ability to discriminate between two white wine varieties (Solaris and Melon de Bourgogne) to evaluate the potential of fast 2D NMR methods to overcome 1D ^{1}H NMR limitations like overlapping signals while improving metabolite identification.

In our study a 1D ^{1}H NMR experiment was compared to two accelerated 2D NMR methods: Non-Uniformly Sampled TOCSY and ultrafast COSY. These fast methods record the entire 2D spectrum in approx. 90 min (NUS TOCSY) and 20 min (uf COSY), while all methods apply water and ethanol suppression.

From multivariate statistical analysis, the possibility to discriminate and separate wines will be evaluated based on enhanced peak separation and metabolite identification. This will be put into perspective of the respective acquisition time of each method.

This comparison demonstrates the enhanced discrimination of metabolites by 2D NMR and offers tailored fast acquisition methods for wine, which can readily be adapted to other metabolomic areas e.g. biofluids analysis or high-throughput screenings.

1.Godelmann, R., et al., Journal of Agricultural and Food Chemistry, 2013. 61(23): p. 5610-5619.

2.Dumez, J.N., Chem Commun (Camb), 2022. 58(100): p. 13855-13872.

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TECHNOLOGY ADVANCEMENTS

1279 A — Neonicotinoids and their metabolites in honey bees

PRESENTING AUTHOR: *Nenad Zaric, BOKU University, Austria*
CO-AUTHORS: *Ratko Pavlovic, David Ruso, Rainer Schuhmacher*
SUB THEME: *Non-targeted and semi-targeted methods*

There are several factors contributing to honey bee colony death. One of the factors are neonicotinoid insecticides. Their toxicity is not limited to the parent compound, but their metabolites as well. Until now metabolites of only three of the seven neonicotinoids were described.

The aim of this study is to investigate the metabolism of orally administered neonicotinoids in honey bees. For this purpose, a stable isotope-assisted tracer approach is being used.

Honey bees (30 bees) were deprived of food for 12 hours and fed once (acute dose) with 500 g litre^{-1} feeding sucrose solution containing 1 + 1 (v/v) non-labelled and uniformly ^{13}C-labeled neonicotinoids, namely thiacloprid (1000 ng/bee), imidacloprid (3 ng/bee) and acetamiprid (10 ng/bee). Bees were sampled at six time points.

Approximately 20 frozen bees (~2 g) were homogenized and spiked with selected neonicotinoids and their metabolites to evaluate and select extraction and clean up procedures. Several different extraction procedures were tested using different solvents with or without a combination of ultrasonication or extraction salts (QuEChERS). For clean-up SPE cartridges and different dSPE were tested. Analyzes were performed by LC-HRMS using an Orbitrap IQ-X Tribrid mass spectrometer.

The best suited extraction and clean up procedure were the use of acetonitrile, water and hexane in combination with MgSO4 and sodium acetate as extraction salts, followed by a clean up step using dSPE with MgSO4. Recoveries for majority of the neonicotinoids and their metabolites were acceptable for complex matrixes, with rates above 60%.

The neonicotinoid transformation products were automatically searched for by the software tools MetExtract II and CPExtract based on their labelling-specific isotopologue patterns, and the biotransformation products were further evaluated manually. The detailed annotation of metabolite structures is an ongoing process.

1280 A — A novel dual-ionization GC-EI&CI-TOFMS – a comparative performance study for targeted and untargeted metabolomics

PRESENTING AUTHOR: *Stefanie Wernisch, Bruker Daltonics, Germany*
CO-AUTHORS: *Mohamed Elsadig, Stefanie Wernisch, Matthew Lewis, Arnd Ingendoh, Volker Behrends, Pablo Castañera Estrada, Blanca Lorente Torres, Michal Letek Polberg, Eliška Eliška Ceznerová, Steffen Bräkling, Mohamed Elsadig, Sonja Klee*
SUB THEME: *Non-targeted and semi-targeted methods*

GC-MS is a routine tool for the analysis of volatile metabolites. For the generation of ions, two approaches exist in the routine, each with own advantages. Electron impact ionization (EI), is the gold standard for GC-MS. It is a high-energy ionization method that produces detailed, highly reproducible fragmentation patterns, facilitating structural elucidation and compound identification through spectral libraries. Chemical ionization (CI) on the other hand is a soft ionization technique that reduces spectral complexity and preserves molecular ions, aiding in molecular weight determination.

We present data from a new instrument generating EI- and CI-information within a single GC-run. This approach enhances identification by providing simultaneously complementary structural and molecular information. Using both methods in a single GC run on one mass analyzer improves annotation confidence, reduces false identifications, minimizes instrument downtime, and streamlines data analysis.

Dried cell extracts of A549 cells were generated, derivatized with methoxyamine and MTBSTFA, and analysed using both a routine GC-EI-SQMS (Agilent 7890 - 5975C, Agilent Technologies Inc., Santa Clara, USA) and the novel dual-ionization, high-resolution GC-EI&CI-TOFMS (ecTOF, Bruker, Bremen). Various CI reactant ions, including N2H+, H3O+ and NH4+, were tested for optimal molecular ion generation, improving identification certainty. Data processing was performed using the vendor-neutral AnalyzerPro XD (SpectralWorks, Runcorn, UK).

The GC-HRMS system was successfully applied for untargeted metabolomics. A new workflow utilizing simultaneous EI and CI data is presented, showing that false positives in EI-only approaches are easily identified using additional CI data, significantly reducing the potential candidate lists. Structural analysis, using additional software-tools, leads to high confidence tentative compound identification. Several examples show the system's ability to expand the chemical space for unknown metabolites and marker discovery e.g., for PPP activity or urea cycle metabolites. This novel approach offers a deeper insight into various biological fields from cancer therapy to infection biology.

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TECHNOLOGY ADVANCEMENTS

1281 A — A Systematic Analysis of Untargeted LC-MS Methods for Human Metabolomics Studies Using the GNPS Public Spectral Library

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CO-AUTHORS: ***Francesc Puig-Castellví, Inés Castro-Dionicio, Philippe Froguel, Marc-Emmanuel Dumas***
SUB THEME: ***Non-targeted and semi-targeted methods***

Although untargeted metabolomics using LC-HRMS is a powerful tool in various research domains, including large-scale clinical studies, standardized guidelines for untargeted method validation are not specifically addressed. Current validation criteria for untargeted methods typically rely on a limited set of representative analytes, mainly due to their high cost and limited availability, introducing bias given the hundreds of detectable metabolites. This study proposes a data-driven strategy for evaluating untargeted LC-HRMS methods by incorporating metabolite coverage as an additional assessment criterion. It leverages the GNPS public spectral database as an accessible alternative to chemical standards to enhance method evaluation.

Commercial human serum samples were first assessed under a total of 23 LC-HRMS methodologies proven for high-throughput analyses from 6 metabolomic platforms using a HPLC Vanquish Duo coupled to an Orbitrap Exploris™ 240 Mass Spectrometer. Iterative Data-Dependent Acquisition (DDA) workflow was implemented in addition to the DDA method. The metabolites annotated with the GNPS public spectral library were used for the evaluation of the chemical coverage, reproducibility and linearity of the methods. The reliance of GNPS for non-targeted annotation was validated with chemical standards in the methods presenting the best performance. As a proof of concept, these methods were applied to characterize the metabolome of human serum clinical samples.

A total of 1482 unique metabolites were annotated using all the methods. Iterative DDA allowed us to increase the number of unique compounds with high-quality fragmentation spectra up to 50%. GNPS annotations were validated using chemical standards, achieving over 80% spectral similarity with a false-positive rate of less than 5%. This robust evaluation strategy allowed us to identify the most informative complementary methods (RP and HILIC) while maintaining the confidence and reliability of the results in a cost-effective scenario and broadening their applicability and feasibility of the proposed strategy across diverse research contexts.

1282 A — FunMN: Functional Group Molecular Networking Applied to Mouse Gut Microbiota-Associated Polar Metabolites

PRESENTING AUTHOR: ***Zdenek Kamenik, Institute of Microbiology Czech Acad Sci, Czechia***
CO-AUTHORS: ***Tommaso Stefani, Anna Jelinkova, Martin Schwarzer***
SUB THEME: ***Non-targeted and semi-targeted methods***

We will present the Functional group Molecular Networking (FunMN) concept that integrates metabolite chemical derivatization with molecular networking[1]. While conventional chemical derivatization is primarily applied in targeted analysis, we extend this approach to non-targeted metabolomics. Using the GNPS platform[1], FunMN provides molecular networks based on the presence of a functional group targeted by the derivatization agent rather than overall structural similarity, as in traditional molecular networking.

Polar metabolites, including amino acids, organic acids, monoamines, nucleotides, phosphates, thiols, and sugars, pose challenges for LC-MS/MS analysis due to their high polarity. Chemical derivatization enhances their compatibility with reverse-phase chromatography by modifying their physicochemical properties. We applied various derivatization agents, 3-nitrophenyl hydrazine (3-NPH), dansyl chloride, S-NIFE, and N-ethylmaleimide, to target key functional groups (carboxyls, hydroxyls, amines, phosphates, thiols) and assessed their universality using several hundreds of metabolite standards spiked in solvent and biological matrices.

To demonstrate the applicability of this approach, we analyzed metabolites associated with host-gut microbiota, which are essential for host physiology and disease. Using feces from three groups of C57BL/6 mice (germ-free, oligoMM12-colonized, conventional), we evaluated the ability of different derivatization techniques to distinguish microbiota colonization types. This study highlights the potential of FunMN as a tool for enhancing non-targeted metabolomics through functional group-based molecular networking with only one prior analogous example reported.[2]

References:

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[2] Wang Y, et al. Uncovering the Carboxylated Metabolome in Gut Microbiota-Host Co-metabolism: A Chemical Derivatization-Molecular Networking Approach. Analytical Chemistry. 2023; 95 (30), 11550.

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TECHNOLOGY ADVANCEMENTS

1283 A — Comparison of Untargeted LC–MS Coverage with Different Resuspension Solvents in Reversed-Phase and Hydrophilic Interaction Liquid Chromatography Modes in Mouse Matrices

PRESENTING AUTHOR: ***Seung Ju Lee, Seoul National University, South Korea***

CO-AUTHORS: ***Kim Yumi, Do Yup Lee***

SUB THEME: ***Non-targeted and semi-targeted methods***

Various molecular classes present in biological samples differ in polarity and solubility, making the selection of an appropriate resuspension solvent critical for comprehensive metabolomic analysis.

In this study, untargeted LC-MS profiling of mouse brain, serum, and fecal extracts was performed under both reversed-phase (RP) and HILIC conditions. For RP separations, samples were reconstituted in water, 70% acetonitrile with 5% methanol, and the original extraction solvent (MeOH:IPA:H_2O, 3:3:2, v/v). For HILIC separations, we compared 70% acetonitrile with 5% methanol, 95% acetonitrile, 80% acetonitrile, and the extraction solvent.

Across both chromatographic modes, 70% acetonitrile with 5% methanol yielded the highest total feature count, while solvent composition selectively influenced the detection of specific molecular classes such as azoles, carboxylic acids and derivatives, fatty acyls, imidazopyrimidines, organooxygen compounds, organonitrogen compounds, phenylpropanoic acids, pyrimidine nucleosides, and steroids and steroid derivatives.

These results demonstrate that, depending on whether the goal is semi-targeted analysis of particular compound families or broad, global metabolome coverage, the resuspension solvent should be chosen and tailored to both the analytical objective and the sample matrix.

1284 A — Formic Acid Treatment Enhances Biomarker Discovery in Untargeted Plasma Metabolomics

PRESENTING AUTHOR: ***Tereza Kacerova, University of Oxford, United Kingdom***

CO-AUTHORS: ***Elisabete Pires, A. David Smith, Fay Probert, Daniel C. Anthony, James S. O. McCullagh***

SUB THEME: ***Non-targeted and semi-targeted methods***

Untargeted plasma metabolomics by liquid chromatography–mass spectrometry (LC–MS) is a powerful tool for biomarker discovery in clinical and translational research. Yet, its performance remains highly dependent on sample preparation protocols, with subtle changes often leading to significant shifts in analytical outcomes. Here, we introduce a simple but impactful modification: pre-treatment of plasma with 1% formic acid (FA) prior to protein precipitation with acetonitrile (MeCN). This acidification step markedly improves both the reproducibility of metabolite extraction and the diversity of compound-feature detection, ultimately driving more accurate and robust supervised multivariate modelling.

This sample preparation protocol was validated using two complementary LC–MS approaches: 1) reversed-phase LC–MS (positive and negative ion modes) on a UPLC–QTOF system, and 2) ion chromatography–MS (negative mode) using a Hybrid Quadrupole-Orbitrap system. We compared standard MeCN-based extractions with those incorporating FA pre-treatment, across both technical replicates and biological samples from two independent clinical cohorts: VITACOG, a nutritional intervention study in adults with mild cognitive impairment, and OPTIMA, a memory clinic cohort designed to distinguish early-stage Alzheimer's disease from vascular dementia. In both cohorts, where metabolic differences were modest, FA-based extraction improved diagnostic model performance (OPLS-DA, Random Forest), increasing classification accuracy and revealing larger fold changes in diagnostically relevant features.

Mechanistic investigations suggested that pre-acidification lowers plasma pH, disrupting metabolite–protein interactions, enhancing solubilisation, and normalising matrix effects. Additional experiments across multiple LC–MS platforms confirmed that acidification did not introduce detrimental effects on data quality, compound stability, or instrument performance.

These results demonstrate that plasma acidification is a highly effective strategy for enhancing analytical sensitivity and reproducibility in untargeted metabolomics. This simple yet powerful modification improves the standard analytical pipeline, enabling more reliable detection of subtle metabolic alterations. As such, it offers a valuable tool for strengthening biomarker discovery efforts in clinical research.

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TECHNOLOGY ADVANCEMENTS

1285 A — Parallel targeted and untargeted metabolite analysis of mouse plasma samples using a benchtop multi-reflecting time of flight mass spectrometer

PRESENTING AUTHOR: *David Heywood, Waters Corporation, United Kingdom*
CO-AUTHORS: *Adam King, Lee Gethings, Richard Lock, Ian Wilson*
SUB THEME: *Non-targeted and semi-targeted methods*

Intro: Metabolomic profiling is routinely performed under two categories; targeted, where metabolites are quantified, and untargeted, where unknown molecules of significance are identified. These types of analyses are performed independently on different platforms using different workflows. This is due to the attributes of the instruments employed. Instrument advancements have narrowed the gap on sensitivity between high resolution and tandem quadrupole instruments; coupled with faster scanning capabilities and the reduction of interscan delays, both approaches on a single HRMS platform become a practical solution for metabolic profiling. Combining both within a single sample injection dramatically reduces the impact on instrument resourcing and sample volume requirements.

Aim: To acquire both targeted and untargeted metabolomic data in parallel from a single injection, generating high-quality data from limited sample volumes.

Methods: Plasma samples (n=48) from mice treated with fenclozic acid, sitaxentan, or vehicle were analyzed using the Waters Kairos amino acid kit. Data were acquired on the Xevo MRT mass spectrometer in positive mode, with chromatographic separation over a 9-minute reversed-phase gradient. The method included Tof MSMS functions for each amino acid (n=65). Untargeted data were collected with low (6 eV) or elevated (20-45 eV) collision energy ramps.

Results: Of the 65 amino acids, 20 were fully quantifiable against stable isotopically labelled standards (SILS), while the remaining 40 were quantified against the SILS based on retention time. Quantification ranged from 5 to 1000 µM with R2 >0.99, and 67% of QC standards were within 15% of the nominal concentration. Untargeted data underwent extensive statistical analysis, including PCA and OPLS-DA, highlighting significant distinguishing features between sample groups.

Conclusions: Combining targeted and untargeted approaches in a single analysis showed minimal compromise on data quality. The fast-scanning MSMS capabilities provided a high number of data points for quantitation whilst maintaining high-resolution accurate mass for untargeted data.

1286 A — NIST® SRM® 1950 LC-MS Lipidomic Database Enriched by Determination of the C=C Double Bond Position using EIEIO-MS

PRESENTING AUTHOR: *Sara Martínez López, Centro de Metabolómica y Bioanálisis (CEMBIO), Spain*
CO-AUTHORS: *Miguel Fernández-García, Sara Londoño-Osorio, Hana Cermakova, Ana Gradillas, Michael Witting, Coral Barbas*
SUB THEME: *Novel approaches in lipidomics*

Humans contain a vast diversity of lipid species, with each lipid class incorporating multiple fatty acyl chains (FAC) combinations. The structural properties of unsaturated lipids, particularly the number and position of carbon-carbon double bonds (C=C), influence their functionality, impacting membrane fluidity, signaling pathways and lipid-protein interactions. Given the complexity of the lipidome, assigning biological relevance to individual lipids requires considering the broader lipid context. Therefore, understanding the collective behavior of lipid subclasses and FAC distribution is essential for accurately interpreting lipid alterations. Developing high-quality, matrix-specific lipidome databases is a key strategy to accomplish this.

Here, we present the development of a highly curated lipidomic database for NIST® Standard Reference Material for Human Plasma using UHPLC-ESI(+/–)-CID-QTOF-MS (Agilent 6545 QTOF-MS). Encompassing 592 lipids, it integrates stringent quality assurance criteria, including adduct formation patterns, within-method m/z evaluation, retention behavior of lipid chain isomers, and expert-driven resolution of isomeric and isobaric interferences, thereby improving data consistency and the reliability of untargeted lipidomic workflows.

To improve structural characterization, we employed UHPLC-ESI(+)-EIEIO-QTOF-MS (SCIEX ZenoTOF 7600 QTOF-MS), where electron impact excitation of ions from organics (EIEIO)-MS enabled C=C localization in unsaturated glycerophospholipids (GP) within our database. Manual curation of EIEIO-MS/MS spectra allowed unambiguous determination of C=C positions in 120 unsaturated GP, revealing a predominance of ω-6 and ω-3 esterified fatty acids.

This study establishes a comprehensive framework for lipid annotation by integrating a CID-MS/MS-based lipid database with advanced fragmentation techniques such as EIEIO-MS/MS for C=C localization. As both analyses were performed using NIST® SRM® 1950, its composition closely reflects that of plasma samples and it is available to all laboratories, facilitating database transfer. In that way, improving lipid identification and structural characterization of unsaturated lipids enhances the accuracy and reliability of lipidomic analyses providing a solid foundation for future research on metabolic regulation, inflammation, and disease pathophysiology.

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TECHNOLOGY ADVANCEMENTS

1287 A — Optimalization of quantitative lipidomics method using UHPSFC-HRMS with a diol column

PRESENTING AUTHOR: *Vit Kosek, University Of Chemistry And Technology Prague, Czech Republic*
CO-AUTHORS: *Kamila Bechynska, Martin Maly, Jana Hajslova*
SUB THEME: *Novel approaches in lipidomics*

Quantitative lipidomics is a well-established field, but there is still a search for more sensitive and effective methods to quantify individual lipid species. Many column chemistries are available for the separation of lipids for their quantification, typically unmodified silica phases for HILIC and normal phase type, but diol phase is rarely used.

Traditional unmodified silica and diol phases were tested for chromatographic separation. Mobile phase modifiers and make-up solvents were also tested. We present an optimized method capable of quantifying many lipid species in plasma using single-point calibration with the SPLASH lipidomic mixture as internal standard, also using in-house lipid mixture for quality control. A wide range of lipid classes are extracted from plasma using a methyl tert-butyl ether/methanol procedure and then separated on a diol column based on their polarity. Different individual lipids from a single lipid class coelute closely together but are partially separated, allowing for better limits of quantitation. Although the diol column acts as a HILIC phase regardless of whether water is added, up to 7% water was found to be beneficial for lipid separation and peak shape, resulting in narrower peaks. The diol phase outperforms unmodified HILIC phases in peak shape for most lipid classes except phosphoserines, but allows better chromatographic parameters for phosphatidic acids. The acquired data are processed in MZmine software and then quantified using LipidQuant software.

The developed method was demonstrated on plasma samples from patients after two different acute endpoints of atherosclerosis: acute coronary syndrome and ischemic stroke, to see how the lipidome changes after each event. In total, 229 plasma lipids were quantified. Using univariate and multivariate tools, we found that the main differences between these two groups were expressed in triacylglycerols, ether-linked phosphocholines, and the individual lipids SM 34:2 and PI 38:4.

1288 A — Data Analysis Methods to Enable 10-second Tissue Pathology Determination with Laser Mass Spectrometry Using Metabolic Features

PRESENTING AUTHOR: *Darah Vlaminck, University Of Toronto, Canada*
CO-AUTHORS: *Alexa Fiorante, Lan Anna Ye, David Munoz, Sunit Das, Gelareh Zadeh, Howard Ginsberg, Arash Zarrine-Afsar*
SUB THEME: *Novel approaches in lipidomics*

Each central nervous system cancer type possesses its own optimal extent (aggressiveness) of resection. To improve surgical outcomes based on this information, the subjectivity of intraoperative tissue diagnosis methods (currently heavily depending on the expertise level of the neuropathologist on call) must be minimized. Picosecond infrared laser mass spectrometry (PIRL-MS) generates a fingerprint of cancer-specific tissue lipids and metabolites under ambient conditions for non-subjective diagnosis in only 10 seconds.

To tease out cancer-specific spectral features in complex datasets, a hierarchical differential diagnosis (decision tree) model optimizing evidence-based adjustment of the extent of resection for lymphoma (no resection), metastatic carcinomas (aggressive resection) and gliomas (maximum safe margin resection), the latter comprised of isocitrate dehydrogenase-1 (IDH1) positive and negative mutational status was created. This tree flows in a logical order based on what a neuropathologist would use for diagnosis, albeit utilized for automated non-subjective diagnosis based on 10-second PIRL-MS lipid analysis. The goal was to optimize the best machine learning (ML) method (from a list of 6 different algorithms) at each discrimination point utilizing dedicated metabolic features optimal for the node and to assess the reproducibility of the extracted metabolite features at each node. The hierarchical method delivered diagnosis of lymphoma, glioma and metastatic tumours in node 1 with sensitivity and specificity of 87% and 94%, respectively and was able to differentiate between IDH1 mutant and WT gliomas with the sensitivity and specificity of >89%, using ‘stable’ features extracted via linear ML methods. The utility of this optimized hierarchical approach with PIRL-MS data is manifested in the users’ ability to adjust the diagnosis depth commensurate with desired performance criteria utilizing node-specific metabolites, enabling incorporation of the clinical ‘cost’ of total resection of one tumour class compared to another. The extracted feature list, accordingly, forms a metabolite array for 10-second diagnosis with PIRL-MS.

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TECHNOLOGY ADVANCEMENTS

1289 A — Towards sustainable lipidomics: chemometrics and experimental workflow for green solvent for lipid extraction

PRESENTING AUTHOR: *Andrea Venturi, ZML at TU Dresden, Germany*
CO-AUTHORS: *Michele Wölk, Sider Penkov, Gabriele Cruciani, Maria Fedorova, Laura Goracci*
SUB THEME: *Novel approaches in lipidomics*

Lipid extraction represents one of the most crucial steps in sample preparation for lipidomics analyses. The selection of an appropriate solvent or solvent mixture strongly impact the lipid extraction efficiency and coverage. Chloroform is one of the most popular solvent in used for lipid extraction due to its superior physicochemical properties (e.g., the capacity to dissolve both polar and apolar lipids, good volatility, and formation of two-phase systems with aqueous solvents) which ensure high recovery of endogenous lipids. Nevertheless, chloroform poses serious risks to human health and the environment. In response, over recent years, there has been a growing interest in finding more sustainable solvents capable of replacing chloroform in common lipidomics protocols while maintaining high extraction efficiencies.

Here we used a combination of chemometrics and experimental approaches to identify and validate “greener” alternatives to chloroform in common lipidomics extraction protocol. In silico analysis, based on the Hansen solubility and Abraham solvation parameters, identified cyclopentyl methyl ether (CPME), 2-methyltetrahydrofuran, and iso-butyl acetate as promising alternatives to chloroform. The selected solvents were experimentally validated for the recovery of synthetic lipid standards and human blood plasma lipids in one- and two-phase extraction protocols. CPME-containing one-phase extraction method provided the highest lipid extraction efficiency, even superior to the conventional chloroform-based Folch protocol. The results emphasize not only the utility of in silico screening for solvent selection but also showcase that more sustainable solvents can be employed in lipidomics without compromising analytical performance. It would be worthwhile to investigate proposed solvent systems for potential application in metabolomics, thus providing comprehensive and sustainable analytical workflow.

1290 A — Development of imine-based covalent organic framework as an adsorbent for per- and polyfluoroalkyl substances detection by on-line SPE LC-MS/MS

PRESENTING AUTHOR: *Hui Ling Lee, Fu Jen Catholic University, Taiwan*
SUB THEME: *Novel samples/matrices*

Perfluoroalkyl Substances (PFASs) have unique chemical properties and are widely used in food packaging, household goods and fire extinguishing agents. PFASs are environmental persistent organic pollutants by the Stockholm Convention, as it can induce various diseases in organisms. In this study, focuses on the development of imine-based covalent organic frameworks as adsorbents. Especially, a fluorinated covalent organic framework (F-COF) was synthesized with 2,4,6-trimethylphloroglucinol (Tp) and 2,2’-bis(trifluoromethyl)benzidine (BD-CF3) under acidic conditions and named as Tp-BD-CF3. This study intends to introduces special fluorine-fluorine interaction into micro-dispersive solid phase extraction (μ-dSPE) to improve the extraction efficiency. The main experimental parameters that affect the extraction efficiency were investigated, such as extraction and desorption time, pH and amount were optimized. Especially, the excellent reusability extraction in 5 consecutive cycles demonstrated cost-effective and eco-friendly material in sample preparation. Finally, to develop the UHPLC–MS/MS method, good linearity within 0.05–5.00 ng/mL was obtained (R2 > 0.9968). The detection limits (LOD) was between 0.002 and 0.15 ng/mL, and the limit of quantification (LOQ) was between 0.006 and 0.500 ng/mL. These results indicate the great potential of fluorinated covalent organic framework as suitable sorbent for the extraction of 11 PFASs from complex real samples.

1291 A — Exploring finger sweat as a proxy biofluid for investigating drug distribution between interstitial fluid and blood

PRESENTING AUTHOR: *Michael Wolf, University of Vienna, Austria*
CO-AUTHORS: *Veronika Pfoser-Poschacher, Daniel Wasinger, Samuel Matthias Meier-Menches, Michael Wolzt, Christopher Gerner*
SUB THEME: *Novel samples/matrices*

Most drug effects take place in the interstitial fluid (ISF), but limited sampling techniques leave this compartment understudied. Importantly, sweat is derived from ISF and may therefore serve as a proxy for ISF drug levels. This study investigated the capabilities of finger sweat (FiS) analysis, a novel non-invasive at-home sampling technique collected within a minute, to analyze distribution of two drugs with distinctive compartmentalization, Dexamphetamine and Zolpidem, between matched FiS and blood samples.

In a clinical trial (EudraCT: 2021-005381-17), 40 participants were randomly assigned to receive either 30 mg Dexamphetamine or 5 mg Zolpidem. EDTA-plasma and FiS were collected at baseline, 3, and 8 hours post-administration. Plasma was extracted by protein precipitation followed by solid phase extraction and FiS by a dilute-and-shoot approach. Absolute drug levels in plasma and FiS were determined by matrix matched external calibration curves. Both sample materials were subjected to the same analytical method using liquid chromatography-mass spectrometry.

Three hours post-administration, Dexamphetamine showed low interindividual variability in plasma (median = 70.5 ng/ml; CV = 14.4%) and large variabilities in FiS (median = 2.06 ng/sample; CV = 85.5%). At the same time point, Zolpidem showed high variability in both plasma (median = 39.9 ng/ml; CV = 51.8%) and FiS (median = 0.11 ng/sample; CV = 108.2%). Remarkably, the average finger sweat sample contained drug levels equivalent to those found in 32.8 µl and 5.4 µl plasma for Dexamphetamine and Zolpidem, respectively.

In agreement with the literature, our data indicate Dexamphetamine to readily distribute into the ISF while Zolpidem remains predominantly in the bloodstream, presumably by its high albumin affinity. The considerable CVs of Zolpidem indicate highly individual compartmentalization, which may be connected to individual drug response and side effects. These findings suggest that FiS holds significant potential as a proxy biofluid to estimate interstitial drug distribution.

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TECHNOLOGY ADVANCEMENTS

1292 A — Investigating the Diabetic Lipidome: A DBS-Based High-Resolution Mass Spectrometry Approach

PRESENTING AUTHOR: ***Jayden Lee Roberts, Australian National Phenome Centre / Murdoch Unive, Australia***
CO-AUTHORS: ***Luke Whiley, Nicola Gray, Melvin Gay, Elaine Holmes, Julien Wist, Jeremy Nicholson, Nathan Lawler***
SUB THEME: ***Other***

Introduction: Comprehensive lipidomic analyses have identified distinct lipid signatures in diabetics, indicating novel lipid markers may aid personalised approaches to monitoring disease. Dried blood spot (DBS) microsamples offer a minimally-invasive and cost-effective method for collecting and storing small sample volumes (typically <50 µL) for this purpose. Additionally, DBS can enable increased sampling frequency and simplified transport via the mail, suitable for remote or resource-limited settings. However, translation of lipidomic profiling of DBS for the diabetic context is limited, and further research is required before routine application of DBS can occur in longitudinal, decentralised, and remote settings.

Methods: Participants (n = 17 diabetic, n = 17 non-diabetic controls) performed DBS self-collection using advanced microsampling devices (10 µL, Capitainer B®). Samples were analysed using an optimised untargeted 4D (timsTOF) method for semi-quantitative lipid analysis using ultra-high-performance liquid chromatography and high-resolution mass spectrometry. The 15-minute workflow enables analysis of 96 samples per day, covering 25 lipid subclasses spanning fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, and sterols.

Results and Discussion: DBS lipid profiling revealed significant differences between diabetic individuals and non-diabetic controls ($R^2Y = 0.77$, $Q^2 = 0.41$, $p = 0.05$). The lipid species driving this difference were consistent with those in matched traditional venous plasma samples ($R^2Y = 0.83$, $Q^2 = 0.49$, $p = 0.05$) and capillary plasma samples ($R^2Y = 0.72$, $Q^2 = 0.49$, $p = 0.05$), highlighting self-collected DBS as a reliable alternative to venous phlebotomy. Associations between lipid species and diabetes-related measures (Problem Areas in Diabetes scale - PAID-20, and Patient Activation Measure - PAM-13), indicate lipidomics may provide insight into relationships between lipid profiles, psychosocial factors, and health-management behaviors in diabetes.

Conclusion: Taken together with our previously demonstrated stability of DBS lipids, preliminary findings indicate the feasibility for longitudinal and remote self-sampling with DBS, enabling personalised health monitoring for diabetes.

1293 A — Enhancing NMR-Based Metabolomics Analysis by Suppressing Sugar Signals in Sugar-Dominated Samples such as Rice, Honey, and Date Using Band-Selective Excitation

PRESENTING AUTHOR: ***Abdul-Hamid Emwas, KAUST, Saudi Arabia***
CO-AUTHORS: ***Upendra Singh, Ruba Al-Nemi, Fatimah Alahmari, Renad Al Ahmadi, Ayindrila Dutta, Amani A. Albalaw, Mariusz Jaremko***
SUB THEME: ***Other***

Nuclear Magnetic Resonance (NMR) spectroscopy stands as a cornerstone in the field of metabolomics, especially when it comes to analyzing intricate mixtures found in nature, such as plants, honey, milk, and biological fluids. Yet, the challenge of identifying metabolites present in low concentrations remains a significant obstacle. Their signals are often overwhelmed by the overpowering peaks from more abundant metabolites, particularly sugars. In this groundbreaking method, we confront the abundant intensity of sugar signals obscuring critical metabolite signals in NMR spectra. We introduce an innovative pulse sequence featuring band-selective excitation designed to suppress signals from sugar's moiety (SSMS). This advancement is not merely a technical adjustment; it is a crucial breakthrough that significantly reduces interference and enhances the detection of metabolites. We tested the SSMS technique across an array of NMR methods, including 1D 1H presaturation (presat), 2D J-resolved (RES), 2D 1H-1H Total Correlation Spectroscopy (TOCSY), and 2D 1H-13C Heteronuclear Single Quantum Coherence (HSQC). The samples analyzed encompassed dates, honey, a glucose stock solution, nine amino acids, and commercial fetal bovine serum (FBS). The results were remarkable, showcasing a substantial reduction in high-intensity sugar signals (1,2). The SSMS technique significantly improved the visibility and sensitivity of metabolite signals in complex mixtures, enabling the identification of a broader range of metabolites. Reducing sugar signal interference enhances NMR's analytical power in metabolomics, opening new possibilities for research and practical applications.

References

1-Upendra Singh, Ruba Al-Nemi, Fatimah Alahmari, Abdul-Hamid Emwas, Mariusz Jaremko. Improving quality of analysis by suppression of unwanted signals through band-selective excitation in NMR spectroscopy for metabolomics studies. Metabolomics (2024) 20:7.

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TECHNOLOGY ADVANCEMENTS

1294 A — Automated Tool for Optimal Quantitative Analysis Conditions Using mzCloud Spectral Library

PRESENTING AUTHOR: *Gergő Bodnár, HighChem s.r.o., Slovakia*
CO-AUTHORS: *Juraj Lutišan, Marynka Ulaszewska, Tim Stratton, Michal Raab*
SUB THEME: *Quantitation in metabolomics*

The mzCloud spectral library has introduced a swift and handy tool that automates the determination of optimal spectral conditions for quantitative analysis of compounds. This tool identifies the five most intensive fragment ions of MS2 HRAM spectra and the best collision energies for each compound, streamlining the analysis process. Utilizing a database of 32,000 compounds and 200,482 MS2 ion tree experiments, it ensures only highest-quality spectral tree are considered. It significantly reduces time and cost of compound optimizations in laboratory settings.

The tool employs an algorithm to search the mzCloud library, performing rigorous quality checks on available MS2 HRAM spectra. Key parameters, such as the number of collision energy steps and the breakdown curve shape of each fragment ion, are evaluated. By scrutinizing the selected parameters, the algorithm ensures that all off the potential fragment ions are provided to the user. For high-quality data, normalized collision energy (NCE) values are recalculated to collision energy (CE). Users can upload lists of compounds via InChIKey for batch processing.

The mzCloud database comprises 32,000 compounds, sourced from both reference and auto-processed libraries. From an initial 3,462,578 of MS2 spectra belonging to 200,482 ion tree experiments, 168,486 met highly stringent quality criteria and were selected for further analysis. Only experiments featuring a sufficient number of collision energy levels, well-distributed between the collision-induced dissociation (CID) and higher-energy collisional dissociation (HCD) axes, were considered for advanced evaluation. The quality of the breakdown curve (BDC) was a pivotal parameter, ensuring well-defined ion kinetics. The current version of the tool recalculates normalized collision energy (NCE) to collision energy (CE) for a variety of instruments, including TribridTM Mass Spectrometers, HybridTM Mass Spectrometers, extending compatibility to triple quadrupole mass spectrometers. This broad applicability enhances the tool's utility across diverse MS platforms.

1295 A — Quantitation of sugar-phosphate metabolites from the pentose phosphate pathway in leaves using liquid chromatography-mass spectrometry with multiple fragmentation techniques

PRESENTING AUTHOR: *Thusitha Rupasinghe, SCIEX, Australia*
CO-AUTHORS: *Ling Ling Chen, Kosala Ranathunge, Hans Lambers, Paul Baker, Thusitha Rupasinghe*
SUB THEME: *Quantitation in metabolomics*

The metabolites in the pentose phosphate pathway (PPP) play a vital role in plant metabolism. Those metabolites play a crucial role in cellular processes, such as biosynthesis of five-carbon sugar metabolites and balancing cellular energy. However, the detection and quantification of phosphorylated metabolites is challenging due to low abundance, high polarity, and chemical instability in plant tissues. With liquid chromatography-mass spectrometry (LC-MS/MS), using a multiple reaction monitoring (MRM) approach, most of the metabolites have similar MRM transitions based on the collision-induced dissociation (CID) fragmentation technique. In this study, we have demonstrated that using an additional fragmentation technique, electron-activated dissociation (EAD), provides unique fragments for specific phosphorylated metabolites which provides additional MRMs for the quantification of sugar metabolites.

Here, we demonstrate, using a single LC-MS experiment with multiple reaction monitoring (MRM) scans using EAD and CID fragmentation for quantitation and detection of metabolites in the pentose phosphate pathway. The limit of quantification (LOQ) was achieved at a low pico-molar (pM) level for all studied metabolites with five orders of linear dynamic range. Using multiple fragmentation techniques, accurate quantification of sugar molecules, when co-eluted with structural homologs, has been successfully achieved in leaf metabolomic analysis.

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TECHNOLOGY ADVANCEMENTS

1296 A — Mapping Metabolic Heterogeneity with a Single-Cell Metabolomics Pipeline Built for Multi-Omics Integration

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CO-AUTHORS: ***Arzu Tuğçe Güler, Jeannet Riley Brook, Naama Kanarek, Boryana Petrova***
SUB THEME: ***Single-cell metabolomics and lipidomics***

Understanding metabolism at the single-cell (sc) level is critical for resolving functional heterogeneity in complex biological systems. While sc transcriptomics and proteomics have advanced rapidly, sc metabolomics remains technically limited due to the chemical diversity, instability, and non-amplifiable nature of metabolites. These impede accurate quantitation and cross-omics integration, limiting broader application in systems biology and disease research.

We present a high-throughput sc metabolomics workflow designed for scalability and multi-omics compatibility. The platform combines stable isotope-based quantitation, gentle cell isolation, and direct-injection mass spectrometry (DIMS) on a Thermo Exploris 480 system to capture native metabolic states in individual cells. Specifically, 13C-labeled K562 cells serve as internal standard to identify biologically derived metabolites and correct for ion suppression. Single cells are isolated using the cellenONE platform—already established in sc proteomics—which enables rapid and gentle dispensing. DIMS combined with our machine learning (ML) data analysis approach removes the need for chromatographic separation, substantially increasing throughput without sacrificing sensitivity.

We are concurrently optimizing protocols for cell isolation and data acquisition, and are streamlining our computational pipeline to ensure robust detection and quantification. DIMS signal intensities are validated using bulk-cell chromatography, which also provides the reference framework for metabolite annotation. Extension of the pipeline will further support integration with transcriptomic and proteomic datasets. All tools are being developed as open-source resources to enhance reproducibility and community adoption.

Ongoing proof-of-concept studies are benchmarking sensitivity, dynamic range, throughput, and reproducibility. These also assess the platform's ability to resolve metabolic heterogeneity across single cells and to integrate additional omics modalities. Ultimately, we aim to demonstrate that this approach offers a practical and scalable means of investigating cell-to-cell metabolic variation—one that not only complements but also integrates seamlessly with existing sc omics platforms.

1297 A — A Highly Sensitive and Selective Targeted Method for Single Cell

PRESENTING AUTHOR: ***Susan Bird, Thermo Fisher Scientific, United States***
CO-AUTHORS: ***Rahul Ravi Deshpande, Ying Liu, David A. Gaul, Facundo Fernandez, Cristina Jacob***
SUB THEME: ***Single-cell metabolomics and lipidomics***

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

Lipids were extracted from one million RK13 and Vero cells and separated on a 75-micron RP column connected to a Vanquish Neo UHPLC system. Data was acquired on the Orbitrap Ascend Tribrid MS using an untargeted multi-stage strategy for fragmentation with HCD and CID both utilized for MS2 followed by CID based MS3 fragmentation of specific product ions generated by the neutral loss of fatty acyl chains. This template for lipid analysis provides individual fatty acids for multiple lipid classes including co-eluting TGs. CD 3.4 with the LipidSearch node was used for data processing and a targeted list of around 1100 confidently annotated lipid species, combining both the cell lines, was generated. A targeted method for the 1100 lipids was created using Parallel reaction monitoring (PRM) on the Stellar MS and the same activation types, HCD and CID MS/MS and MS3 capabilities as on the discovery MS. PRM of the targets significantly enhances specificity and sensitivity for isomeric lipids and structurally similar species.

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

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TECHNOLOGY ADVANCEMENTS

1298 A — Less is more: Low-Volume Plasma Analysis with nanoLC-TIMS-MS/MS

PRESENTING AUTHOR: *Klidel Fae Rellin, Helmholtz Munich, Germany*
CO-AUTHORS: *Lisa Abel, Viola Jeck, Aiko Barsch, Michael Witting*
SUB THEME: *Small volume metabolomics technologies*

Lipidomics is essential for understanding disease mechanisms and progression, identifying biomarkers, and advancing precision medicine and diagnostics. However, the large sample volumes required by conventional LC-MS workflows limit their use in minimally invasive studies, including pediatric research and longitudinal microsampling. To address this challenge, we present a pipeline leveraging nano-liquid chromatography (nanoLC) coupled with trapped ion mobility spectrometry and time-of-flight mass spectrometry (TIMS-TOF) to enable comprehensive lipid profiling from just one microliter of plasma.

We compared two commonly used single-phase lipidomics extraction protocols (MeOH:MTBE versus IPA:H_2O) and evaluated their scalability to 1 µL of NIST SRM 1950 plasma, along with their effects on lipid coverage. Additionally, the impact of mobile phase buffer concentration (10 mM, 5 mM, and 2 mM NH_4HCO_2) on detected lipid species was assessed, including its influence on overall nanoLC performance. We applied multivariate statistical analysis to determine the differences in lipid coverage and distribution and manually verified lipid annotation and mobility values with those from literature and databases. All analyses were conducted using a nanoElute 2-timsTOF HT setup in both positive and negative DDA-PASEF acquisition modes.

Using NIST SRM 1950 plasma as a benchmark, our optimized workflow demonstrated comparable lipid class coverage with just 1 µL of starting material while improving sensitivity for low-abundance lipids and enhancing the separation of structurally similar species. Compared to high-flow LC-MS lipidomics protocols, similar coverage was achievable at lower buffer concentrations, though significant shifts were observed in lipid species dependent on ammonium adduct formation.

These findings highlight the feasibility of utilizing nanoLC-TIMS-TOF for robust lipid profiling from minimal sample volumes, offering an alternative, scalable, and high-sensitivity platform for sample-limited applications, including biomarker discovery and clinical research.

1299 A — Microbore 1.0 mm I.D. UHPLC-HRMS for low input sample analysis as robust and sensitive approach for untargeted metabolomics

PRESENTING AUTHOR: *Danila La Gioia, University Of Salerno, Italy, Italy*
CO-AUTHORS: *Eduardo Sommella, Pietro Campiglia*
SUB THEME: *Small volume metabolomics technologies*

In untargeted metabolomics, enhanced sensitivity is crucial for analyzing small sample volumes and low-abundance metabolites. While 2.1 mm I.D. columns are standard, the potential of 1.0 mm I.D. microbore columns in high-resolution mass spectrometry (HRMS) remains underexplored. This study introduces a microbore UHPLC-HRMS strategy that improves sensitivity, reduces solvent consumption, and broadens metabolome coverage, making it ideal for challenging biological samples.

A Vanquish Neo UHPLC system coupled with an Orbitrap Exploris 120 mass spectrometer was used to evaluate reversed-phase (RPLC) and hydrophilic interaction liquid chromatography (HILIC) separations with 1.0 mm I.D. sub-2 µm columns. Optimized parameters included flow rate, gradient conditions, injection volume, and ionization settings. The method was benchmarked against conventional 2.1 mm I.D. workflows using human plasma, dried blood spots (DBS), and colorectal cancer patient-derived organoids (PDOs) undergoing chemotherapy.

With an optimal flow rate of 100 µL/min, the microbore approach demonstrated excellent reproducibility (≤0.5% CV for retention time, ≤2.3% CV for peak area) and a significant sensitivity gain, with limits of detection (LOD) and quantification (LOQ) of 0.95 ng/mL and 3.18 ng/mL, respectively. Compared to conventional workflows, it yielded a twofold increase in MS1 spectral features and metabolite annotations (MSI level 2), particularly enhancing the detection of low-abundance metabolites. The method proved highly robust over 300 injections while reducing solvent consumption by 80% (300 mL vs. 1.5 L).

The improved detection of metabolic perturbations in PDOs after chemotherapy, including changes in polyamine metabolism, the citric acid cycle, and aromatic acid pathways, highlights the transformative potential of this microbore LC-HRMS method. Offering superior sensitivity and sustainability, it represents a powerful solution for studies with limited or complex sample matrices.

TECHNOLOGY ADVANCEMENTS

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TECHNOLOGY ADVANCEMENTS

1300 A — Cutting Through the Noise: Standardised QC-Centred Feature Curation for Untargeted LC-MS, Proven Across >1 000 Studies

PRESENTING AUTHOR: ***Thorsten Klaus Otto Gravert, Cmbio, Denmark***

CO-AUTHORS: ***Lea Giørtz Johnsen***

SUB THEME: ***Standardization, Reproducibility, QA/QC and ring trials***

Untargeted LC-MS preprocessing software routinely exports thousands of signal features per sample, yet many do not carry meaningful biological information and confound subsequent statistics. They represent background, artefacts, redundant peaks or compounds with poor analytical performance on the applied system.

We developed a vendor-agnostic, quality-control-centred curation pipeline that turns these wide-coverage tables into concise, interpretable metabolite lists with transparent annotation confidence.

Samples are acquired on high-resolution Orbitrap or qToF systems with liquid chromatography in a deliberately structured sequence – randomised injections bracketed by procedural blanks, reference standards, pooled-QC replicates and a three-level QC dilution series. After conventional preprocessing in CompoundDiscoverer, MS-Dial, mzMine, MetaboAnalyst or MetaboScape, the exported matrix is filtered in a MATLAB app using six quantitative rules: QC precision ≤ 20% CV; blank-to-QC signal ratio ≥ 5; representativeness 0.5 ≤ QC: sample mean ≤ 2.0; dilution linearity $\rho \geq 0.80$; missingness in QCs < 25%; and duplicate removal based on m/z–RT proximity combined with intensity correlation. Ambiguous isomers that cannot be chromatographically resolved are reported jointly, and every surviving feature receives an MSI-compliant confidence score.

Applied to more than 1000 commercial and academic studies, this pipeline routinely eliminates 40 – 60 % of initially presented 'compound detections', i.e. faeces/cecum 4 200 ➔ 2 400, culture media 4 400 ➔ 2 000, and plasma/serum 2 700 ➔ 1 200 - while reducing median QC variability drastically. Semi-automatic curation can be performed on a standard laptop, with limited expert knowledge and in a reasonable time frame. Although strict curation narrows chemical coverage, it markedly lowers false-positive findings and clarifies biochemical interpretation.

Inserting this rapid, standardised step after any preprocessing software allows laboratories to harmonise processing outputs, meet QA/QC benchmarks and accelerate the translation of untargeted metabolomics into clinical, environmental and industrial practice.

1301 A — Facing the Challenges of Untargeted Metabolomics: Our Path to Workflow Development and Data Integration

PRESENTING AUTHOR: ***Tommaso Stefani, Institute Of Microbiology, Czech Republic***

CO-AUTHORS: ***Anna Michl, Ondřej Hřebíček, Martin Schwarzer, Zdeněk Kameník***

SUB THEME: ***Standardization, Reproducibility, QA/QC and ring trials***

Untargeted metabolomics requires robust workflows that ensure data quality while handling the complexity of large datasets generated by advanced LC-MS instrumentation. Over the past year, we have developed a comprehensive workflow encompassing chromatographic and mass spectrometry method development, system suitability and quality control (QC) strategies, and multi-platform data processing.

In this work, we share our experience tackling the chromatography dilemma for polar metabolites - HILIC or chemical derivatization? Then, we cover our first-year experience with trapped ion mobility (TIMS) in terms of isomer separation, sensitivity, and MS/MS spectra quality, and the advantage of VIP HESI ion source over conventional ESI. These aspects will be demonstrated on datasets of diverse matrices, including feces and microbial culture broths.

Our data processing workflow combines outputs from Bruker´s MetaboScape, and open-source GNPS, SIRIUS, and MetaboAnalyst into a single network visualized in Cytoscape, providing a more comprehensive view of the metabolome. The workflow's modular design and integration across platforms set the foundation for future automation, aiming to streamline data processing and enhance reproducibility.

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TECHNOLOGY ADVANCEMENTS

1302 A — Quantification of underivatized acylcarnitines and carnitine intermediates using RP chromatography and ion funnel triple quadrupole in fecal samples

PRESENTING AUTHOR: *Daniel Cuthbertson, Agilent Technologies, United States*

CO-AUTHORS: *Bianca Silva, Ruben Ramos, Justin Cross, Karen Yannell*

SUB THEME: *Targeted methods*

Acylcarnitine profile analysis is a commonly requested analysis in genetic disease research laboratories due to their wide metabolic involvement. Thus, acylcarnitine detection can be important markers for numerous disorders as they are intermediates of fatty acid oxidation and amino acids metabolism in tissues and body fluids. Currently, acylcarnitines are often tested using flow injection analysis, which compromises their quantification because various species occur as isomers and/or have very low concentrations. To address many issues inherent to such analysis, a new comprehensive LC-MS/MS method was developed to quantify 32 acylcarnitine species, with acyl-chain lengths from C0 to C20 in fecal samples without derivatization step. Samples were injected into an ion funnel triple quadrupole mass spectrometer coupled to a biocompatible LC system, which is coated with an iron free alloy. Optimal source conditions and MRM transitions for all compounds were determined individually using commercial optimization software. The method achieved the desired separations of the main isomers, such as: (C4) butytryl- and isobutytryl-L-carnitine; (C5) isovaleryl-, valeryl-, and 2-methylbutyryl-L-carnitine; and (C6DC) 3-methylglutaryl and adipoyl-L-carnitine without ion pairing additives, which can cause ion suppression. This separation used a standardized configuration intended for discovery analysis and workflow to allow flexibility for different studies. The chromatography was reproducible, and the figures of merit evaluated for each analyte were linearity, detection and quantification limits, recovery, and precision. Method reproducibility showed RSD < 14%. Limits of quantification ranged from 0.01 to 1 ng/L for all metabolites. All the compounds showed a great linearity (R2 > 0.994) and RSD below 12%. This data shows the method is capable of quantifying acylcarnitines and carnitines intermediates at levels relevant to fecal for monitoring metabolic alterations. The method developed on the standardized system can be easily transferred and applied to perform different experiments from pathway discovery metabolomics to absolute quantitation.

1303 A — Validated method for simultaneous quantification of a broad range of tryptophan metabolites in multiple matrices shows promise in explaining the dietary-microbiome-immune system link.

PRESENTING AUTHOR: *Ulrike Brüning, BIH Metabolomics platform Berlin, Germany*

CO-AUTHORS: *Jenny Meinhardt, Belen Millet Pascual-Leone, Morgan Essex, Jennifer Nazat Martinez Medina, Victor Hugo Jarquin Diaz, Bastian Opitz, Sofia Forslund, Helena Radbruch, Jennifer Kirwan*

SUB THEME: *Targeted methods*

Tryptophan (Trp) and its metabolites play key roles in the immune system, heart and brain health. The eventual fate of dietary Trp involves an interplay between the host and its gut microbiome. Characterization, including identification and quantification, of a broad range of Trp metabolites provides important information about the microbiome-immune regulation link.

We demonstrate the development and application of a robust, accurate, reproducible targeted method for the quantification of 34 compounds related to the Trp pathway for the investigation of inflammatory conditions, especially Covid-19.

The method uses an ultrahigh performance liquid chromatography tandem mass spectrometry technique and was validated for serum and plasma samples following European Medicines Agency guidelines. Additionally, the method was successfully tested and adapted for other matrices such as feces, mucus, interstitial fluid and cerebral spinal fluid (CSF). The novel method was successfully used in both an acute and a long COVID study.

We report on the analytical linearity, limit of detection, limit of quantification, matrix effect, selectivity, carryover, recovery and precision of the method. We also discuss the biochemical limitations of using plasma for certain tryptophan measurements. Stability of standards at various temperature conditions were also investigated. At 10 degrees, all tested compounds were stable for up to 36 hours. For long-term storage, Trp metabolites in samples and extracts were stable for up to 6 months at -80 degrees. In a study of hospitalized COVID patients, tryptophan metabolites seemed to be a crucial link between the microbiome and immune system activation. In a second study of long COVID patients, metabolites associated with microbiome tryptophan metabolism were increased in CSF in subsets of patients. Interestingly, additional tryptophan metabolites not normally seen in blood e.g. oxoadipic acid were also detected, highlighting the need for a broad panel and to investigate beyond plasma and serum in clinical studies.

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TECHNOLOGY ADVANCEMENTS

1304 A — A Comprehensive Targeted Metabolomics Workflow for Efficient Profiling Across Diverse Biological Matrices

PRESENTING AUTHOR: *Mohamad Saoud, Heidelberg University, Germany*
CO-AUTHORS: *Erwin Kupczyk, Hagen Gegner, Rafael Duenas-Sanchez, Roland Palkovacs, Rüdiger Hell, Gernot Poschet*
SUB THEME: *Targeted methods*

Targeted metabolomics workflows are often constrained by limited analyte coverage and high per-sample costs of commercial kit-based assays, leading to incomplete metabolic insights. Here, we present a comprehensive and cost-efficient targeted metabolomics platform that combines broad analyte coverage with high throughput across diverse biological matrices. Utilizing the MetaSci Complete Library - encompassing >1,200 metabolites spanning central carbon, lipid, amino acid, and nucleotide pathways - our method can be tailored from focused analyses of specific compound classes to broad screening of several hundred metabolites per sample. Analytical measurements employ a QTRAP 6500+ mass spectrometer with dual HILIC and reversed-phase chromatography to accommodate polar and nonpolar analytes. We demonstrate the workflow's versatility and robustness through metabolic profiling of mammalian tissues (e.g., mouse liver), plant tissues (e.g., Arabidopsis thaliana leaves), biofluids (e.g., human plasma) and cultured cells (e.g., cancer cell lines). Each matrix yields detection and quantification of hundreds of metabolites within a single analytical experiment. Compared to commercial kit-based assays or untargeted approaches, our platform reduces per-sample costs significantly while maintaining high sensitivity and reproducibility. This user-friendly workflow offers a scalable and modular solution for targeted metabolomics in academic and clinical research settings.

1305 A — Development of a Cost-Effective Targeted Mass Spectrometry Method for plasma quantification of 27 Bile Acids in Large Study Populations

PRESENTING AUTHOR: *Alice Passoni, Istituto Ricerche Farmacologiche Mario Negri IRCCS, Italy*
CO-AUTHORS: *Federica Maggi, Antonio Multari, Matteo Preziati, Laura Brunelli*
SUB THEME: *Targeted methods*

Bile acids (BAs) play essential biological roles, ranging from facilitating lipid and fat-soluble vitamin absorption to acting as signalling molecules through dedicated cellular receptors. Dysregulation of bile acid synthesis or signaling pathways, often driven by dietary patterns, has been increasingly linked to various human diseases, including metabolic disorders, type 2 diabetes, and even neurodegenerative diseases. Understanding the complex interplay between diet, bile acid metabolism, and disease pathology provides insights into disease mechanisms but also offers promising therapeutic opportunities through targeted dietary interventions. The BAs' diverse physicochemical properties make quantitative analysis in biological matrices particularly challenging, requiring careful optimization to ensure accuracy and reproducibility. Most existing methods achieve the required sensitivity through solid-phase extraction but are often limited to a reduced panel of metabolites. Here, we present the development and validation of a liquid chromatography-mass spectrometry method, using a triple quadrupole instrument (LCMS8060, Shimadzu) for the quantification of 27 BAs, including cholic acid, deoxycholic acid, chenodeoxycholic acid, glycochenodeoxycholic acid, taurocholic acid, lithocholic acid, ursodeoxycholic acid, and several oxo and keto derivatives —using a cost-effective sample preparation in a 96-well Isolute PPT+ protein precipitation plate (Biotage, USA). The method was validated according to the European Medicines Agency (EMA) guidelines, ensuring the complete isomer separation within a 17-minute chromatographic run. he chromatographic run and the automatic sample preparation guarantee the extraction and analysis of the samples within 24 hours, which is well-suited to the low stability of some BAs in both matrix and extracts. Validation results confirmed high accuracy, precision, recoveries over 90% for all tested analytes, and linearity ranges established based on physiological concentrations and current literature. The method achieves a Lower Limit of Quantitation (LLOQ) between 12.5 and 25 ng/mL. In conclusion, this method is designed for high-throughput analysis, providing a robust and reproducible approach for large-scale epidemiological and clinical studies.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2000 B — Enabling FAIR Comparative Lipidomics with LipidCompass

PRESENTING AUTHOR: ***Nils Hoffmann, Forschungszentrum Juelich, Germany***

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SUB THEME: ***Collaborative and open data science***

Lipids are vital to biological systems, with disturbances in lipid homeostasis linked to various diseases. Despite the advancements in lipidomics, the field lacks a central reference point for the occurrence and concentration of lipids. We introduce LipidCompass, a community-driven repository, aligned with the FAIR principles, to simplify discovery, comparison and visualization of lipidomic data from various sources, opening up new perspectives and potentially advancing the field of lipidomics research.

The input data format, mzTab-M, allows study data to be mapped to standardized terminology defined by controlled vocabularies such as NCIT and PSI-MS. The embedded lipid nomenclature converter, Goslin, translates lipid names into the latest lipidomics shorthand nomenclature, facilitating cross-linking to public databases like LIPID MAPS, SwissLipids, and ChEBI. Additionally, data is represented according to the hierarchical levels of the shorthand lipid nomenclature, enabling comparisons of studies measured at different structural resolutions at the most common level.

The initial studies included as a starting point encompass more than 11,000 distinct lipids across all hierarchical lipid levels, highlighting LipidCompass's potential for large-scale comparative data analysis. Consequently, LipidCompass serves as a comprehensive knowledge base on qualitative and quantitative lipid diversity across different species, tissues, and conditions.

As a central component of the Lipidomics Informatics for Life-Science (LIFS) project, LipidCompass will integrate with services like LipidXplorer and LipidSpace. Collaborations with the International Lipidomics Society will further enhance standardization, integrating support for the lipidomics checklist, leveraging a community-guided reviewing and curation model and thereby solidifying LipidCompass as a reference resource in comparative lipidomics.

2001 B — Leveraging open science cheminformatics tools for non-targeted metabolomics in C. elegans

PRESENTING AUTHOR: ***Gianfranco Frigerio, San Raffaele Scientific Institute, Italy***

CO-AUTHORS: ***Yunjia Lai, Emma L. Schymanski, Gary W. Miller***

SUB THEME: ***Collaborative and open data science***

The nematode Caenorhabditis elegans is an established model to study biological responses to genetic and environmental perturbations. In this study, we present a workflow for the non-targeted analyses of C. elegans samples, particularly leveraging the latest state-of-the-art open science tools.

We applied two distinct metabolite extraction schemes, monophasic and biphasic, to extract metabolites from C. elegans samples belonging to a wildtype strain, two knockout strains of xenobiotic-metabolising enzymes, and two transgenic strains associated with neurodegeneration. Separated pooled quality control samples were prepared for each sample group. LC-MS/MS analyses were performed in HILIC with positive polarity and in RPLC with negative polarity. Data processing was performed using patRoon and MS-DIAL. patRoon enabled the integration of IPO, XCMS, CAMERA, and MetFrag, with the latter using PubChemLite as chemical database. Moreover, we expanded the existing WormJam database with additional C. elegans-specific metabolites from PubChem and from the literature, which was then integrated into the workflow via MetFrag. Moreover, in silico spectral libraries were generated for the expanded WormJam database using CFM-ID and then used to build MSP libraries that were used in MS-DIAL, and compared with the processing with the publicly available MSP libraries.

By comparing monophasic and biphasic extraction schemes, we found that the former provided higher metabolite coverage in HILIC analyses, while the latter yielded more features in RPLC. Data processing with patRoon and MS-DIAL highlighted their respective strengths, with MS-DIAL enabling broader annotation, particularly via public MSP libraries; however, the custom in silico MSP libraries improved annotation quality by reducing unreliable candidates. Finally, we identified several significant metabolites in knockout and transgenic strains compared with the wildtype.

In conclusion, by leveraging cheminformatics open science tools, we provide a scalable approach for metabolite annotation to characterise C. elegans metabolome.

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★ AWARD WINNERS

COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2002 B — White Noise Normalization (WiNN): a pipeline for reducing technical variation in high-dimensional metabolomics datasets

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CO-AUTHORS: *Malte Londschien, Anja Sjöström, Antoine Jeanrenaud, Franco Giulianini, Yanyan Liu, Heike Luttmann-Gibson, Samia Mora, Olga Demler*

SUB THEME: *Data processing & software tools*

Untargeted mass spectrometry enables the detection of thousands of metabolite concentrations in biological samples, but often suffers from substantial technical variability such as batch effects, instrument drift, and experimental inconsistencies. We introduce White Noise Normalization (WiNN), a multi-step preprocessing method designed to reduce technical noise in metabolomics data while preserving underlying biological signals.

WiNN systematically identifies metabolites with temporal drift in their intensities using autocorrelation analysis and applies penalized generalized additive models (GAMs) to conservatively correct drifts along sequential measurements. If explicit batch (plate) information is available, detrending is batch-specific, and residual batch effects are corrected using ANOVA-based residualization or ComBat algorithms. In the absence of explicit batch identifiers, WiNN incorporates a modified Pruned Exact Linear Time (PELT) algorithm to segment the data based on measurement order, with the resulting segments serving as surrogate “batches”.

To further minimize technical variability across samples, WiNN can perform median adjustments using either shrinkage or normalization approaches, based on deviations from a reference spectrum derived from control samples or global metabolite medians. Outlier metabolite values are winsorized rather than trimmed, preserving information about metabolite rank-order even at extremes of detection limits. WiNN is available as an R package (https://github.com/ratschlab/winn), offering flexible parameters including automated optimization based on provided control samples.

Evaluation of WiNN on a melanoma patient-derived clinical dataset with randomized sample measurement orders, replicate measurements, and control samples demonstrated significant improvements in data quality metrics (intraclass correlation coefficients and signal-to-noise ratios) compared to conventional preprocessing methods such as moving median correction. Further, data preprocessed with WiNN showed superior performance in classifying biologically distinct patient groups using Elastic Net regression classifiers.

These findings show that WiNN reduces technical variability and enhances biological signal fidelity, demonstrating its utility for accurate and reliable metabolomics analysis.

2003 B — Automated integration and quality assessment of chromatographic peaks in LC-MS data using TARDIS

PRESENTING AUTHOR: *Pablo Vangeenderhuysen, Ghent University, Belgium*

CO-AUTHORS: *Matthijs Vynck, Beata Pomian, Kimberly De Windt, Emile Callemeyn, Ellen De Paepe, Lindsey De Commer, Jeroen Raes, Tim Nawrot, Johannes Rainer, Lieselot Y. Hemeryck, Lynn Vanhaecke*

SUB THEME: *Data processing & software tools*

Introduction: Within the field of computational metabolomics, significant efforts have been made in the development of open-source software to (pre) process untargeted LC-MS data. However, advances in tools for targeted preprocessing have been comparatively limited and the few existing open-source options are often not maintained nor thoroughly tested on biological data. Furthermore, reliance on vendor-specific software can hamper the translation and transparency of methods and results while remaining time consuming. We therefore present TARDIS, an open-source R package with graphical user interface (GUI) for targeted LC-MS data analysis.

Aim: To develop and benchmark an R package that enables high-throughput, reliable and reproducible LC-MS peak integration.

Methods: A total of 547 saliva, 329 urine and 292 fecal samples from three different cohorts (FAME, ENVIRONAGE and FGFP) were in-house analyzed (LC-MS metabolomics and lipidomics) and preprocessed using TARDIS and Xcalibur™. Preprocessing time and coefficients of variation (CV) of targets reported by each software were compared and correlation between peak areas was quantified by Spearman’s rank correlation (ρ) and linear regression (R2). Integrated peaks were labelled as “Bad”, “Ambiguous” or “Good” and a machine learning classifier was trained using TARDIS-derived quality metrics to predict these labels.

Results: TARDIS succeeded to detect 215 targets in 700 LC-MS runs in less than four hours. CV of target peaks was comparable between TARDIS and Xcalibur™ and ρ and R2 between reported areas were high (mean ρ = 0.96, mean R2 = 0.94). A random forest classifier trained on multi-matrix data predicted 98.57% of peaks labelled “Bad” correctly, allowing to filter out low-quality targets without falsely disregarding high-quality targets.

Conclusion: Our results show that TARDIS provides comparable results to state-of-the-art vendor software while enhancing reproducibility, transparency and time efficiency. Included quality metrics revealed the potential of machine learning to enhance peak filtering. TARDIS is freely available at https://github.com/UGent-LIMET/TARDIS.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2004 B — Interactive Metabolomics Exploration in mzmine

PRESENTING AUTHOR: *Robin Schmid, mzio GmbH, Germany*

CO-AUTHORS: *Steffen Heuckeroth, Ansgar Korf, Simone Zuffa, Corinna Brungs, Roman Bushuiev, Niek F. de Jonge, Pieter Dorrestein, Mingxun Wang, Tomas Pluskal*

SUB THEME: *Data processing & software tools*

Untargeted high-resolution mass spectrometry (HRMS) drives the discovery of metabolites, transformation products, and novel natural products. While established explorative analysis strategies convert raw MS data into feature lists for statistical prioritization and annotation, Feature-based Molecular Networking (FBMN) enhances this process by propagating annotations through MS2 similarity networks. However, current workflows often split into multiple tools, disconnecting raw data from features and downstream results.

We introduce an mzmine pipeline that integrates interactive Molecular Networking with statistical prioritization and compound annotation in graphical dashboards. This system links feature results from mzmine or external tools like metaMASST for comprehensive data inspection. When selecting statistically significant features, mzmine automatically displays feature shapes and annotations while zooming into nearest-neighbor molecular networks, improving visualization of complex datasets that typically produce dense, uninterpretable hairball-like networks. The mzmine workflow incorporates PyTorch models (DreaMS and MS2DeepScore) for spectral similarity scoring. Our implementation includes the metaMASST tool, which performs repository-scale MS searches against public studies, mapping matches to various ontologies (Global FoodOmics) and taxonomies (plants or microbes). These matches provide insights into potential sources of features and the uniqueness of fragmentation patterns across study types.

We validated our approach using public datasets from human gut microbiome and Piper plant extracts. Feature detection, annotation, and molecular networking using modified cosine and DreaMS similarity generated complex networks that were effectively navigated through interactive dashboards. Each selected node was automatically searched against billions of spectra in public repositories including MetaboLights, GNPS/MassIVE, and MetabolomicsWorkbench. As expected, microbiome features were predominantly detected in microbial and clinical datasets, while plant extract features appeared mostly in plant and food studies.

The web service fasst.gnps2.org and open-source mzmine code are available on GitHub, providing a robust platform for comprehensive metabolomics exploration in multi-modal MS datasets including those from LC-MS, GC-EI-MS, ion mobility-MS, and MS imaging.

2005 B — Evaluation of New Computational Approaches for the Analysis of Metabolomic Data Generated by GC×GC-TOFMS

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CO-AUTHORS: *Thibaut Dejong, Kinjal Bhatt, Meriem Gaida, Pierre-Hugues Stefanuto, Jean-François Focant*

SUB THEME: *Data processing & software tools*

Study Background:

Non-targeted screening of small molecules is essential in metabolomics, allowing comprehensive characterization of complex biological samples. Gas chromatography coupled with comprehensive two-dimensional mass spectrometry (GC×GC-MS) offers high separation resolution, peak capacity, and detectability making it the preferred method for such analyses. However, data processing remains a challenge due to the complexity of GC×GC datasets, retention time drifts and instrumental fluctuations. Benchmarking computational solutions is particularly difficult. To address this, we applied recent software tools for simultaneous sample processing, generating composite peak tables to simplify data review and improve consistency.

Sample Description and Analytical Approaches:

We analyzed human stool samples from omnivorous (O) and vegetarian (V) subjects preserved as liquid (Liq) or lyophilized (Lyo), with three biological replicates per class (OLiq, OLyo, VLiq, VLyo). Samples were analyzed using GC×GC-TOFMS (Pegasus BT 4D, LECO) with a normal orthogonality column set. Volatile organic compounds (VOCs) were extracted using solid-phase microextraction (SPME) with three different fibers to assess dietary and storage effects.

To further examine compound volatility, we applied two derivatization methods targeting lipids (Lip) and metabolites (Met): sodium methoxide and boron trifluoride (BF3) for lipids, and methoxyamine hydrochloride with MSTFA for metabolites, yielding eight additional classes.

Software Approach and Translational Methods:

GC×GC data were processed using three computational tools: (1) the Tiles approach for targeted chromatogram segmentation, (2) peak regions defining structured areas of interest, and (3) the 2D trace for collective dataset processing. These approaches enabled VOC, lipid, and metabolite comparisons across sample classes, improving trend identification and reducing processing time.

These computational strategies enhance our understanding of diet- and storage-related biochemical variations, supporting advancements in microbiome research and personalized medicine.

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★ AWARD WINNERS

COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2006 B — Development of an automated processing pipeline for LC-IM-QToF raw data

PRESENTING AUTHOR: ★ *Jessica O'Loughlin, The University Of Edinburgh, United Kingdom*
CO-AUTHORS: *Karl Burgess, Louise Horsfall, Catherine McNay, Sarah Windsor*
SUB THEME: *Data processing & software tools*

Liquid Chromatography (LC), Ion Mobility (IM), and Quadrupole Time of Flight (QToF) mass spectrometer methods - separating ions based on their chemical, physical and structural properties – offers valuable metabolic insights into complex biological samples. However, the scientific understanding that can be derived from these results is only as reliable as the steps used to process the raw data. Especially for data collected with an IM dimension, where very few existing pipelines can process this information. Currently, vendor software packages used to achieve this are time consuming to use - limiting their throughput - and their proprietary algorithms have not caught up with state-of-the-art methods and are closed source, preventing thorough verification of their accuracy.

An open source, programmatic pipeline can resolve these issues, combining all the processing steps together whilst allowing fine-grained control over the full process. Here, we present a Python-based workflow using the Data Extraction for Integrated Multidimensional Spectrometry Conda environment (DEIMoS, Colby et al. 2022 DOI: 10.1021/acs.analchem.1c05017), ipaPy2 module (Del Carratore et al. 2023 DOI: 10.1093/bioinformatics/btad455) metabolite annotation capabilities, and additional mathematical functions to achieve this. DEIMoS picks peaks from MS1 and MS2 raw spectra before they undergo MS2 Extraction, Isotope Detection, Retention Time Alignment, CCS Calibration, and Cross-sample Agglomerative Clustering. A novel Gap Filling algorithm – using m/z, Retention Time, and IM derived Drift Times to integrate missing peaks - is applied to filter the feature table that can then be annotated using ipaPy2, creating a dataset ready for statistical analyses.

With this workflow, the time taken to process this raw MS data is substantially reduced compared to vendor software. The range of outputs available at each processing stage as well as the ability to customise this pipeline allows performance quality to be critically evaluated and optimised to any research group or facility's needs.

2007 B — Reducing False Annotations in Lipidomics

PRESENTING AUTHOR: *Ralf Tautenhahn, Thermo Fisher Scientific, United States*
CO-AUTHORS: *Rahul Ravi Deshpande, Giorgis Isaac, Aaron Robitaille, Bashar Amer, Thomas Moehring, Susan Bird*
SUB THEME: *Data processing & software tools*

Confident annotation is essential for translating untargeted lipidomics data into meaningful biological information. False positives, false annotations and unintended MS1 fragmentation are critical challenges in lipidomics, affecting data accuracy and interpretation. False annotations arise when lipids are misidentified due to spectral similarities, database limitations, or incorrect adduct assignments, leading to erroneous biological conclusions. Unintentional MS1 fragmentation occurs when lipids break down within the ion source or the ion path of the mass spectrometer, generating fragment ions that may be mistaken for distinct lipid species. This complicates lipid identification and quantification, increasing false discovery rates. Here we show the utility of Compound Discoverer software for grouping of unintentional MS1 fragments to avoid false annotations and confident annotation of lipids. Experiments were done using bovine liver extracts and lipid standards. Lipids were separated on the C30 column. The presence of large number of isobaric species requires the use of high resolution.

On a modified Orbitrap Hybrid, a deep scan workflow was used, resulting in over 70% of the detected compounds being fragmented. Compound Discoverer 3.4 with an integrated LipidSearch node was used for data processing. The software can group unintentional MS1 fragments with its precursor, leading to fewer false annotations. The use of LipidSearch and mzCloud spectral library leads to confident annotation of lipids with strategies to classify the confidence of annotation using spectral matching as well as the structural information of MS2 fragments being observed. Using this strategy, the number of compounds identified fell by more than 50% when all the unintentional MS1 fragments were grouped together. The mild trapping option also reduced the number of MS1 fragments.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2008 B — QC4Metabolomics: Real-time and Retrospective Quality Control of Metabolomics Data

PRESENTING AUTHOR: *Jan Stanstrup, University Of Copenhagen, Denmark*
CO-AUTHORS: *Lars Ove Dragsted*
SUB THEME: *Data processing & software tools*

Robust results and sound conclusions can only be achieved when high quality data are available. In metabolomics this is particularly difficult to achieve since the large datasets Necessarily require long acquisition times – typically weeks to months.

Because of the production time, critical problems can remain hidden until initial data analysis, at which point many hundreds of samples might have been analyzed. At this time it will be expensive and time-consuming, if at all possible, to re-analyze the samples. The common practice of spot-checking during the run is unreliable for identifying issues that, while non-obvious, can have detrimental effects on data consistency. As metabolomics is maturing to the point where it can form the basis of clinical diagnostic platforms, a definitive requirement will be tight control of data quality.

To this end, we have developed an automated system that tracks quality parameters and generates dynamic online reports in real-time. Common problems can be detected as the samples are being analyzed, thereby giving the operator the ability to identify and correct any issues immediately and the report can be used to document data quality after the analysis. The system tracks selected compounds for retention time drift and line broadening, loss of m/z calibration and sensitivity loss. It is also possible to concurrently monitor the levels of known contaminants, so that the operator is warned if contamination reaches unacceptable levels. The system is fully open source, modular, extendable and based on R, allowing bioinformaticians to tune or extend the system to specific needs.

URL: https://github.com/stanstrup/QC4Metabolomics

2009 B — CcpNmr AnalysisMetabolomics: A versatile graphical tool for reference-based profiling of 1D1H NMR-based Metabolomics data.

PRESENTING AUTHOR: *Morgan Hayward, University Of Leicester, United Kingdom*
SUB THEME: *Data processing & software tools*

The AnalysisMetabolomics program is a recent development of the CcpNmr Analysis Version 3 package: a freely-available software suite for biomolecular NMR for visualisation and interpretation of NMR data, written in the Python programming language. AnalysisMetabolomics contains tools for interactive graphical identification and quantification of metabolites, aka profiling, of 1D1H NMR spectra via reference to accurate simulated metabolite standards.

Metabolite profiling in AnalysisMetabolomics is flexible and fully interactive and can be performed manually and semi-automatically. It is supported by the Ccpn Analysis Simulated Metabolomics DataBase (CASMDB), a remediated collection of simulated 1D1H metabolite standards data for 1938 distinct metabolites, gathered from three publicly available repositories: the HMDB, BMRB and GISSMO libraries. AnalysisMetabolomics also provides tools for users to quickly build their own simulated references for custom libraries to support profiling, in addition to the references provided in the CASMDB.

Furthermore, AnalysisMetabolomics is also backed up with extensive user support through the CCPN user forum, clear graphical tutorials and YouTube Video tutorials.

2010 B — Using machine learning and metabolomics to redefine cardiometabolic risk phenotypes in children with obesity

PRESENTING AUTHOR: *Mahsa Naghavi Sheikholeslami, IISPV, Spain*
CO-AUTHORS: *Camilla Guerrini, Mariona Gispert, Natalia Ferré, Verònica Luque, Joaquín Escribano, Noelia Ramirez*
SUB THEME: *Machine Learning and AI*

Childhood obesity represents a significant public health challenge associated with complex clinical consequences. The prevalence among children and adolescents has escalated dramatically from 8% to 20% over the past three decades. Cardiometabolic diseases are the predominant comorbidities linked with childhood obesity; however, the existing parameters utilised for assessing cardiometabolic risk frequently underestimate the associated hazards and fail to support effective preventive strategies. This study introduces a refined approach to cardiometabolic risk assessment by integrating ^{1}H-NMR metabolomics and machine learning analyses. The study population included 267 children with obesity (aged 8–13 years) from the COLOBEPED cohort. ^{1}H-NMR spectroscopy was used to characterize their metabolic profiles, identifying 59 metabolites, including 17 polar metabolites, 23 lipoproteins, 5 glycoproteins, and 14 lipids. An unsupervised K-means clustering algorithm was applied to identify children into metabolically distinct subgroups. The association between these clusters, individual metabolites, and cardiometabolic risk was assessed using advanced statistical methods. Two distinct clusters emerged from the analysis. Cluster 1 exhibited a metabolically unhealthy profile, characterized by atherogenic dyslipidemia, elevated low-density lipoproteins, and increased inflammatory biomarkers, with a strong association with cardiometabolic risk. Cluster 2 represented a more metabolically healthy profile, with higher high-density lipoprotein levels, lower inflammatory biomarkers, and reduced cardiometabolic risk. When comparing these findings with traditional classifications, 30.3% of children were misclassified, demonstrating the limitations of clinical-based risk assessment. Our results highlight that relying exclusively on conventional clinical parameters overlooks critical metabolic variations linked to long-term disease risk. By incorporating metabolic profiling and machine learning, we identified children classified as metabolically healthy under traditional methods but who, based on their metabolic signatures, exhibit profiles indicative of increased cardiometabolic risk. This integrative approach provides a more precise risk stratification, allowing for earlier and more targeted interventions to prevent chronic disease progression in children with obesity.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2011 B — Structure-enabled enzyme function prediction unveils elusive terpenoid biosynthesis in archaea

PRESENTING AUTHOR: *Tomáš Pluskal, IOCB Prague, Czech Republic*

CO-AUTHORS: *Raman Samusevich, Téo Hebra, Josef Sivic, Tomáš Pluskal*

SUB THEME: *Machine Learning and AI*

The exponential growth of uncharacterized enzyme sequences in genomic repositories demands novel tools for functional annotation. Here, we combined alignment-driven structural domain analysis with protein language models to create EnzymeExplorer, a machine-learning pipeline for enzyme function prediction. We applied this approach to terpene synthases (TPSs), which present an ideal model case because they catalyze complex carbocationic rearrangements whose product outcomes cannot be predicted from active site residues alone. TPSs are responsible for the biosynthesis of the largest class of natural products, including widely used flavors, fragrances, and first-line medicines. EnzymeExplorer analysis of the UniRef90 database revealed previously undetected structural domains and identified TPSs overlooked by current computational methods. Remarkably, we uncovered and experimentally validated the widespread biosynthesis of terpenoids in Archaea. Our approach offers a powerful framework for characterizing the unannotated “dark matter” of protein sequences in the rapidly expanding genomic and metagenomic datasets.

Preprint: https://doi.org/10.1101/2024.01.29.577750

2012 B — Oversampling of plasma metabolomics data to improve machine learning-based classification models. A case-control study on brain tumors.

PRESENTING AUTHOR: *Adrian Godlewski, Medical Univ. of Bialystok, Poland*

CO-AUTHORS: *Krzysztof Solowiej, Patrycja Mojsak, Joanna Godzien, Julia Zelkowska, Magdalena Rybaczek, Tomasz Lyson, Joanna Reszec, Karol Kaminski, Marcin Moniuszko, Adam Kretowski, Michal Ciborowski*

SUB THEME: *Machine Learning and AI*

Differences in the number of cases and controls in biomarker-oriented studies can lead to misleading or limited conclusions. Such imbalances may distort the biomarkers’ sensitivity, specificity, and clinical value assessment. Oversampling algorithms can enhance predictive coefficients by artificially increasing the number of observations of the minority group. However, in the case of metabolomics studies, variability in human biological samples and fluctuations in spectral measurements introduce noise into the data, which may introduce artefacts into oversampled data. Therefore, our study aimed to evaluate the impact of oversampling on the developed classification models obtained for plasma metabolomics data of controls and glioma and meningioma patients. The diversity of metabolomic data was addressed using untargeted GC-MS and LC-QTOF-MS and targeted LC-QQQ-MS data.

The study groups consisted of 65 glioma and 68 meningioma patients and 500 controls. Patients’ metabolomics data were randomly undersampled, followed by oversampling back to their original sizes using the Synthetic Minority Oversampling Technique (SMOTE) algorithm. Classification models were developed using support vector machine (SVM) and random forest (RF) algorithms, with evaluations based on predictive power, model overfitting metrics, and revalidation results.

The calculations were repeated 100 times, randomly selecting samples each time to validate the oversampling process. After implementing the SMOTE algorithm, the classification model utilizing 10 glioma samples across all analytical platforms showed enhancement. However, the models developed for the 10 meningioma patients and the synthetic samples exhibited a low Jaccard index (less than 0.5), indicating a significant difference between the artificially generated and original datasets. Models developed on targeted data were revalidated, revealing an average increase of 1.5% in accuracy and F1-score values for the SVM algorithm, while the values for RF increased by 6% and 4%, respectively.

Funding: Ministry of Education and Science within the project “Excellence Initiative - Research University”, Medical University of Bialystok (B.SUB.24.117).

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2013 B — SpectruMS: A Multi-modal Foundation Model for Better Generalizability on Tandem MS2 Data

PRESENTING AUTHOR: *Yoann Gloaguen, Pangea Bio, Germany*

CO-AUTHORS: *Daniel Crusius, Tornike Onoprishvili, Jui-Hung Yuan, Vijay Ingalalli, Lila Khederlarian, Gustavo Bremo, Kamen Petrov, Niklas Leuchtenmuller, Sona Chandra, Aurelien Duarte, Andreas Bender*

SUB THEME: *Machine Learning and AI*

Mass spectrometry (MS) is able to effectively determine the metabolite fingerprint of a biological sample, such as a plant, and hence to provide information about its constituent compounds. Although MS is considered a primary method for structure elucidation, its power is restricted by the scarcity of available annotated tandem MS data. Recent machine- and deep-learning approaches, including Graph Neural Networks and Transformers, aimed to bridge this gap by predicting structures from spectra, and vice versa, without relying on reference spectral databases. Despite the competitive performance of these models, their debatable generalizability on unseen chemical spaces hampers their reliability in real-world applications.

We present a series of large transformer models and tokenizers incorporating semi-supervised learning to mitigate data scarcity, enabling better generalization across unseen molecular structures and spectra. Our state-of-the-art multi-modal foundation model along with its tokenizer are pre-trained on tens of millions of chemical compounds and unlabeled MS2 spectra data independently, leveraging robust translation between these learnt languages. Moreover, extra attention is given to chemical space exploration across the models' predictions ensuring the generalizability of our models in downstream applications such as spectra-to-structure and structure-to-spectra translation.

In order to evaluate our models' performances, we first filtered out any chemical compounds from the training data that belong to the benchmark dataset of interest. This filtering step ensures that the model is able to predict new molecular structures. Our primary models are able to generate over 90% valid molecular structures with up to 5% of the time ranking the correct prediction as the top 1 candidate, 14% and 20% in the top 10 and 50 candidates, respectively. Our work shows that utilising the large available unlabeled data for pre-training is essential for transformer models to learn MS language, stepping forward from database dependent identification to de novo structural elucidation of unknown compounds.

2014 B — Automated Bitter Compound Screening and Molecular Docking Reveal TAS2R14 as Primary Target & Triterpenoids as Key Bitter Compounds in Sweet Basil (Ocimum basilicum)

PRESENTING AUTHOR: *Shashikant Kotwal, Rutgers University-New Brunswick, United States*

CO-AUTHORS: *Utsav Kumar, James Simon, Qing-li Wu*

SUB THEME: *Machine Learning and AI*

Bitter taste in some sweet basils (Ocimum basilicum) can limit its sensory appeal and consumer acceptance, despite its widespread culinary popularity. In this study, we performed deep untargeted metabolomics profiling using UHPLC-QTOF/MS iterative data dependent acquisition (DDA) to investigate the metabolome associated with bitterness of three sweet basil cultivars: 'Aroma2', 'Obsession', and 'Thunderstruck'. PubChemR package was used to convert InChIkey of tentatively identified compounds by MS/MS library spectral matching to SMILES. A python script was developed to automate the resulting metabolites to a bitter-ligand molecular docking tool, BitterX, against 25 human bitter taste receptors to identify potential contributors to bitterness. BitterX screening resulted in TAS2R14 as the primary receptor with the highest number of ligand interactions. Molecular docking using SwissDock & CB-Dock of potential bitter candidates found a triterpenoid saponin, trachelosperogenin A1, to have similar binding affinity in TAS2R14 and TAS2R46 pockets as reported from other major bitter compounds in sweet basil such as sagerinic acid. Targeted analysis by UHPLC-QQQ/MS of major predicted bitter compounds in O. basilicum shows that 'Obsession' and 'Thunderstruck' share relatively similar abundance of phenolic compounds including chlorogenic acid, rosmarinic acid, umbelliferone, and caftaric acid, which are comparatively less abundant in 'Aroma2'. Rutin and trachelosperogenin A1 were the highest in 'Thunderstruck' and lower in 'Obsession' and 'Aroma2'. These findings provide new insights into the molecular basis of bitterness in sweet basil and a potential roadmap for breeding less bitter varieties and further downstream bitter-ligand receptor assays. Combined with screening targeted metabolites and known volatiles that contribute to the overall aroma and flavor but which may also contribute in part to bitterness could provide a more complete profile.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2015 B — Integrating volatile and non-volatile metabolomics for predicting wine quality and consumer liking

PRESENTING AUTHOR: ***Beatriz Quintanilla-Casas, University of Copenhagen, Denmark***

CO-AUTHORS: ***Rasmus Bro***

SUB THEME: ***Machine Learning and AI***

Wine quality is a complex attribute influenced by multiple chemical and sensory factors. Quality assessment has conventionally relied on sensory evaluations, which, while valuable, are time-consuming. Moreover, these assessments are inherently subjective, particularly in consumer studies evaluating hedonic quality. With advancements in analytical techniques and data science, there is an opportunity to develop more objective and reliable methods for predicting wine quality and consumer liking based on their chemical composition.

Volatile organic compounds, characterized by small molecular size and propensity to vaporize at room temperature, have a significant contribution to the aroma profiles of foods. Consequently, the volatile metabolome serves as an ideal marker for an instrumental assessment of flavour attributes and overall food quality [1]. In this study, we show how tensor methods - namely parallel factor analysis 2 (PARAFAC2) - allow to efficiently perform untargeted GC-MS data analysis in a more robust and automated way [2]. On the other hand, non-volatile metabolites are determined by fast spectroscopic techniques e.g., Fourier transformed infrared spectroscopy (FT-IR), since they are key for taste perception [3]. Therefore, the integration of volatile and non-volatile wine metabolome by means of data fusion, and the use of machine learning methods is essential for developing robust predicting models of wine quality, while elucidating relationships between wine's chemical makeup and its perceived qualities.

1. Quintanilla-Casas, B., et al. Current Opinion in Food Science 2025, 61, 101235.

2. Johnsen, L. G., et al. Journal of Chromatography A 2017, 1503, 57-64.

3. Basalekou, M., et al. Beverages 2020, 6, 30.

2016 B — MetaboT: AI-based agent for interaction with metabolomics knowledge graphs

PRESENTING AUTHOR: ***Madina Bekbergenova, University of Cote d'Azur, University of Antwerp, France***

CO-AUTHORS: ***Lucas Pradi, Benjamin Navet, Yousouf Tagzhouti, Emma Tysinger, Jean-Luc Wolfender, Wout Bittremieux, Fabien Gandon, Louis Felix Nothias***

SUB THEME: ***Machine Learning and AI***

Mass spectrometry metabolomics generates vast data requiring advanced methods for interpretation. Knowledge graphs (KGs) address these challenges by structuring mass spectrometry data, metabolite information, and their relationships into a network [PMID:38559298]. However, effective use of KGs demands detailed semantic modeling. To overcome this, we designed MetaboT, an AI system utilizing large language models (LLMs) to translate user questions into SPARQL, the query language of the KG. We demonstrated its effectiveness using the public KG endpoint for the largest open metabolomics dataset for plants [PMID:36649739].

MetaboT employs specialized AI agents for handling user queries and interacting with the KG. The multi-agent system is constructed using the LangChain and LangGraph libraries, which facilitates the integration of LLMs with external tools. Its structured query generation process begins with specialized agents extracting necessary details such as entities and chemical taxonomies from user inputs. Next, a dedicated agent, informed of the KG ontology, crafts SPARQL query using these identifiers. The system then executes the query against the KG and returns structured, context-aware results.

We assessed MetaboT's performance on 50 metabolomics-related questions with expected answers. In addition to submitting these questions to MetaboT, we evaluated a baseline by submitting them to GPT-4o with a prompt that incorporated the KG ontology but did not provide specific entity IDs. This baseline achieved only 8% accuracy, compared to MetaboT's 84%, underscoring the necessity of our multi-agent system for accurately retrieving entities and generating correct SPARQL queries.

MetaboT shows promising performance, functioning as a conversational question-answering assistant. By enabling researchers to retrieve structured metabolomics data using natural language, MetaboT can facilitate data-driven discoveries. It automates SPARQL query generation and execution, which removes technical barriers that have traditionally limited access to KGs. MetaboT can be accessed on [https://metabot.holobiomicslab.eu/] and the code repository is available at [https://github.com/HolobiomicsLab/MetaboT].

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2017 B — Computational Metabolomics Approaches for Siderophore Discovery in Deep-Sea Actinobacteria

PRESENTING AUTHOR: ***Alexandros Polyzois, National and Kapodistrian University of Athens, Greece***
CO-AUTHORS: ***Nikola Milic, Christina Koufali, Christina Stamataki, Venetia Varvara Karveli, Fernando de la Calle Verdu, Nikolas Fokialakis***
SUB THEME: ***Metabolic networks***

Deep-sea environments are home to a wide variety of microorganisms, including actinobacteria and other extremophiles. Particularly, extremophilic actinobacteria are known for their prolific production of secondary metabolites, which show promising therapeutic potential due to their antimicrobial and cytotoxic properties. Notably, siderophores—iron-chelating compounds—present unique opportunities for drug discovery and biomedicine applications.

Our aim is to isolate and identify siderophores using computational mass spectrometry metabolomics approaches. Our goal has two main objectives: a) discover new producing microorganisms for known siderophores, b) identify novel compounds with siderophore activity that could serve as potential drug leads.

We isolated Mediterranean actinobacteria from the Spanish coast grown in liquid cultures, under the project SECRETed. Following a multi-step liquid-liquid extraction, the supernatant was separated into two phases, while the biomass was extracted to obtain progressively polarity extracts. The obtained extracts were subjected to LC-MS/MS analysis, which served two purposes: semi-automatic dereplication of siderophore-related features, and method development predicted metabolites isolation. For the computational aspect, data acquisition was followed by processing through MZmine and comparative metabolomic analysis to annotate the chemical drivers distinguishing active from non-active siderophore extracts. These prioritized features were examined through library matching and supported by SIRIUS in silico fragmentation studies. We propagated this data through GNPS molecular networks incorporating bioactivity metadata and ferric ion identity, highlighting siderophore clusters. This guided the purification process, which was monitored by TLC FeCl3 assays, and led to isolates, dereplicated by HRMS and NMR.

Key compounds included hydroxamate siderophores from the “ferrioxamine family” and spore germination inhibitors from the “germicidin family”. Additionally, carboxylic acid derivatives and dipeptides with notable application potential were identified.

This advanced analytical workflow, enhanced by state-of-the-art cheminformatics, led to novel bioactive compounds and was established as a pipeline for computationally-assisted high-throughput siderophore identification. The project was funded by the European Community’s H2020 Programme, No.101000794.

2018 B — Correlation Networks and Conditional Independence Graphs: A Case Study of Metabolomic Response to Fusarium culmorum in the Grass Family

PRESENTING AUTHOR: ***Marta Kańczurzewska-Staszak, Poznan University Of Technology, Poland***
CO-AUTHORS: ***Aneta Sawikowska***
SUB THEME: ***Metabolic networks***

Correlation Networks (CN) and Conditional Independence Graphs (CIG) are powerful tools for analyzing complex biological systems and revealing relationships beyond pairwise associations. Their application in plant science enhances understanding of biochemical regulation and stress responses. As the number of experimental factors, tissue types, and time points increases, understanding multifactorial relationships becomes more challenging.

This study demonstrates the utility of CN and CIG by analyzing the metabolomic response of grasses to Fusarium culmorum infection, revealing critical interactions in plant-pathogen dynamics. By constructing and comparing CN and CIG, we assess their effectiveness in identifying key metabolites and pathways involved in plant defense mechanisms. We also explore differential correlation networks to compare correlations across different levels of an experimental factor or combinations of factor levels, providing a nuanced view of complex biological interactions.

Metabolomic data from infected and non-infected grasses were analyzed using Weighted Gene Co-expression Network Analysis (WGCNA) in R to detect relationships between metabolites and highly correlated metabolite groups. Gaussian graphical models, implemented via the rags2ridges package, inferred conditional dependencies, distinguishing direct from indirect interactions. Obtained networks were visualized in Cytoscape and R, respectively. We extended this approach by constructing networks with both unique and shated edges for four distinct conditions, revealing condition-specific and common regulatory patterns.

CN and CIG identified broad co-regulation patterns and core regulatory metabolites. Significant shifts in lipid, amino acid, and secondary metabolite interactions distinguished resistant from susceptible genotypes. Moreover, biotic stress increased correlations between selected metabolites, particularly within unsaturated fatty acids and phenylpropanoids, suggesting their key role in the immune response. Differential network analysis highlighted dynamic changes in correlation and direction across conditions, shedding light on context-dependent metabolic adjustments.

These results highlight CN and CIG as effective tools for uncovering key metabolic interactions in plant-pathogen systems, supporting applications in plant resilience studies and crop improvement strategies.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2019 B — **High-Throughput MS/MS Profiling of Bioactive Compounds: A Foundation for Accelerated Natural Product Research**

PRESENTING AUTHOR: ***Aninda Mazumdar, Mikrobiologický Ústav Av Čr, V. V. I., Czech Republic***

CO-AUTHORS: ***Zdenek Kamenik***

SUB THEME: ***Metabolite and mass spectral databases***

The discovery of novel bioactive compounds from natural sources such as soil, marine environments, and other ecological niches remains a labor-intensive and costly process. A major challenge lies not only in identifying new compounds but also in distinguishing them from known entities, which often incurs substantial analytical effort and expense. To address this, we aim to develop a comprehensive, high-resolution MS/MS spectral library comprising over 13,000 bioactive compounds with well-established biological relevance. These compounds, chemically diverse, are complementary to existing repositories such as the COCONUT natural product database. These compounds will be analyzed using ultraperformance liquid chromatography (UPLC) coupled with a Bruker timsTOF HT system, utilizing a reverse-phase C18 column.

The resulting spectral dataset will serve as a valuable addition to public spectral databases, significantly enriching the chemical and biological diversity of available reference data. This high-quality, biologically annotated resource is expected to support a broad range of applications, from dereplication and compound identification to guiding future computational strategies. In particular, the library lays a critical foundation for the development of advanced artificial intelligence-driven tools aimed at accelerating bioactivity prediction and natural product discovery.

2020 B — **Fragmentation-Based Characterization of Oxidized Phosphatidylethanolamines: Development of a Comprehensive Diagnostic Fragment Library**

PRESENTING AUTHOR: ***Adrian Brun, University of Tübingen, Germany***

CO-AUTHORS: ***Michael Laemmerhofer***

SUB THEME: ***Metabolite and mass spectral databases***

Metabolomics and lipidomics are among the fastest-growing fields in molecular life sciences. Precise structural characterization of metabolites is essential, particularly in untargeted LC-MS studies where constitutional isomers, diastereomers, and isobaric molecules must be reliably distinguished. This challenge becomes more complex when such molecules co-elute, requiring a deep understanding of their fragmentation behavior. Incorrect characterizations can severely impair the translation of analytical data into biologically meaningful insights, thereby compromising the quality of metabolic phenotyping, which is critical for biomarker discovery.

This study focuses on the development of a comprehensive database dedicated to the detailed fragmentation behavior of oxidized phosphatidylethanolamines specifically those with epoxy, oxo, hydroxy, and hydroperoxy modifications. Fragmentation was investigated using collision-induced dissociation (CID) with a stepwise increase in collision energy (10–80 eV). All relevant oxidation species and underlying radyl chains, including ester, ether, and vinyl ether species, were included. The aim was to systematically determine the specificity of oxidized modifications and different radyl moieties in order to derive diagnostic fragments that enable robust structural annotation of these oxidized lipids.

Since lipids within a lipid class follow consistent fragmentation rules, we created a comprehensive library encompassing all biochemically relevant side chains and oxidation species. This enables the differentiation not only of radyl isomers but also of regioisomeric oxidation modifications through diagnostic fragment ions. As existing libraries often contain only a limited number of fragments and rarely address fragment specificity, our library facilitates targeted fragment matching under realistic analytical conditions.

2021 B — **A De-Novo Recursive Lipid Annotation strategy using Retention Time Dependencies**

PRESENTING AUTHOR: ***Trisha Arora, Fundacio Eurecat, Spain***

CO-AUTHORS: ***Nicholas Ly, Rafael Montenegro-Burke, Xavier Domingo-Almenara***

SUB THEME: ***Metabolite identification/annotation***

Reversed-phase liquid chromatography-mass spectrometry (RPLC-MS) is relatively ubiquitous in lipidomics with the elution order of lipid species showing characteristic patterns based on the fatty acyl chain and degree of unsaturation. Conventionally used as a validation strategy for end-stage lipid annotation, we utilize this inherent order to introduce an approach to de-novo annotate species at the MS1 feature level. The lipids are annotated in a recursive manner by first annotating anchor lipid subclasses that serve as a base for subsequent annotations. Chosen heuristically on the basis of abundance, the anchor subclasses are initially annotated using optimization functions that model linear regression. Parallel lines are fit across successive degrees of unsaturation whose parameters are guided by in-source fragmentation information and isotopic patterns. The regression parameters optimized on the anchor subclasses are consequently propagated to other lipid subclasses, including ether counterparts by changing a single regression parameter and relying on intra class relationships. Results showed that regression parameters are easily extrapolated to different subclasses falling within a specific gradient range allowing such class based retention time projection. The relative errors of the optimization differed depending on the subclass, with the overall performance remaining consistent, showing the approach to be robust across the majority of lipid classes. A reduction in false positive annotations is achieved, aiding differentiation between isobaric species thereby allowing higher coverage even in the absence of standards and tandem MS data. These annotations can further be used to optimize MS/MS data acquisition. The tool will be made available as an R package with a graphical interface where users can annotate pre-processed LC-MS data to adjust the regression lines, visualize lipid feature plots and retrospectively annotate features following recognizable retention time profiles.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2022 B — Machine Learning-based Algorithm for the Absolute Quantification of Metabolites from Nuclear Magnetic Resonance Spectra

PRESENTING AUTHOR: *Angela Maennel, Hannover Medical School, Germany*
CO-AUTHORS: *Franziska Görtler, Michael Altenbuchinger, Wolfram Gronwald, Helena U. Zacharias*
SUB THEME: *Metabolite identification/annotation*

The measurement and evaluation of metabolite data is essential for researching disease-specific metabolic connections and for the development of new biomarkers. Nuclear Magnetic Resonance (NMR) spectroscopy is a valuable tool for this purpose, as it can absolutely quantify a large number of metabolites employing only one standard reference substance, making it much more cost-effective than mass spectrometry measurements. However, NMR spectra often suffer from significant signal overlap, complicating the accurate quantification of individual metabolites. To address this challenge, we developed a machine learning algorithm that can automatically deconvolute overlapping signals and accurately quantify metabolites.

We adapted a deconvolution algorithm (1) originally designed for digital tissue deconvolution to NMR data. We trained our algorithm once on simulated plasma spectra to achieve a higher generalizability and once on a small sample of real-world plasma specimens for a higher accuracy. We compared the resulting two models to state-of-the-art methods for automatic NMR metabolite quantification. Our method outperformed the other methods on the average correlation between predicted and true concentrations, with both the weights learned on simulated data and on real data. We aim to test our algorithm on samples from other biofluids starting with a dataset of urine samples. Our goal is to develop a tool which allows groups to train their own models fitted to their measurement setup, and enables them to share their learned weights and spectral reference libraries to ensure transparency and reproducibility.

(1)Görtler, Franziska et al. Loss-Function Learning for Digital Tissue Deconvolution, Journal of computational biology: a journal of computational molecular cell biology, 2018, DOI: 10.1007/978-3-319-89929-9_5

2023 B — CSF acylcarnitine profiling in paediatric tuberculous meningitis: Correlation with clinical findings

PRESENTING AUTHOR: *Ms Ontefetse Neo Plaatjie, North West Univeristy, South Africa*
CO-AUTHORS: *M Tutu-van Furth, Martijn Van der Kuip, Shayne Mason*
SUB THEME: *Metabolite identification/annotation*

The poor outcomes of tuberculous meningitis (TBM) are largely due to dysregulated neurometabolism, and the difficulty of diagnosis caused by non-specific symptoms and the absence of specific and sensitive tests. Carnitines are involved in the regulation of energy metabolism and their elevation have been consistently reported in CSF of TBM. We aim to characterise the CSF acylcarnitine metabolome in paediatric TBM to explore its role and diagnostic potentials.

We used a targeted LC-MS/MS approach to analyse TBM (n=56) and compare to controls (n=57) and viral meningitis (VM; n=31) in a South African paediatric cohort. We evaluated the diagnostic potential of significant acylcarnitines and investigated their correlations with clinical meta-data: Clinical and neuroimaging findings.

Free carnitine and three short-chain acylcarnitines (acetylcarnitine, propionylcarnitine, butyrylcarnitine) were significantly elevated in TBM. Acetylcarnitine, propionylcarnitine and butyrylcarnitine showed great diagnostic potential between TBM and control with 80-90% specificity and 70-80% sensitivity but did not differentiate TBM from VM and controls from VM. Both acetylcarnitine and propionylcarnitine were positively correlated with CSF lymphocytes while butyrylcarnitine showed no correlation with the meta-data.

Free carnitine emerged as the strongest potential marker for TBM, differentiating TBM from control with high specificity (100%) and sensitivity (90%) and differentiating TBM from VM with 70% specificity and 80% sensitivity. Free carnitine did not differentiate VM from control, underscoring its specificity to TBM. Furthermore, free carnitine strongly correlated with both brain basal enhancement and hydrocephalus, key radiological markers of TBM.

Our results suggest that acetylcarnitine and propionylcarnitine, could contribute as potential markers for TBM diagnosis, with free carnitine serving as a potential biomarker for TBM and assessment of TBM severity. Furthermore, these results suggest fatty acid and energy metabolism disruptions in TBM cases are important to consider because free carnitine plays a crucial role by transporting long-chain fatty acids into the mitochondria for energy-production.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2024 B **Bioactivation of tetrahydrofurofuranoid lignans in human liver microsomes and characterization of their glutathione conjugates**

PRESENTING AUTHOR: ***Hye Suk Lee, The Catholic University Of Korea, South Korea***
CO-AUTHORS: ***Min Seo Lee, Eui-Hyeon Kim, Kwang-Hyeon Liu, Hye Suk Lee***
SUB THEME: ***Metabolite identification/annotation***

Tetrahydrofurofuranoid lignans exhibit a broad spectrum of pharmacological activities, including anti-inflammatory, antioxidant, anticancer, antiviral, antiallergic, antibacterial, anti-diabetics, and antihypertensive effects. Among them, lignans containing 1,3-benzodioxole moiety (e.g., aschantin, fargesin) have been reported to act as mechanism-based inhibitors. Furthermore, numerous 1,3-benzodioxole-containing compounds are known to induce cytochrome P450 (CYP)-mediated toxicities and enzyme inactivation. This study aimed to elucidate the formation of reactive metabolites by analyzing the glutathione (GSH) conjugates of tetrahydrofurofuranoid lignans formed in human liver microsomes. CYP-mediated metabolism of aschantin, fargesin, and kobusin, which possess a 1,3-benzodioxole moiety, led to the formation of catechol metabolites, which subsequently conjugated with GSH to yield two GSH conjugates per compound. GSH conjugates were also detected from 3-methoxycatechol metabolites derived from guaiacol-containing tetrahydrofurofuranoid lignans, including three conjugates from pinoresinol and four from sylvatesmin. Notably, piperitol formed five GSH conjugates in total, originated from both catechol and 3-methoxycatechol metabolites. In contrast, no GSH conjugates were observed for lignans bearing only methoxy-substituted phenyl groups, such as magnolin, epimagnolin A, and eudesmin. These findings provide valuable insights into the bioactivation potential of tetrahydrofurofuranoid lignans and highlight the critical role of structural features, particularly the 1,3-benzodioxol and guaiacol moieties, in the formation of reactive metabolites. Moreover, these results contribute to a deeper understanding of the mechanisms underlying CYP enzyme inactivation associated with tetrahydrofurofuranoid lignans.

2025 B **MassGenie: a transformer model to predict compound fragmentation spectra**

PRESENTING AUTHOR: ***Ivayla Roberts, University of Liverpool, United Kingdom***
CO-AUTHORS: ***Royston Goodacre, Warwick Dunn, Douglas Kell***
SUB THEME: ***Metabolite identification/annotation***

Introduction: Many metabolites of interest when observed as features in untargeted liquid chromatography-mass spectrometry studies are of an unknown structure. This is due to the fact that only a small percentage of the detected compounds have a reference fragmentation spectrum in in-house, public or commercial libraries. Although fragmentation spectra offer the most accurate identification option as they directly reflect the molecule's structural properties, including bond strengths, large-scale chemical purchasing, when available, can be expensive, and MS/MS mass spectra can differ between instruments. De novo approaches to bypass these limitations are therefore required. To facilitate compound identification, numerous computational tools have been developed; however, structural annotation continues to present significant challenges. Consequently, unidentified compounds of interest are often disregarded, and important findings remain unpublished. The work presented here aims to develop a novel machine learning-based tool to support unknown compound annotation by leveraging the information available from sample acquired fragmentation spectra.

Approach: To this end, a deep learning architecture, specifically a transformer model, was employed to predict fragmentation spectra from structure. While such models rely on large datasets to extract patterns, reference fragmentation spectra are rare. To circumvent this challenge, we used the transfer learning capabilities of those models, i.e., the model was first trained on a large synthetic dataset (26M compounds) and subsequently fine-tuned on experimental spectra.

Results and discussion: Early results demonstrated good performance in matching synthetic data with matching experimental spectra, indicating the capacity of this approach to learn the complex relationship between precursor structure and resulting fragments with small volumes of experimental data. In conclusion, we propose a novel deep learning approach to assist in unknown compound annotation and thereby make a step forward in addressing a key limitation of untargeted metabolomics studies.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2026 B — Xula: A tool for annotating in-source fragments without spectral libraries.

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CO-AUTHORS: ***Sara M. de Cripan, Nuria Canela, Adrià Olomí, Iris Samarra, Antoni del Pino, Xavier Domingo-Almenara***
SUB THEME: ***Metabolite identification/annotation***

In-source fragments (ISFs) significantly contribute to peak redundancy. in untargeted MS1 metabolomics, potentially representing up to 70% of detected signals. Several strategies have been proposed for annotating ISFs, including the comparison of LC-MS1 signals with low collision energy MS/MS reference data. However, public MS/MS spectral libraries currently lack low-energy (0 eV) reference spectra, limiting their effectiveness. While commercial libraries like NIST and METLIN offer low-energy spectra, their utility is restricted to metabolites already present in these libraries.

Recently, our Substructure-Based Fragment Annotation (SBFA) approach has successfully annotated ISFs based on potential substructure matches, circumventing limitations of available reference spectra. ISFs can offer valuable insights when combined with known m/z differences related to adduct formation, aiding in tracking protonated or deprotonated species. Moreover, ISFs provide structural insights into metabolites due to their specificity toward molecular substructures.

In Stable Isotope Tracing (SIT) metabolomics, ISFs of metabolites synthesized using labeled precursors (e.g. [U-13C1-6] glucose) demonstrate compatible 13C isotopic shifts analogous to their protonated or deprotonated counterparts. This phenomenon significantly enhances confidence in the annotation of labeled metabolites.

Here we introduce Xula, a new computational tool utilizing SBFA to annotate monoisotopic masses alongside their ISFs, and to identify compatible metabolite structures exhibiting characteristic 13C incorporation patterns. By applying Xula to mass spectra from metabolites of E. coli K12 grown on unlabeled and fully labeled glucose, we annotated 1683 parent isotopologue groups, each corresponding to one or more candidate metabolites, containing at least two fragments. Among these, 193 groups contain fragments with one or more peaks (either monoisotopic or isotopic) appear in the NIST library, 238 in GNPS, and 65 groups overlap between both libraries.

2027 B — Simultaneous Metabolomic, Lipidomic, and Proteomic Extraction for a Multi-Omics Approach: A Case Study on Arabidopsis thaliana

PRESENTING AUTHOR: ***Simon Duthen, CNRS - Laboratoire De Recherche En Sciences Végétale, France***
CO-AUTHORS: ***Anya Bennaï, Anne Gonzalez De Peredo, Guillaume Marti***
SUB THEME: ***Multi-omics/Integrative omics***

Integrating proteomic, lipidomic, and metabolomic datasets, often acquired independently, presents significant hurdles for comprehensive systems biology investigations. This collaborative project between metabolomics and proteomics core facilities tackles this challenge through two primary objectives: (1) optimizing and validating biphasic extraction protocols for concurrent multi-omics analysis (proteins, lipids, metabolites) from single samples; and (2) validating bioinformatics strategies for robust multi-omics data integration and interpretation. To validate these approaches, we employ the model organism Arabidopsis thaliana, contrasting the wild-type (Col-0) ecotype with a p35S:CBF1 mutant overexpressing the CBF1 transcription factor. The well-documented role of CBF1 in modulating cold response pathways anticipates distinct alterations across the proteome, lipidome, and metabolome between these genotypes. This controlled biological variation offers an ideal framework to rigorously benchmark the performance of the extraction protocols across different molecular classes and subsequently evaluate the effectiveness of various bioinformatics strategies for multi-omics data integration and biological interpretation. Establishing optimized protocols for both concurrent extraction and integrative analysis will facilitate more holistic systems biology studies, enabling a deeper understanding of molecular crosstalk in complex biological processes.

This initial phase allowed us to evaluate two simultaneous extraction protocols and their impact on the recovery efficiency across protein, metabolite, and lipid fractions, highlighting the advantages of one particular protocol. Current work now focuses on the interpretation of the resulting multi-omics data and establishing links with the known molecular differences between the Col-0 and p35S:CBF1 genotypes

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2028 B — Multi-Omics Approach Reveals the Protective Role of Pre Exercise in Immobilization-Induced Muscle Weakness

PRESENTING AUTHOR: *You Mee Ahn, Korea Institute Of Oriental Medicine, South Korea*
CO-AUTHORS: *Sarah Shin, Jeeyoun Jung*
SUB THEME: *Multi-omics/Integrative omics*

While the protective effects of regular exercise on muscle health are well known, it remains unclear whether these benefits persist during periods of disuse, such as immobilization or post-operative recovery. This study used a multi-omics approach to examine whether pre-exercise can mitigate immobilization-induced muscle weakness.

Young C57BL/6 mice completed 8 weeks of high-intensity treadmill exercise followed by 10 days of left hindlimb immobilization. Mice were divided into control (CON), immobilization only (IM), and exercise plus immobilization (E+IM) groups.

Significant atrophy was observed in immobilized muscles of both IM and E+IM groups, but histological analysis showed that muscle fiber integrity was better preserved in the E+IM group compared to IM.

Plasma analysis revealed elevated GDF-8 and reduced IGF-1 in the IM group, changes that were normalized in the E+IM group, indicating systemic benefits of pre-exercise.

Muscle metabolomics showed shared alterations in energy metabolism (TCA cycle, glycolysis, pyruvate metabolism) in both IM and E+IM groups. However, the IM group showed additional activation of stress-related pathways (e.g., glutathione metabolism), while the E+IM group selectively modulated amino acid pathways (e.g., lysine degradation), suggesting metabolic reprogramming.

Transcriptomic analysis supported these findings, with the E+IM group maintaining expression of energy-related genes and limiting stress-induced responses.

These results suggest that pre-exercise enhances metabolic resilience during disuse by preserving energy metabolism and preventing stress-related damage.

2029 B — Deepening Diabetes Research Through Expanded Multi-Omics Capabilities and an Integrated Metabolic Atlas at HumanIslets.com

PRESENTING AUTHOR: *Yao Lu, McGill University, Canada*
CO-AUTHORS: *Han Sun, Seth Seth, Cara Ellis, Charles Viau, Miriam Hoyeck, Jennifer Bruin, Anna Gloyn, Patrick E MacDonald, Jianguo Xia*
SUB THEME: *Multi-omics/Integrative omics*

The study of human pancreatic islets is essential for understanding the pathophysiology of diabetes and developing novel therapeutic strategies. However, the accessibility, integration, and exploration of high-quality islet datasets remain limited. To address this challenge, the first version of HumanIslets.com was developed as a vital resource for diabetes research, offering a comprehensive platform for accessing, analyzing, and downloading human islet data from the Alberta Diabetes Institute IsletCore program. Building on this foundation, HumanIslets.com 2.0 introduces significant advancements, including expanded omics datasets, enhanced multi-omics integration to support a more detailed investigation of islet biology and disease mechanisms. This latest version significantly expands transcriptomics, proteomics, and single-cell datasets while incorporating metabolomics and environmental contaminant data. It also enriches donor phenotype information with type 1 and type 2 diabetes genetic risk scores, genetic ancestry, and insulin processing (proinsulin, C-peptide) measurements, further enhancing the resource's depth and utility. To fully leverage the diverse omics data, a dedicated multi-omics analysis module has been incorporated, allowing researchers to explore and integrate different layers of molecular data. Additionally, HumanIslets.com 2.0 features a human islet metabolic atlas built using human genome-scale metabolic models. This atlas facilitates the integration of gene, protein, and metabolite data within a unified metabolic framework, allowing researchers to investigate donor-specific metabolic patterns and compare differences across diabetes groups. Together, these enhancements make HumanIslets.com 2.0 a more robust and comprehensive resource for the scientific community, providing improved tools and datasets to advance research on islet function, diabetes mechanisms, and potential therapeutic targets.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2030 B — A comprehensive workflow for exposomics data processing, dose response modeling and causal analysis

PRESENTING AUTHOR: *Jianguo Xia, McGill University, Canada*
CO-AUTHORS: *Zhiqiang Pang, Fiona Hui, Jianguo (Jeff) Xia*
SUB THEME: *Multi-omics/Integrative omics*

Exposomics aims to comprehensively investigate the etiology of diseases by formally integrating individuals' environmental exposures and associated biological responses into the traditional genotype-phenotype framework. High-resolution LC-MS plays a key role in exposomics studies. However, current bioinformatics tools are mainly designed for untargeted metabolomics data analysis which emphasize biological responses but lack key features required for exposomics data analysis.

Here we present a novel, comprehensive workflow that addresses three important challenges in exposomics data analysis, including 1) comprehensive data processing and compound annotation for endogenous and exogenous compounds; 2) advanced dose response analysis to identify exposure biomarkers and estimate their benchmark doses (BMDs) from 14 statistical models; 3) causal analysis through Mendelian randomization by leveraging summary statistics obtained from compatible public GWAS data.

We demonstrate the workflow's efficacy through application to a large-scale exposomics study analyzing blood and urine samples from e-waste workers in the GEOHealth II project. Our analyses reveal significant disruptions across multiple metabolic pathways, including steroid hormone biosynthesis, bile acid biosynthesis, vitamin metabolism, and prostaglandin biosynthesis. Importantly, we have identified distinctive exposure biomarkers with characteristic dose-response relationships. Causal analysis investigations are still ongoing.

This integrated workflow provides a powerful platform for advancing exposomics research and environmental health assessment. The complete workflow will be freely available in our GitHub as well as the MetaboAnalyst web-based platform.

2031 B — Decoding the molecular information within extracellular vesicles cargo: a multi-omics approach combining lipidomic, metabolomic and proteomic data

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CO-AUTHORS: *Francesca Benelli, Clarissa Braccia, Radmila Pavlovic, Denise Drago, Massimo Moro, Cristina Borzi, Annapaola Andolfo*
SUB THEME: *Multi-omics/Integrative omics*

Extracellular vesicles (EVs) are key mediators of intercellular communication and play a crucial role in cancer. Indeed, enclosed by a phospholipid bilayer, EVs carry a variety of biomolecules such as proteins, lipids, RNA and metabolites, enabling them to influence cancer progression even at distant sites. Thanks to the complexity and heterogeneity of their cargo, EVs represent an ideal resource for multi-omics integration, offering the potential to uncover significant biological signatures that may not be apparent when analyzing each omics layer individually. However, existing analytical workflows are mainly optimized for genomics and transcriptomics, thus limiting their ability to incorporate non-genomic layers like metabolomics and lipidomics. In this study, we explore cargo of EVs derived from LKB1-wildtype (WT) and knockout (KL) NSCLC cells to test our multi-omics integration approach. We processed EV samples to extract small molecules and proteins separately. LC-MS/MS analysis enabled the identification of approximately 60 metabolites, 300 lipids and 150 proteins. We started by performing single-omics analysis to ensure data quality and proper normalization. Before integration, data from the three omics layers underwent harmonization. Omics data integration was carried out using the MixOmics R package, revealing strong correlations (> 0.9) across the three layers. We designed DIABLO model to reduce the data complexity and extract key features able to maximize the differences between the two experimental conditions. Multi-omics pathway analysis was then performed using tools such as multi-GSEA and LipidSig2.0 to identify commonly modulated processes across the integrated omics. Various visualization strategies were tested to better interpret and present the integration results. We propose a multi-omics integration strategy that can help to uncover hidden biological information from proteomic, metabolomic and lipidomic data. This approach offers a powerful framework for exploring omics data and revealing their underlying biological significance.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2032 B — Multi-Omics Integration Uncovers Rac GTPase Dysregulation and Lipid Metabolic Disruptions in Vascular Cognitive Impairment

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SUB THEME: ***Multi-omics/Integrative omics***

Background: Vascular cognitive impairment (VCI) is a major contributor to dementia, accounting for 20–40% of cases, yet its molecular mechanisms remain poorly understood. While metabolomics plays a crucial role in characterizing disease-associated biochemical changes, traditional single-omics approaches fail to capture the complex interactions driving VCI pathogenesis. Multi-omics integration bridges the gap between genotype and phenotype, offering a powerful framework to uncover interconnected molecular pathways and their impact on VCI pathogenesis.

Objectives: This study aimed to leverage multi-omics integration to uncover VCI-associated molecular disruptions by analyzing genomic, epigenomic, transcriptomic, and metabolomic alterations, enabling a deeper understanding of disease mechanisms beyond single-layer analyses.

Methods: Brodmann area 7 brain tissue samples from 19 VCI cases and 21 cognitively normal controls were analyzed using multi-omics approaches, including genomics, epigenomics, transcriptomics, and targeted metabolomics. Single-layer analyses were performed for each omics dataset, followed by integrative computational modeling to identify cross-omics associations and key molecular interactions driving metabolic dysregulation in VCI.

Results: Multi-omics integration revealed widespread molecular disruptions in VCI, with significant shifts in association networks. Genomic analysis pointed to the Rac GTPase pathway as a key player in VCI, with epigenetic changes disrupting the expression and splicing of related genes. Transcriptomic data showed disruptions in lipid metabolism, oxidative stress, and GTPase activity, while metabo-epigenomic analysis linked Rac GTPase dysregulation to altered diacylglycerol and phosphatidylethanolamine metabolism. These findings provide new insights into the complex interactions driving VCI pathophysiology.

Conclusion: These findings emphasize the power of multi-omics integration in revealing complex molecular interactions and metabolic alterations in VCI that remain hidden in single-omics studies. By uncovering Rac GTPase-mediated lipid dysregulation, this study provides novel insights into VCI pathogenesis, paving the way for biomarker discovery and potential therapeutic strategies.

2033 B — Harmonization of a Comprehensive Life Fluids Metabolome and Chemical Elements DBs for Human Blood: Applying the Metabolites Merging Strategy (MMS) for Enhanced Data Integration

PRESENTING AUTHOR: ***Annabel Margalef Burgos, IISPV, Spain***

CO-AUTHORS: ***Maria Llambrich, Héctor Villalba, Pau Bordoy, Raquel Cumeras***

SUB THEME: ***Other***

BACKGROUND:

Metabolites identification remains a hot topic in metabolomics, differences in analytical techniques and low protocols adoption is causing ambiguity and difficulties in data interpretation, sharing, and complicating collaborative efforts. To address this, we developed the Metabolites Merging Strategy (MMS) to harmonize metabolite data across studies (Villalba et al., Metabolites 2023,13:1167). Here, MMS is applied to harmonize the Life Fluids Metabolome (LFMB) and the Life Fluids Chemical Elements (LFCE) databases for human blood (https://lifefluids.com/blood-composition/).

METHODS:MMS consists of three steps: translating and merging datasets using InChIKeys, retrieving attributes from these InChIKeys, and manual curation to ensure data quality.

LFMB RESULTS: 44,871 blood-associated metabolites from HMDB(36,710), BinVestigate(651), MarkerDB(578), Blood ExposomeDB(15,597), ExposomeExplorer(465), and curated literature compounds(185). Additionally 44,759 metabolites are uniquely found in the Blood ExposomeDB and stored separately. LFMB includes MSMS spectra from open-access sources when available. Attributes include name descriptors (PubChem title; RefMet), chemical properties (MW, monoisotopic mass, molecular formula, xlogp), systematic identifiers (InChI, InChIKey, SMILES), non-systematic identifiers (44,603-CID; 44,559-HMDB; 5,717-KEGG; 6,404-CheBI; 2,593-LipidMaps; 2,076-DrugBank; and 651-BinID), along with ontology classification.

LFCE RESULTS: 98 blood-associated chemical elements from HMDB(55), MarkerDB(2), Blood ExposomeDB(87), ExposomeExplorer(8). Attributes include descriptors (name, symbol), chemical properties (MW, monoisotopic mass), systematic identifiers (InChI, InChIKey, SMILES), non-systematic identifiers (98-CID; 44-CheBI; 75-HMDB; 21-KEGG; 4-DrugBank), and chemical element classification. Results as periodic table of elements format: https://public.flourish.studio/visualisation/20050753/.

CONCLUSIONS: The harmonized LFMB and LFCE databases, built using MMS, represent a valuable resource for computational metabolomics, biomarker discovery, and exposome research. The databases will be made publicly available alongside other Life Fluids databases, including proteins and associated genes(15,763), and transcripts(1,456) in blood, expanding their applicability for multi-omics research. Furthermore, the MMS is being applied to other biofluids such as urine, breath, saliva and cerebrospinal fluid. During the Metabolomics Conference we will show blood results and the different pathways where they are found.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2034 B — Bioorthogonal Click Chemistry in LC-MS lipidomics to trace lipid metabolism: from experimental data to high-throughput computational analysis

PRESENTING AUTHOR: *Stefano Bonciarelli, Mass Spec Analytica Sl, Spain*

CO-AUTHORS: *Palina Nepachalovich, Gabriele Lombardi Bendoula, Jenny Desantis, Michela Eleuteri, Christoph Thiele, Laura Goracci, Maria Fedorova*

SUB THEME: *Other*

Investigating lipid metabolism is crucial for understanding its role in health and disease. Recently, lipid metabolic tracing using bioorthogonal click chemistry has emerged as a powerful alternative to stable isotope tracers. [1,2] Thiele and colleagues showed that ω-alkyne-functionalized fatty acids, derivatized with azido-quaternary ammonium reporters, offer advantages like enhanced ionization and specific neutral loss, enabling high-throughput, sensitive lipid analysis. However, direct injection MS faces challenges, such as resolving isomeric species and possible occurrence of false positives from unaccounted in-source fragmentation. [3]

Therefore, here we present an integrated LC-MS and bioinformatics platform for high-throughput lipid tracing using bioorthogonal click chemistry. [4] Synthetic standards and endogenously produced alkyne lipids were used to explore the LC-MS behavior of “clicked” lipid species with the C171 azido-quaternary ammonium reporter. Key factors like preferential adduct formation, in-source fragmentation (ISF), and MS/MS fragmentation patterns were annotated across 23 lipid subclasses. Fragmentation rules for each subclass were implemented in Lipostar2 software, enabling high-throughput annotation and quantification of 224,514 “clicked” lipids from 15 lipid classes.[4]

We validated the platform by tracing palmitic and oleic acids in HT1080 fibrosarcoma cells under fatty acid overload. High-throughput analysis identified 479 “clicked” lipids in palmitic acid-treated cells and 379 in oleic acid-treated cells, with species labeled singly, doubly, or triply. Distinct incorporation patterns were observed, including isomeric sphingolipid species, where the tracer incorporated into either the fatty acyl or sphingoid base. LC-MS revealed ISF products that would be indistinguishable from endogenous lipids by other methods. [4]

In conclusion, this integrated platform enables efficient, high-throughput analysis of “clicked” lipid tracers in LC-MS lipidomics.

[1] C. Thiele, et al., ACS Chem. Biol. 2012, 7, 2004–2011.

[2] C. Thiele, et al., Nat. Methods 2019, 16, 1123–1130.

[3] F.-F. Hsu, Anal Bioanal Chem 2018, 410, 6387–6409

[4] P. Nepachalovich, et al., Angew. Chem. Int. Ed. 2025, e202501884.

2035 B — Limitations of software-based analysis of fecal bile acid amidates

PRESENTING AUTHOR: *Stephen Barnes, University Of Alabama At Birmingham, United States*

CO-AUTHORS: *Landon Wilson, Youngmee Kim*

SUB THEME: *Other*

Introduction: Bacterially produced bile acid amidates (BAAs) are a fascinating group of metabolites in LC-mass spectrometry metabolomics. In this study we have examined the various publicly versions of MS-DIAL used to extract annotated BAAs. The switch from the use of profile data in versions up to 4.90 to centroid data in versions 5.XX, initially lost all annotations of bacterially produced BAAs, leaving annotations of only hepatic BAAs. Adjustments to lower the reverse dot product from the default parameters recovered a similar number of annotations to those obtained with version 4.90; however, the annotated BAAs were different. When using version 5.5.250404, there were a significant number of “low score” annotations. The goal of this study was to manually evaluate these low score annotations.

Results: Using MS-DIAL 5.5.250404 on the fecal dataset and not restraining retention times to those observed with an in-house BAA database, there were 23 ion features with low annotation scores. Allegedly, many were amidates of lithocholate and 7-oxo-lithocholate. To remove artifacts in low score BAAs, the ratios of the mean peak area of the 12 pooled samples processed with the Phree cartridges to mean peak areas of the 12 blank Phree cartridges were calculated. Those with ratios less than 100:1 (4) were discarded from further analysis. The remainder (19) had ratios from 223-12,856. Of these, seven had retention times less than 4 min, distinct from any of those that were fully annotated. Six (6) had mean peak areas greater than 10,000. Careful examination of the MSMS spectra of these could only satisfactorily annotate glyco-7-oxolithocholate. Indeed, it was the only one with a prominent m/z 337.253 product ion, characteristic of other BAAs.

Conclusions: The ion features, such as BAAs, that are annotated by MS-DIAL exhibit software version-dependence and this maybe a bellwether for other metabolite annotations.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2036 B — Predicting Trait Inheritance in Dandelion Species Combinations using NMR Metabolomics, Multivariate and Machine Learning Methods

PRESENTING AUTHOR: *Christine Drießlein, Technical University of Munich, Germany*
CO-AUTHORS: *Andreas Krumpel, Clemens Thielen, Fred Eickmeyer, Roland Geyer*
SUB THEME: *Statistical methods*

The significance of this work lies in the potential for dandelions to become an alternative source of natural rubber production, mitigating environmental concerns associated with traditional rubber production while creating significant regional value chains. To date, only molecular approaches have been applied to identify relevant genetic markers in dandelion for rubber content and root morphology, while the metabolome has not been considered. This project aims to use multivariate and machine learning methods to establish a relationship between certain properties of dandelion species, such as high rubber content, and their metabolite profiles. The calculated models are expected to provide insights into relevant individual metabolites and metabolite networks and, thus, help to improve breeding efforts.

Plant material from leaves and roots underwent optimized sample preparation, and 1D-1H-NMR measurements were conducted using a 600-MHz Bruker NMR spectrometer. Metabolites were automatically identified and quantified from spectra using a self-written identification algorithm, nonlinear optimisation methods, and an extensive database. Based on the concentrations obtained, different statistical methods were applied, including PCA, PLS-DA, t-SNE, UMAP, and a variety of Random Forest models.

As part of this project, 142 metabolites were measured in dandelion matrices. Among these, 34 known metabolites were confirmed, while 23 new metabolites were identified. Together, these 57 metabolites account for most signals in dandelion spectra, while the remaining identified metabolites are present in smaller intensity and, thus, concentration. Automated identification processes demonstrated high accuracy. All statistical methods show a clear differentiation of the plant varieties consisting of Russian dandelion, common dandelion, and hybrid crosses. However, as of submission, predictive models have not yet been fully developed. This project represents a crucial step towards rubber yield enhancement in dandelion. The use of advanced data analysis methods highlights the importance of continued research in this field for further advancements in biotechnology and sustainable agriculture.

2037 B — Leveraging SLOPE Regularization for Variable Clustering in Metabolomics

PRESENTING AUTHOR: *Krystyna Grzesiak, University of Wrocław, Poland*
CO-AUTHORS: *Michał Burdukiewicz, Małgorzata Bogdan*
SUB THEME: *Statistical methods*

Metabolomic datasets often consist of hundreds to thousands of interrelated metabolites, many of which belong to the same biochemical pathways or functional groups, such as lipids, amino acids, or organic acids. Identifying biologically meaningful patterns within this high-dimensional space is a key challenge in metabolomics. To address this, we apply SLOPE (Sorted L-One Penalized Estimation), a regularization technique that encourages both sparsity and grouping of variables with similar effect sizes.

We demonstrate that SLOPE is well-suited for metabolomic data: it not only selects relevant metabolites for predictive modeling but also tends to cluster structurally or functionally related compounds together. In our study, SLOPE successfully grouped metabolites from the same chemical classes and pathways, offering insight into the biological organization of the data. These results suggest that SLOPE can serve as a powerful tool for both dimension reduction and biological interpretation in metabolomic research.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2039 B — Untargeted Metabolomics Identifies Metabolites Associated with Mammographic Breast Density

PRESENTING AUTHOR: ***Adetunji Toriola, Washington University School Of Medicine, United States***
CO-AUTHORS: ***Myung Sik Jeon, Chongliang Luo, Jingqin Luo***
SUB THEME: ***Systems biology***

Background: A high mammographic breast density (MBD) is a strong risk factor, and an intermediate phenotype for breast cancer. Identifying biomarkers of MBD will provide insights into the underlying biology and guide towards those that can be targeted in breast cancer prevention. Metabolites are associated with breast cancer risk, but a detailed evaluation of metabolites in relation to MBD is lacking. We, therefore, comprehensively investigated the associations of metabolites with MBD.

Methods: We performed untargeted metabolomic analysis (Metabolon (Durham, NC®) in 705 women who were recruited during their annual screening mammogram at the Joanne Knight Breast Health Center at Washington University School of Medicine in St. Louis. We used Volpara (Volpara Health®) to determine volumetric percent density (VPD). We investigated the associations of metabolites with VPD using multivariable linear regression models, adjusted for multiple confounders. We corrected for multiple testing using the Bonferroni method and metabolites were considered statistically significant at a corrected p-value<0.05.

Results: We profiled1074 metabolites in 10 super pathways, but analysis was limited to 828 metabolites with <300 missing values: lipid I (n=217 metabolites), amino acid (n=211), xenobiotics (n=138), lipid II (n=54), peptide (n=43), nucleotide (n=38), cofactors and vitamins (n=37), carbohydrate/energy (n=34), lipid III (n=34) and partially characterized molecules (n=22). We identified 15 metabolites: 5 amino acid (glutamate, 2-pyrrolidinone, picolinate, s-methyl cysteine sulfoxide, and n2, n5-diacetyl ornithine), 2 carbohydrate (ribitol and n-acetylglucosamine-n-acetylgalactosamine), 3 cofactors and vitamins (beta-cryptoxanthin, carotene diol 2 and gamma-tocopherol), and 5 steroid (cortolone glucuronide (1), androstenediol (3beta,17beta) monosulfate (2), androstenediol (3beta,17beta) disulfate (1), 5alpha-androstan-3alpha,17beta-diol monosulfate (2), and 5alpha-androstan-3alpha,17beta-diol disulfate) metabolites that were associated with VPD at a Bonferonni p-value <0.05.

Conclusions: We identified several novel metabolites that are associated with VPD. Our findings provide new insights into biomarkers that could be targeted in breast cancer prevention and warrant confirmation in other study populations.



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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2050 B — A Biochemical Roadmap to Brain Metabolic Health - Molecular Influences of Chemical Exposome, Diet, Lifestyle and Gut Microbiome.

PRESENTING AUTHOR: ***Rima Kaddurah-Daouk, Duke University, United States***

SUB THEME: ***Ageing research***

It has become increasingly clear that the genome, gut microbiome (GM), diet, lifestyle, socioeconomic disadvantages, and environmental exposures all affect an individual's metabolic state, contributing to brain health and disease. The 2024 Lancet Commission on dementia identified 14 modifiable risk factors that account for 45% of the global dementia burden. Molecular mechanisms of neurodegenerative and neuropsychiatric diseases are not well understood, nor is variation in treatment response, but it is clear there are influences beyond genome. We will share our experiences leading large NIH funded initiatives (Alzheimer Gut Microbiome Project, Alzheimer's Disease Metabolomic Consortium, and Mood Disorder Precision Medicine Consortium) on how big data generation with the metabolome as a readout is starting to transform our understanding of these diseases and their heterogeneity. This exemplifies how peripheral changes are connected to brain changes. We will address how the exposome, diet, and GM influence the human brain metabolome highlighting which chemicals have long-lasting effects. In addition, we will present evidence from large community studies on a role for the: 1) gut-brain axis and gut metabolites in AD pathogenesis, 2) exposome in cognitive function, and 3) diet in major depression. We will present initial molecular insights into the impact of socioeconomic disparities and geographical location on health and cognition. The effect of diet (ketogenic and Mediterranean) and lifestyle interventions on the metabolome will be shared illustrating the impact on disease related pathways and processes. Novel therapeutic approaches have emerged from our studies which could tackle the interconnected influences of genome, exposome, diet, and GM on brain metabolic health.

2051 B — Lipidomic Signatures of Brain Aging and Alzheimer's Disease Across Key Brain Regions

PRESENTING AUTHOR: ***Maria Tretowicz, Laboratory Genetic Metabolic Diseases, Amsterdam., Netherlands***

CO-AUTHORS: ***Sofie Lautrup, Johannes Frank, Maria Tretowicz, Frédéric Vaz, Georges Janssens, Riekelt Houtkooper, Evandro Fang***

SUB THEME: ***Ageing research***

Alzheimer's disease (AD) and aging both profoundly impact brain lipid composition, yet their individual contributions to regional lipid remodeling remain unclear. To investigate these effects, we conducted lipidomic profiling on post-mortem brain tissues from 90 individuals, spanning young, middle-aged, and elderly adults, as well as AD cases categorized by Braak stages (1–2, 3–4, 5–6). Samples were analyzed from three brain regions that differ in their vulnerability to aging and AD: the entorhinal cortex, hippocampus, and prefrontal cortex.

Our results show that lipid alterations in AD are multifactorial—partly reflecting features of accelerated aging, while also revealing region- and class-specific disruptions. Across both aging and AD, the entorhinal cortex exhibited the most extensive lipid changes, followed by the hippocampus and prefrontal cortex, with AD-related shifts layering upon age-associated trends. Aging was notably marked by the accumulation of bis(monoacylglycero) phosphates (BMPs), lipids closely associated with lysosomal function. Further analyses revealed distinct trends across brain regions. In the entorhinal cortex, lipid changes in AD appeared to follow a different trajectory than those seen with normal aging, which may point to disrupted autophagic processes. In contrast, changes observed in the hippocampus and prefrontal cortex more closely resembled those that occur with aging, suggesting that AD in these regions may involve an acceleration of typical aging-related lipid shifts. Notably, several lipid classes, such as cardiolipins (CLs), exhibited a consistent shift to more saturated species across all regions and conditions.

Together, these findings highlight the complex nature of lipid dysregulation in Alzheimer's disease, revealing how aging-related processes and disease-specific mechanisms interact to drive regional lipid changes.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2052 B — Bile acids and their interaction with the musculoskeletal system

PRESENTING AUTHOR: *Giovanny Rodriguez Blanco, Med Uni Graz, Austria*

CO-AUTHORS: *Zoltan Mezei, Marco Balasso, Gunter Almer, Hans Juergen Gruber, Maria Donatella Semeraro, Sabine Pailer, Guenter Fauler, Harjit Bhattoa, Markus Herrmann, Nerea Alonso*

SUB THEME: *Ageing research*

Bile Acids (BAs) are endocrine molecules that can play a role in the development of osteosarcopenia, although the link is not well understood. To investigate the role of bile acids in bone and muscle deterioration, a well-characterised cohort of 95 females >50 years of age (nosteosarcopenia=23), and a rodent female model (n=20) for high fat diet (HFD)-induced bone loss were used. We applied targeted and untargeted approaches using triple quadrupole or orbitrap mass spectrometers to quantify fifteen free, taurine- and glycine-conjugated BAs, as well as sulphated (s-), glucuronidated (g-), and microbially-conjugated (MCBAs) species. Patients with osteosarcopenia presented lower concentrations of primary BAs (2.3[1.3-4.4] vs 1.4[0.5-3.2] uM, p = 0.0098), than those without osteosarcopenia. Patients with sarcopenia and osteosarcopenia had elevated g-UDCA (1.5 fold change, p=0.01) and s-bile acids (fold change range=1.7-11, prange=0.01-1.8x10-4) compared to non-sarcopenia patients. They correlate positively with age, P3NP, SARC-F questionnaire and severity of sarcopenia (rrange=0.208-0.358, prange=0.04-3.9x10-4). Sulfated BAs negatively correlated with handgrip score and gait score, CTx and BSAP (rrange=-0.207--0.261, prange= 0.04-0.01). In the rat model, HFD reduced the BA concentration in serum by 63% (p=4.65x10-6) and increased it almost 3-fold (p=3.69x10-9) in stool compared to control. microCT analysis showed 28-46% reduction in both trabecular and cortical parameters (p=3.41x10-9-1.57x10-6) in HFD, which correlate with bile acid dysregulation. Extended untargeted BA analysis identified elevated levels of eight MCBA (FC>2.2, prange=0.049-0.0006) in stool and reduced levels of g-BAs (FC=10, p=0.0003) and s-BAs (rangereduction=81.7-97.4%, prange=0.001-0.0003) in serum of HFD animals compared to controls. These BA species correlated with cortical bone parameters (MCBA rrange=0.697-0.823, prange=0.0006-8.4x10-6; g-BAs rrange=0.697-0.823, prange=0.0006-8.4x10-6, s-BA rrange=0.677-0.821, prange=0.0007-9.14x10-6). Similar results were found for urine and liver. Our results suggest that bile acids, including underappreciated species sulfated, glucuronidated, and MCBAs) play a key role in bone and muscle health in both osteosarcopenia patients and obesity-driven osteoporosis.

2053 B — Challenges in the progeria animal model metabolomics research

PRESENTING AUTHOR: *Michal Ciborowski, Medical University of Bialystok, Poland*

CO-AUTHORS: *Patrycja Mojsak, Joanna Godzien, Julia Zelkowska, Karolina Pietrowska, Adrian Godlewski, Adam Kretowski, Elena Martin, Miguel de la Fuente, María González-Amor, Beatriz Dorado, Vicente Andres*

SUB THEME: *Ageing research*

Hutchinson-Gilford progeria syndrome (HGPS) is an ultrarare genetic disorder caused by the mutant progerin protein expression. HGPS patients develop ageing-like symptoms at 1–2 years and die at an average age of 14.5 years. Robust molecular biomarkers for monitoring disease progression or therapy are lacking. The HGPS mouse model is valuable for studying this disease; however, biological material is limited, as only up to 300 µl of blood can be collected from advanced-stage animals. This study aimed to determine the minimal amount of biological samples required for robust and high-coverage MS-based untargeted metabolomics in progeroid mice (G609G). LC-MS- and GC-MS-based metabolomics was performed on arteries, PBMCs, and “conventional” and “ficoll” plasma left after PBMCs isolation. A higher number of features (metabolites for GC-MS), was detected in “conventional” vs “ficoll” plasma, >110 vs <100 for GC-MS and >1100 vs <900 for LC-MS. PBMCs isolated from the blood of one or the pooled blood of two animals were used. A higher number of metabolites/features was obtained for the pooled samples, >60 vs <50 for GC-MS and >800 vs <300 for LC-MS. From 1 to 3 arteries were used. Each time, a similar number of features was detected using LC-MS. In GC-MS, twice as many metabolites were detected when 2 or 3 arteries were pooled. To perform robust and information-rich metabolomics of PBMCs or arteries, pooling blood from two progeroid mice to obtain PBMCs and combining at least two arteries is needed. Plasma obtained from a single animal is sufficient to yield comprehensive metabolomics results.

The project is funded by the National Center for Research and Development under the 4th competition of the European Joint Programme Rare Diseases from the state budget; the EJP RD program received funding from the Horizon 2020 Framework Program. Contract no: EJPRD/IV/78/ProgerOmics/2023.

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2054 B — Impact of a PROtein enriched MEDiterranean diet and EXercise on the plasma metabolite profile of older adults.

PRESENTING AUTHOR: ★ *Claire Connolly, University College Dublin, Ireland*
CO-AUTHORS: *Lorraine Brennan, Ya Gao, Nicola Ann Ward, Claire McEvoy*
SUB THEME: *Ageing research*

Aging is often associated with the deterioration of cognitive health, influenced by factors such as genetics, lifestyle and nutrition. Research suggests that adherence to a mediterranean diet and exercise may provide a protective effect against cognitive decline.

This research examines the effect of a protein enriched mediterranean diet and exercise intervention on the metabolite profile.

PROMED-EX is a 6-month, single-blind, parallel-group randomised control trial examining the effect of a protein enriched mediterranean diet, with and without exercise, on cognitive decline and risk of under-nutrition in adults aged 60 and over. Plasma samples were obtained from participants (N=92) at baseline and 6 months, in both control and intervention groups. Metabolomic analysis of plasma samples was performed with a targeted LC-MS/MS metabolomic approach, measuring 447 metabolites across 26 metabolite classes. Prior to statistical analysis, metabolomics data acquired from both intervention groups was combined into one group. Univariate statistical analysis between control and intervention was performed at baseline and 6 months using Metaboanalyst (version 6).

The mean age of participants was 67± 5 years and 71.4 % were female. No significant differences were observed in the metabolite profiles between control and intervention groups in baseline samples (FDR < 0.1). A total of 42 metabolites including triglycerides, fatty acids, a phosphatidylcholine and an amino acid were significantly different between control and intervention groups in 6-month samples (FDR < 0.1). Changes observed in various metabolites were indicative of the mediterranean dietary pattern, which is rich in mono and poly-unsaturated fatty acids. Interestingly, the metabolite tryptophan-betaine which is associated with cognitive health was significantly higher in the intervention group at month 6.

In conclusion, an altered metabolite profile was observed following the consumption of a protein enriched mediterranean diet. Future research will examine the relationship of this altered metabolomic profile and cognitive health.

2055 B — Cracking the Code: Reproducibility Challenges in Metabolomics for Asthma Models

PRESENTING AUTHOR: *Pierre-Hugues Stefanuto, Liège University, Belgium*
CO-AUTHORS: *Thibaut Dejong, Virginie Bertrand, Thibault Massenet, Anais Rogrigues, Jean-François Focant, Florence Schleich*
SUB THEME: *Biomarker validation and clinical translation*

Context: Asthma represents the most common chronic respiratory disease, affecting 235 million people worldwide. Asthma is a non-curable complicated disease. Currently, asthma monitoring relies on invasive sputum analysis or empirical approaches. The development of personalized medicine strategies would positively impact patients' quality of live. In this study, we developed a multi-omics workflow to identify metabolic pathways in in vitro asthma models to identify breath biomarkers origin.

Method: We used A549 lung epithelial cell line as a proof-of-concept in vitro model compatible with a multi-omics workflow. The cells were stressed with H2O2, lipopolysaccharide (LPS), and sputum supernatant to trigger inflammatory responses. Next, we investigated the metabolic profile using headspace monitoring for volatile molecules, metabolite and lipid double derivatizations. The three types of extract were all analysed using comprehensive multidimensional gas chromatography coupled to mass spectrometry (GCxGC-MS). Resulting profiles were processed independently and using data fusion approaches.

Results: In the literature, we observed high variability in the reported stress incubation conditions for H2O2 and LPS challenges. Moreover, day-to-day reproducibility was challenging, especially for H2O2. To gain a better understanding, we developed an in situ H2O2 quantification method for different culture conditions. In the end, only the LPS and the sputum conditions met the replication requirements.

For the three optimized analytical conditions, the resulting metabolite profiles were processed for pathway discoveries and cross-compared with our asthma breath marker data base. From there, we identified specific aldehyde-related pathways.

Conclusion: In this study, we performed a thorough investigation of the reproducibility of oxidative stress conditions for an in vitro asthma model in a multi-omics setting. We developed three analytical workflows, each focusing on a specific group of molecules: volatile organic compounds, semi-volatile metabolites and semi-volatile lipids. The generated data were cross compared with our breath marker data base.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2056 B — Metabolic Endotyping of Pulmonary Hypertension: Novel Lipidomics Biomarkers for Improved Diagnosis and Risk Stratification

PRESENTING AUTHOR: *Natalie Bordag, Medical University of Graz, Austria*
CO-AUTHORS: *Vasile Foris, Thomas Eichmann, Gabor Kovacs, Horst Olschewski, Andrea Olschewski*
SUB THEME: *Biomarker validation and clinical translation*

Addressing the high morbidity and mortality associated with pulmonary hypertension (PH) requires new diagnostic and prognostic approaches. There is an unmet need for sensitive and specific biomarkers for PH diagnosis that can also predict prognosis.

We have identified a specific set of metabolic markers that indicate PH and the prognosis of PH patients fromnon-fasted blood samples (serum, EDTA and heparin plasma) collected from three cities as two independent cohorts (169 training, 64 validation).These markers are based on ratios of fatty acids (FA) and lipids, and have been performing with exceptional diagnostic accuracy (AUC 0.89 training, 0.90 validation). These ratios were also prognostic and synergistic with established clinical scores (Bordag et al. preprint 10.1101/2023.05.17.23289772, patent WO2017153472A1).

To investigate the impact of further metabolites, we advanced our HRMS method, splitting runs by scan polarity and liquid chromatography type (HILIC, RP) as well as incorporating 10 stable isotope labelled reference compounds for enhanced intra- and inter-batch normalization. This novel approach revealed a significant increase in ketone bodies (e.g. 3-hydroxybutyrate) in PH patients in a pilot study. This novel observation suggests a potential role for ketogenesis in PH pathophysiology, beyond the established lipidomic alterations. Therefore a large cohort with over 200 PH patients and controls is currently being analysed.

Our metabolomic analysis suggests lipid and ketone body alterations as novel, blood-based biomarkers for PH, enriching risk stratification complementing established scores. This underlines metabolic pathogenic mechanisms in PH, opening promising avenues for targeted interventions.

2057 B — Evaluation of CYP2D6 Activity: Validation of Solanidine and Discovery of Novel Biomarkers in a Korean Population

PRESENTING AUTHOR: *Ga Yeong Lee, Seoul National University, South Korea*
CO-AUTHORS: *Woori Chae, Sungyeun Bae, SeungHwan Lee, Namyi Gu, Anhye Kim, In-Jin Jang, Joo-Youn Cho*
SUB THEME: *Biomarker validation and clinical translation*

Solanidine, a steroidal alkaloid found in Solanum genus plants—particularly in potatoes, eggplants, and tomatoes—is the primary aglycone of glycoalkaloids. Solanidine and its metabolite, 3,4-Seco-Solanidine-3,4-dioic acid (SSDA), have been proposed as sensitive and specific dietary biomarkers for CYP2D6 activity. Recent studies have highlighted their relevance, particularly in Europeans with potato-rich diets. However, their applicability in populations with different dietary patterns remains uncertain, necessitating further validation.

This study aimed to assess the utility of solanidine as a CYP2D6 biomarker in a Korean population and to identify novel endogenous biomarkers. In this open-label, three-period clinical trial, we evaluated the effects of the CYP2D6 inhibitor, paroxetine, on the pharmacokinetics of venlafaxine and its metabolite, O-desmethylvenlafaxine, in 20 healthy subjects, including 14 normal metabolizers (NMs) and 6 intermediate metabolizers (IMs). In addition, plasma samples collected pre- and post-multiple doses of paroxetine were analyzed for the semi-quantification of solanidine and SSDA, alongside untargeted metabolomics profiling on the same samples. Both analyses were conducted using a Q Exactive Plus Orbitrap mass spectrometer coupled with an Ultimate 3000 UPLC. The results showed that in plasma samples, solanidine levels increased following CYP2D6 inhibition, while SSDA levels decreased. Moreover, the SSDA/solanidine ratio was significantly higher in NMs than in IMs in both pre- and post-paroxetine samples. Additionally, we found multiple features as endogenous biomarker candidates for CYP2D6 activity. Notably, feature A (m/z: 264.01671) exhibited a statistically significant decrease in plasma levels after paroxetine administration compared to pre-administration. Further studies are needed to identify and validate additional endogenous biomarkers in independent cohorts. This study demonstrates that solanidine is applicable as a biomarker for assessing CYP2D6 activity in the Korean population

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2058 B — Quantitative serum NMR spectroscopy and tissue MALDI imaging decipher metabolomic and lipidomic heterogeneity in endometriosis

PRESENTING AUTHOR: ***Sisi Deng, University of Tuebingen, Germany***

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SUB THEME: ***Biomarker validation and clinical translation***

Background:

Endometriosis (EMT) affects around 190 million reproductive-age women worldwide, imposing a substantial economic burden. It causes chronic pelvic pain which can only be temporarily managed, infertility, and a 50% increased ovarian cancer risk, with high recurrence despite treatment. No accurate non-invasive diagnostic tests or biomarkers exist, highlighting the need for early diagnosis and improved disease management.

Methods:

We analyzed 235 serum samples (183 EMT, 52 borderline ovarian tumor [BOT]) and five EMT tissue samples. Metabolite and lipoprotein profiles were obtained using ^{1}H-NMR metabolomics following IVDr standard procedures. A healthy control group (129 serum samples) was included. MALDI imaging of EMT tissue was performed on a Bruker timsTOF fleX system at 10 µm resolution. Uni- and multivariate statistical analyses identified metabolic alterations.

Results:

EMT serum showed increased pyruvic acid, glucose, and VLDL, with decreased lactic acid, glutamic acid, valine, acetic acid, leucine, threonine, and phenylalanine. Key distinguishing metabolites were pyruvic acid and glucose (EMT) versus acetic acid, threonine, and phenylalanine (healthy). Compared to EMT, BOT showed increased LDL, HDL, and specific lipid subfractions (L6AB, L6PN, H4FC, H4A1). BOT was characterized by elevated acetone and citric acid, whereas EMT was more associated with HDL and LDL subfractions. Both EMT and BOT exhibited increased lipid levels (V3TG, H3TG, TPTG), with BOT showing a distinct lipidomic profile. Amino acids such as leucine and valine were downregulated in both EMT and BOT compared to healthy controls.

Conclusion:

NMR serum data suggest altered glycolysis and lipid metabolism support EMT tissue survival in hypoxic, inflammatory conditions, contributing to its pain and inflammatory phenotype. BOT exhibits similar metabolic shifts but with a more tumor-like profile, suggesting a pre-malignant state. MALDI imaging reveals spatial metabolic dysregulation in EMT tissue. Further research should integrate histology and spatial metabolomics to stratify EMT subtypes and uncover underlying mechanisms.

2059 B — Longitudinal lipoprotein and cytokine analysis uncover persisting inflammation and hyperlipidemia following SARS-CoV-2

PRESENTING AUTHOR: ***Gyuntae Bae, University of Tuebingen, Germany***

CO-AUTHORS: ***Georgy Berezhnoy, Zhiqi Yang, Daniele Bucci, Claire Wegner, Hartmut Schäfer, Yogesh Singh, Caterina Lonati, Christoph Trautwein***

SUB THEME: ***Biomarker validation and clinical translation***

Introduction

Most of the individuals recover from acute COVID-19 (AC) within weeks depending on the symptoms and severities. However, 10-20% of individuals are diagnosed as long COVID-19 or post-acute COVID-19 syndrome (LC or LTCS).

Objectives

LC lacks clear and operationalized characterizations that hinder effective interventions. Hence, we aimed at investigating the lipidomic and inflammatory mediator profiles of longitudinal serum collected from LC patients after the initial acute infection.

Methods

We prospectively profiled lipoproteins and glycoprotein inflammatory parameters in long COVID-19 (5 Month (M)=95, 9 M=73, 12 M=95, 16M=78, and 20 M=85) by in-vitro diagnostics research (IVDr)-based nuclear magnetic resonance (NMR) spectroscopy approach. Simultaneously, we measured cytokines with a 13-plex inflammatory cytokine panel by flow cytometry. We then compared the lipoprotein profile with historical data from AC (N=307) and healthy cohorts (prehistoric SARS-CoV-2 infection (N=305), whereas the cytokine profile was correlated with that of the AC cohort.

Results

We identified 31 and 80 significantly altered main and sub lipoproteins, respectively. Long COVID-19 was associated with higher serum levels of very low-density, intermediate-density, low-density, high-density, triglycerides, cholesterols, and apolipoprotein a-I & a-II lipoproteins compared to the healthy cohort, but those triglycerides and very low-density lipoproteins were significantly lower compared to the AC cohort. Moreover, each lipoprotein size and distribution of LC were distinct to healthy and AC cohorts. We observed NMR-based acute inflammatory parameters showed significantly lower concentrations in LC than in AC cohort whilst, proinflammatory mediators (IFN-α2, IFN-γ, TNF-α, CXCL8/IL-8, IL-12p70, IL-17A, and IL-23) displayed significantly higher concentrations in LC than in AC cohort. However, CCL2/MCP-1, IL-6, and IL-18 were significantly higher in AC cohort than in LC cohort.

Conclusion

Our findings provide compelling evidence that hyperlipidemia is a hallmark of long COVID-19 alongside the development of chronic inflammation and lipoprotein metabolism vary in states of acute and chronic inflammation.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2060 B — Atmospheric pressure mass spectrometry spatial analysis of FFPE tissues in pathology. More than expected!

PRESENTING AUTHOR: *Bart Ghesquiere, VIB - KU Leuven, Belgium*
CO-AUTHORS: *Marco Giampa, Gilles Frache, Marthe Verhaert, Dietmar Thal, Raf Sciot*
SUB THEME: *Biomarker validation and clinical translation*

Millions of pathologically well-documented formaldehyde-fixed, paraffin-embedded (FFPE) tissues representing a wide variety of diseases are archived in biobanks all over the planet. Today, however, this wealth of material is almost exclusively employed for various microscopy analyses describing histological differences between health and disease. In the past 30 years also spatial mass spectrometry (MS) technologies have been developed for tissue imaging. Unfortunately, formaldehyde crosslinking —an essential step in samples preservation— historically resulted in analytical chemists neglecting FFPE material for MS based molecular biomarker discovery studies.

Yet, making FFPE samples accessible for molecule specific MS imaging analyses would tremendously help integrating microscopic pathological data obtained by classical histochemical techniques with mass spectrometric molecular information.

We developed a workflow for spatial (bio)molecule analysis of FFPE tissues, using two modern atmospheric pressure (AP) ionization high-resolution MS platforms and demonstrate that endogenous peptides and a subset of small metabolites are readily, i.e. fully 'top-down' detectable, i.e., without the use of enzymes or derivatization or labeling reagents. Analogous to immunohistochemistry (IHC), we designated this technology mass spectrometry histochemistry (MSHC).

We used AP/MALDI fitted to a quadrupole-orbitrap (ThermoFisher Exploris480) instrument, and desorption electrospray ionization (DESI) fitted to a multi-reflectron time-of-flight (Waters Select MRT) system for MSHC on sections of both mouse and human FFPE tissues.

We show that AP/MALDI and DESI HR MSHC multi-omically detects diverse sets of biomolecules both in positive as well as negative ionization mode. Moreover, the high sensitivity of both HR MS instruments allows for FFPE MSHC to be qualified as single-cell resolving method.

Our data validate that MSHC is 100% compatible with the very same material which pathologists are routinely examining in traditional microscopy-based immuno- or other histochemistry analyses. This opens novel perspectives for use of MSHC in biomarker discovery as well as in clinical and pharmaceutical developments.

2062 B — Alterations in Plasma Lipid Profile before and after Surgical Removal of Soft Tissue Sarcoma

PRESENTING AUTHOR: *Jae Hwa Lee, Kyungpook National University, South Korea*
CO-AUTHORS: *Mi-Ri Gwon, Hae Won Lee, Sook Jin Seong, Young-Ran Yoon*
SUB THEME: *Cancer*

Soft tissue sarcoma (STS) is a relatively rare malignancy, accounting for about 1% of all adult cancers. It is known to have more than 70 subtypes. Its rarity, coupled with its various subtypes, makes early diagnosis challenging. The current standard treatment for STS is surgical removal. To identify the prognosis and pathophysiology of STS, we conducted untargeted metabolic profiling on pre-operative and post-operative plasma samples from 24 STS patients who underwent surgical tumor removal. Profiling was conducted using ultra-high-performance liquid chromatography–quadrupole time-of-flight/mass spectrometry. Thirty-nine putative metabolites, including phospholipids and acyl-carnitines were identified, indicating changes in lipid metabolism. Phospholipids exhibited an increase in the post-operative samples, while acyl-carnitines showed a decrease. Notably, the levels of pre-operative lysophosphatidylcholine (LPC) O-18:0 and LPC O-16:2 were significantly lower in patients who experienced recurrence after surgery compared to those who did not. Metabolic profiling may identify aggressive tumors that are susceptible to lipid synthase inhibitors. We believe that these findings could contribute to the elucidation of the pathophysiology of STS and the development of further metabolic studies in this rare malignancy.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2063 B — Differential Abundance of Extracellular Amino Acids in the Bone Marrow Microenvironment Differentiates Between Premalignant and Malignant Clonal Plasma Cell Disorders

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CO-AUTHORS: ***Yogesh Chawla, Puja Dey, Dragan Jevremovic, Taro Hitosugi, Wilson Gonsalves***

SUB THEME: ***Cancer***

Multiple myeloma (MM) is a clonal plasma cell (cPC) malignancy responsible for over 120,000 annual deaths worldwide. It is preceded by premalignant cPCs in conditions like monoclonal gammopathy of undetermined significance (MGUS) or smoldering MM (SMM), both requiring continuous monitoring for malignant transformation. Current biomarkers fail to distinguish premalignant from malignant cPCs, leading to disease progression to MM with organ damage before therapeutic intervention. Identifying novel biomarkers predictive of early malignant transformation of MGUS and SMM is critical for early intervention.

c-Myc overexpression, a key driver of cPC malignancy, alters intracellular metabolic pathways in malignant cells crucial for proliferation and immune evasion. This study utilized untargeted metabolomic profiling of bone marrow (BM) plasma via liquid chromatograph mass spectrometry (LC-MS) to differentiate metabolite signatures in MGUS (N = 50) and MM (N = 50) patients. Key amino acid alterations were identified, including decreased branched-chain amino acids and increased tryptophan catabolism leading to elevated levels of the immunosuppressive kynurenine metabolites and low levels of kynurenine and tryptophan.

Targeted quantification of these metabolites by LC-MS in an independent, prospective validation cohort of MGUS (N = 50) and MM (N = 65) patients confirmed these findings in BM plasma. These metabolites identified SMM patients at high risk of rapid progression to MM. Furthermore, serial in vitro monitoring of these metabolites via LC-MS in human MM cell lines with and without c-Myc modulation validated their biological significance. Finally, these metabolites were evaluated in various in vivo human MM cell lines and patient-derived xenograft models.

This study establishes LC-MS-based targeted metabolite profiling as a clinically relevant tool for distinguishing conditions with premalignant from malignant cPCs. The identified metabolic alterations provide insight into MM pathogenesis and offer a foundation for novel risk stratification strategies in MGUS and SMM patients, potentially enabling therapeutic interventions to prevent MM.

2064 B — From UHPLC-MS to MALDI-MSI: Dynamic Distribution and Detection of Nucleosides in Breast Cancer FFPE Tissue

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SUB THEME: ***Cancer***

Background:

Despite major advances in treatment, breast cancer remains a major therapeutic challenge due to its high metastatic potential, extensive heterogeneity, diverse molecular subtypes, and complex metabolic reprogramming. Nucleosides, as essential components of nucleic acids, reflect underlying tumor metabolism, yet their spatial distribution in archival tissues remains poorly characterized. Understanding their spatial localization can elucidate stage-specific metabolic alterations in tumors, thereby enhancing the discrimination of different stages of tumor progression for diagnostic purposes.

Methods:

We combined ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to analyze nucleosides in FFPE breast cancer tissues. UHPLC-MS enabled sensitive and quantitative profiling of nucleosides, while MALDI-MSI provided in situ, label-free imaging with spatial localization. Methanol extraction was optimized to enhance nucleoside recovery from FFPE tissues for reliable UHPLC-MS quantification. In addition, univariate statistics (Receiver Operating Characteristic, ROC) of MALDI-MSI derived data is used to evaluate the stage-specific discriminatory power of individual nucleosides.

Results:

UHPLC-MS profiling with methanol extraction significantly improved nucleoside recovery from FFPE tissues, yielding results comparable to fresh-frozen samples. MALDI-MSI enabled spatial resolution visualization of nucleoside distribution in both tumor and normal tissues. Among the 11 detected nucleosides, 5-formyl-cytosine showed strong potential for distinguishing early-stage tumors from normal tissues (AUC > 0.7). Additionally, xanthosine and inosine demonstrated AUC values above 0.65, indicating their potential as markers for differentiating middle and advanced-stage tumors from early-stage ones, highlighting their relevance to tumor progression.

Conclusions:

This study demonstrates the feasibility of spatial nucleoside metabolomics in archival FFPE breast cancer samples, with 5-formyl-cytosine, xanthosine, and inosine emerging as potential markers for tumor stage discrimination. These findings highlight the utility of combining quantitative and spatial metabolic profiling in advancing our understanding of tumor metabolism and improving diagnostic accuracy for breast cancer.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2065 B — Urinary Eicosanoid Profiling in Endometrial Cancer Patients - the Impact of Matching Participants' BMI and Age on the Statistical Outcome in a Case-Control Study

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SUB THEME: *Cancer*

Endometrial cancer (EC) is one of the most common malignancies of the female reproductive system. In recent years, metabolomic and lipidomic approaches have gained increasing attention in discovering new biomarkers and to improve our understanding of EC pathogenesis. Comparative studies between EC patients and healthy controls are frequently employed; however, due to the typical occurrence of EC in women over 50 and the strong association between EC risk and obesity, selecting an appropriate matching control group is challenging. As a result, this introduces potential confounding effects that impact data interpretation because of differences in age and body mass index (BMI). In the present study, we investigated this issue, focusing on urinary eicosanoids.

The study groups consisted of 55 EC patients and 52 controls. For all participants, urinary eicosanoids were profiled using LC-MS/MS (Sieminska et al., 2024, DOI: 10.1016/j.prostaglandins.2023.106789). As the groups differ significantly in age (p-value=0.0285) and BMI (p-value=0.0003), statistical analysis was performed for all participants and in a subgroup of 30 EC patients and 30 controls matched for age

(p-value=0.8628) and BMI (p-value=0.6150).

Among 16 analytes, 10 were consistent regarding statistical significance and direction of change in initial and matched group comparisons. However, the amplitude of these differences was noticeably higher in the age- and BMI-matched group. Among the six metabolites without statistical significance in both comparisons, three: 2,3-dinor-8-isoPGF2α, 2,3-dinor-11β-PGF2α, and tetranorPGAM showed opposite change directions dependently of age- and BMI-matching of participants.

Our findings suggest that appropriate age and BMI matching of subjects reveals greater group differences, which enhances the probability of discovering disease related alterations in arachidonic acid metabolism.

2066 B — Structural Identification of Lipids Involved in Pancreatic Cancer using Oxygen Attachment Dissociation

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SUB THEME: *Cancer*

Introduction and Aim

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers with one of the lowest survival rates due to late diagnosis and high resistance to treatment. To aid earlier detection, untargeted metabolomics was applied to reveal phenotypic differences in the serum profiles of human PDAC patients compared to healthy controls with the aim of discovering potential biomarkers. Oxygen Attachment Dissociation (OAD), a technique that enables carbon-carbon double bond specific fragmentation, was used to increase reporting confidence in the identification of significant lipids.

Methods

Human serum samples (30 healthy controls and 30 PDAC) were analysed using a reversed-phase LC-QTOF-MS/MS untargeted metabolomics method. Data were processed with MS-DIAL and significant metabolite and lipids were identified to MSI levels 1 and 2. To increase reporting confidence for putatively identified lipids where standards are less readily available, phenotypic QCs were analysed using simultaneous OAD-MS/MS and CID-MS/MS in positive and negative ion mode to generate sufficient information to structurally characterise lipids.

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

Results

OAD-MS/MS was combined with CID-MS/MS to reveal the head group, acyl chain length and the number and position of double bonds in lipids found to be significant in PDAC through untargeted metabolomics. Structurally characterised lipids diminished by pancreatic cancer included LPC 18:2(n-6,9), LPC 18:3(n-3,6,9), LPC 20:5(n-3,6,9,12,15), LPE 18:1(n-9), LPE 18:2(n-6,9), PC 14:0_18:2(n-6,9), PC 16:0_20:5(n-3,6,9,12,15), PC 18:0_18:2(n-6,9), PC 18:0_20:5(n-3,6,9,12,15), PC 18:1(n-9)_18:2(n-6,9), PC 18:2(n-6,9)/18:2(n-6,9), PC 18:2(n-6,9)_20:4(n-6,9,12,15), PC O-16:1_18:2(n-6,9), SM 18:1(n-14);O2/16:0 and SM 18:2(n-4,14);O2/16:0. Amongst these lipids dysregulated by PDAC, omega-6 linoleic acid was revealed as a common constituent in several of them.

Conclusions

In this research, OAD-MS/MS has been applied with the aim of providing more comprehensive identification needed to understand the biological roles of the lipids identified as potential markers of PDAC.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2067 B — Developing and applying a targeted metabolomics workflow to identify novel metabolites presented by the antigen-presenting molecule, MR1

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SUB THEME: *Cancer*

Introduction: Major histocompatibility complexes (MHCs) present peptide antigens to immune cells known as T lymphocytes. In 2012, the MHC Class I related protein, MR1, was found to present small-molecule metabolite antigens, which could be covalently bound and stabilize MR1 by forming a Schiff base. MR1-restricted T cells have been implicated in immune responses against infections, autoimmune diseases, and cancers. Identifying disease-specific metabolite antigens could disclose novel therapeutic targets for currently challenging conditions, including cancers. A metabolomics approach for discovering novel metabolite antigens presented by MR1 is therefore pertinent. Here, we present a well-characterised metabolomics-based workflow employing ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) to detect endogenously derived MR1-bound small molecule ligands.

Methods: To optimise metabolite extraction, MR1 molecules refolded with a panel of known ligands, of diverse metabolite classes and chemical properties, were subjected to multiple extraction methods. A targeted small molecules assay was developed using standards representative of known MR1-binding metabolite classes. The optimized protocol was then applied to MR1 molecules expressed in mammalian cell lines to identify the spectrum of endogenous ligands.

Results: From the 38 methods tested, an optimal protocol for extracting MR1-bound ligands was established, using methanol. Two UHPLC-MS assays — Reversed-Phase (RP) and Hydrophilic Interaction Liquid Chromatography (HILIC) — were observed to provide comprehensive coverage of the metabolite classes expected to be MR1-bound ligands and were optimised. Applying this developed workflow, novel MR1-presented ligands from mammalian cell lines were putatively annotated.

2068 B — Untargeted metabolic profiling of FFPE samples: proof of concept in gastric cancer biopsies

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SUB THEME: *Cancer*

Globally, gastric cancer is among the top ten cancers with the highest mortality rates. Metabolomics studies in gastric cancer are critical to understanding its high heterogeneity and for biomarker discovery related to clinical outcomes, such as treatment and survivability. However, access to high quality fresh frozen (FF) samples can be difficult in developing countries. Previous findings from our team demonstrated a conservation of up to 30% of the polar metabolome from formalin-fixed paraffin-embedded (FFPE) gastric biopsies samples, in accordance with previous works from prostate and renal FFPE tissues. More importantly, a few numbers of reports were made using rodents (i.e. rats), and weren´t applied on a clinical settings. The main goal of the research is to prove that the polar metabolic alterations found in FF samples are sufficiently present in FFPE samples to distinguish tumor tissue from normal paracancerous tissue.

Gastric biopsies samples were collected from 16 Chilean patients who underwent gastrectomy due to gastric cancer. Tumor and paracancerous tissue were sectioned and preserved both at -80°C and in formalin/paraffin. FF samples were extracted with methanol and FFPE samples were deparaffinized with xylene and extracted with 80% methanol. Polar metabolites were separated using hydrophilic liquid chromatography in a UHPLC.

The results show a clear separation in the PCA of tumoral and paracancerous tissue of FF samples, which is not comparable in FFPE samples. Differences can be attributed to a loss of the polar metabolome due to the preservation process. Near 20% of the total FF sample features are found in both sample types. Moreover, some of the relevant changes includes the increase of proline, hypoxanthine, ornithine and cytidine in tumor tissue. Noteworthy, some features can be attributable to formyl-adducts in FFPE samples due to the preservation process. This result indicates that the metabolome could be more preserved than previous reports

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2069 B — Urine Metabolomics as a Non-Invasive Tool for Prostate Cancer Diagnosis

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SUB THEME: *Cancer*

Prostate cancer (PCa) is the second most commonly diagnosed malignancy and the fifth leading cause of cancer-related death in men worldwide, with an estimated 1.4 million new cases and 375,000 deaths reported in 2020. Although prostate-specific antigen (PSA) testing remains the standard screening tool, its limited specificity has led to overdiagnosis and unnecessary biopsies and colonoscopies. Some patients with low PSA levels may still have PCa, while others with elevated levels may not, underscoring the need for more accurate and non-invasive diagnostic alternatives.

This study aimed to evaluate the potential of urine metabolomics for the early detection of PCa, offering greater sensitivity and selectivity. First-morning urine samples were collected from 20 PCa patients and 20 healthy controls at URONORTE, Colombia. Samples were incubated at 60 °C for 30 minutes to promote the volatilization of organic compounds. The resulting headspace was extracted at a constant flow of 5 mL/min for 10 minutes using an AirChek XR5000 vacuum pump and collected on ORBO™ 402 Tenax® TA sorbent tubes.

The Tenax sorbent tubes were sent to Spain, where the volatile organic compounds (VOCs) were then thermally desorbed and captured using a CAR/PDMS SPME fiber housed in a custom-made holder. The loaded fiber was analyzed via gas chromatography–mass spectrometry (GC-MS) to detect and tentatively identify urinary volatiles. Raw chromatographic data were acquired using vendor software and processed to extract relevant features.

Data analysis revealed a clear distinction between PCa patients and controls, supporting the feasibility of this approach. The classification results suggest that urinary metabolomic profiling can reliably differentiate between groups and may serve as a source for identifying novel PCa biomarkers.

These findings highlight the promise of urine-based metabolomics as a complementary diagnostic strategy to improve early detection and clinical decision-making in prostate cancer management.

2070 B — Characterization of patients on Methotrexate (MTX) based on real-time breath metabolomics

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SUB THEME: *Cancer*

Methotrexate (MTX), an antimetabolite of the antifolate type, is a commonly used medication in oncological patients, high-dose MTX (HD-MTX) combined with other medications is used in the treatment of acute lymphoblastic leukaemia. However, due to its narrow therapeutic window and toxicity, MTX requires frequent monitoring to ensure successful therapeutic management. Gaining further insights into both drug-related and drug-regulated endogenous metabolites could support a more personalized treatment approach. Therefore, in this study we explored whether real-time breath metabolic profiling could assist the therapeutic management of pediatric oncological patients undergoing treatment with MTX.

In this longitudinal study, we recruited eight pediatric oncological patients, each receiving up to four MTX treatment sessions. During each session, a baseline breath measurement (~5 hours prior to HD-MTX administration) and up to five follow-up breath measurements were performed in real-time using secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS), resulting in a total of 77 breath measurements. In parallel, MTX blood concentrations were measured at all time points following the standard clinical protocol.

We applied a novel clustering approach, NAvMix, to group features with similar temporal behaviour and identified nine distinct clusters. Among these, Clusters 7 and 9 exhibited time profiles closely resembling MTX blood concentration patterns. Later pathway enrichment analysis via Mummichog shows enrichment of TCA cycle in these two clusters compared to others.

This is an ongoing study, and preliminary results demonstrate the potential of breath metabolic profiling to provide additional insights into MTX pharmacometabolism - particularly regarding endogenous metabolites like TCA cycle intermediates - which could pave the way for more personalized therapeutic monitoring.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2071 B — Metabolomics and lipidomics to characterize the toxic mixtures effects of a synergistic two anti-cancer drugs combination in 3D cell model of kidney cancer

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SUB THEME: ***Cancer***

Low-dose drug combinations is emerging as a superior alternative to high-dose monotherapy in cancer treatment, mitigating the risks of resistance and adverse effects. The efficacy of these combinations stems from their synergistic interactions, which selectively target cancer cells by disrupting multiple pathways involved in survival, proliferation, and resistance. However, the mechanistic understanding of these complex interactions remains incomplete, necessitating further investigation. Here, we address this knowledge gap through metabolomic analyses.

In a previous study, synergistic drug-drug interactions between erlotinib-HCl (tyrosine kinase inhibitor) and tubacin (histone deacetylase inhibitor) were identified. Here, untargeted metabolomic and lipidomic analyses were performed to elucidate the molecular pathways underlying these interactions.

Human kidney homotypic three-dimensional spheroids were exposed to erlotinib-HCl, tubacin, and their combination for 48h, followed by comprehensive post-exposure metabolome and lipidome profiling. By mapping metabolic perturbations and identifying interconnected metabolic sub-networks, we aimed to uncover the biochemical mechanisms underlying the observed drug synergy. Polar metabolomics was obtained with a zHilic stationary phase (pH=9.3), while lipidomics was obtained on a C18 column, resulting in >100 metabolites (level 1) and >700 high-quality MS2-confirmed lipids, respectively, thanks to an Orbitrap Exploris in negative/positive mode.

Multivariate analysis revealed distinct alteration patterns following exposure to tubacin, erlotinib-HCl and their combination. For both erlotinib-HCl and tubacin, a higher relative concentration in ceramides was observed. Moreover, erlotinib-HCl treatment led to an increase in sphingomyelins and triglycerides accompanied by a decrease in phosphatidylcholines. These changes were similarly observed in the combination treatment, but with enhanced magnitude. The biological interpretation of these lipid shifts, supported by variations in certain metabolites, suggests that those two drugs synergistically inhibit ceramides degradation, acknowledged in the literature as a powerful mechanism to promote cancer cell death and reduce their proliferation.

This study therefore highlights the potential of these -omics in reading phenotypes to deconvolute the mixture effects.

2072 B — Early Detection of Pancreatic Cancer Based on Altered Plasma Lipidome

PRESENTING AUTHOR: ***Michal Holčapek, University Of Pardubice, Czech Republic***

CO-AUTHORS: ***Ondřej Peterka, Zuzana Lásko, Robert Jirásko, Zuzana Dolečková, Mária Dosoudilová, Beatrice Mohelníková Duchoňová, Martin Loveček, Bohuslav Melichar***

SUB THEME: ***Cancer***

Pancreatic cancer has the worst prognosis among all malignancies, primarily due to late-stage diagnosis when curative treatment is no longer possible. The disease remains asymptomatic in early stages, making early detection crucial. In a cohort of 830 human plasma samples, we demonstrated that lipidomic analysis can distinguish pancreatic cancer patients from healthy controls (D. Wolrab et al., Nat. Com. 13 (2022) 124). To achieve this, we developed and validated a high-throughput ultrahigh-performance supercritical fluid chromatography–mass spectrometry (UHPSFC-MS) method for lipid quantitation in human plasma, capable of processing over 2,000 samples per month. Reliable quantitation is ensured by using at least two internal standards per lipid class and regular injections of quality control samples. Semi-automated data processing is performed using our freely available LipidQuant 2.1 software. Comprehensive MS-based lipid profiling reveals significant differences in blood lipid composition between cancer patients and healthy individuals, visualized through multivariate data analysis. The most striking results were obtained for pancreatic cancer, with a diagnostic performance of 95% sensitivity and specificity. Similar lipid dysregulation patterns were observed in kidney, breast, prostate, and other solid cancers, characterized by the down-regulation of very long-chain sphingomyelins, ceramides, and some (lyso)phosphatidylcholines. To translate this methodology into clinical practice, the University of Pardubice and FONS established the spin-off company Lipidica, which is currently conducting a clinical study to validate its clinical utility. Upon successful validation, the lipidomic test is expected to be implemented in national cancer screening programs for high-risk individuals, including those with a family history of pancreatic cancer, genetic mutations, or hereditary pancreatitis. Interestingly, altered lipidomic profiles persist even in patients who remain symptom-free for over five years after radical surgery. Further research aims to elucidate the biological mechanisms underlying these lipidomic dysregulations in pancreatic cancer. The work was supported by the ERC Adv grant 101095860.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2073 B — Targeting Carboplatin-Induced Metabolic Alterations to Sensitize Breast Cancer Cells to Treatment

PRESENTING AUTHOR: ***Shaymaa Dib, Weill Cornell Medicine-Qatar, Qatar***
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SUB THEME: ***Cancer***

Background: Resistance to chemotherapeutic agents remains a significant challenge in breast cancer treatment. Metabolic alterations associated with resistance can contribute to cancer progression and metastasis. Carboplatin, a platinum-based drug, is used to treat various cancers by inhibiting DNA replication and triggering apoptosis. However, resistance to carboplatin remains an issue, highlighting the need for further exploration. We hypothesize that carboplatin may induce metabolic changes contributing to resistance in breast cancer.

This study aims to investigate how carboplatin treatment alters metabolism in resistant breast cancer cells using untargeted metabolomic profiling, and to evaluate whether inhibition of altered pathways can sensitize these cells to carboplatin.

Methods: We conducted untargeted metabolomic profiling on six carboplatin resistant breast cancer cell lines treated with carboplatin for 24 and 48 hours. Based on data analysis, we selected three metabolic inhibitors: CHQ (chloroquine: autophagy inhibitor), EGR240 (BCAT1 inhibitor), and C.968 (glutaminase inhibitor) to evaluate their effect in combination with carboplatin. Cell viability and apoptosis were assessed using MTT, ATP, and Caspase-Glo assays, and autophagy was examined through LC3-I/II expression by western blotting.

Results: Pathway analysis revealed alterations in nucleotide, lipid, branched chain amino acid (BCAA), and glutamine metabolism. Our data suggested that increased lipid catabolism reflects on autophagy activation as survival mechanism. Chloroquine alone already significantly reduced cell viability in resistant cells; however, it did not synergize with carboplatin. Targeting BCAA metabolism with ERG240 and glutamine metabolism with C.968 failed to sensitize the cells to carboplatin, as viability remained above 70%.

Conclusion: CHQ showed potential as a single agent in carboplatin resistant breast cancer cells but lacked synergy with carboplatin. ERG240 and C.968 were ineffective in combination with carboplatin. Although these pathways are altered in resistant cells, further investigation using more potent inhibitors is needed to determine their therapeutic potential.

2074 B — Lipidomic Analysis as a Tool for Discrimination of Symptomatic and Asymptomatic Carotid Atherosclerotic Plaques

PRESENTING AUTHOR: ***Kamila Bechynska, UCT Prague, Czech Republic***
CO-AUTHORS: ***Anna Seidlova, Norbert Svoboda, David Netuka, Jana Hajslova, Vit Kosek***
SUB THEME: ***Cardiovascular diseases***

Atherosclerosis is a chronic inflammatory disease that leads to the accumulation of lipids and the formation of fibrous plaques in the walls of medium and large arteries. In this way, lipids are involved in the development of coronary heart disease and ischaemic stroke, which are among the leading causes of death in western civilisation. In clinical practice, the early diagnosis of incoming carotid plaque rupture remains problematic because the progression of atherosclerosis is nonlinear in time and conventional imaging techniques are unable to recognize changes in the internal structure of the plaque.

The aim of this study was to reveal differences between the plaque lipidome of patients who underwent carotid endarterectomy for excessive plaque volume (asymptomatic) and of patients who had ischemic stroke or its milder alternatives, such as transient ischaemic attack or Amaurosis fugax (symptomatic). Lipidomic analysis of 103 carotid atherosclerotic plaques, each cut up to 15 parts, was performed using MTBE:MeOH extraction and ultra-high performance liquid chromatography coupled to high-resolution tandem mass spectrometry. Overall, 766 lipids from 22 lipid classes were detected and used together with patient clinical parameters to create a classification model. Using AdaBoost with random forest (n=100), the accuracy on test set reached 75%. By far the most important feature was BMI, followed by age, and then lipids, mainly triacylglycerols, cholesteryl esters or plasmalogens.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2075 B — Metabolic adaptations to cardiac rehabilitation through physical exercise: Insights from untargeted metabolomics using dried blood spots in post-myocardial infarction patients

PRESENTING AUTHOR: *Eleonora Bossi, University Of Milano-bicocca, Italy*
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SUB THEME: *Cardiovascular diseases*

Ischemic heart disease remains the leading cause of death from cardiovascular diseases worldwide. The beneficial effects of cardiac rehabilitation (CR) through physical exercise on disease progression in patients after acute myocardial infarction (AMI) are well known, while knowledge of the effects on metabolic processes is still limited. Blood microsampling is a promising tool for blood collection as it is simple, minimally invasive, allows remote and multiple sampling and is optimal for longitudinal studies. For these reasons, it has gained attention and has been applied to metabolomics. This study aims to evaluate the metabolic response to CR using dried blood spots (DBS). 17 male subjects undergoing CR after a first AMI participated in this study. DBS were collected at 6 different time-points during the three CR weeks: before and after the first training, before and after training halfway through the CR protocol, and before and after the last training. Untargeted metabolomics and lipidomics analyses using ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS) were performed to assess training-induced changes in these subjects. Comparisons between pre- and post-training samples showed a long-term effect, mostly on energy metabolism, associated with increased exercise performance after three weeks. On the other hand, time-dependent changes in the lipidome, especially of phosphatidylserine lipid species, were compatible with the waning effect of the previous AMI. Moreover, CR monitoring showed that blood levels of N-acetyl-L-tyrosine (NAT) increased during the rehabilitation time. This metabolite has been shown to induce mitohormesis, a mitochondrial salvage response, in mice and insects under stress stimuli. Mitohormesis has also been observed in humans after physical training, but it has not yet been linked to blood NAT levels. Overall, our results indicate that, along with physical performance, CR affects energy metabolism; moreover, they point to NAT as a potential biomarker of CR efficacy over time.

2076 B — Serum Starvation Accelerates Intracellular Metabolism in Endothelial Cells

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CO-AUTHORS: *Cornelia Bartsch, Angelika Vietzke, Alina Eisenberger, Karl Stangl, Verena Stangl, Jennifer Kirwan, Mario Lorenz*
SUB THEME: *Cellular metabolism*

Cellular metabolic adaptions are vital to cope with diminished nutrient supply. Conditions of limited energy are challenging for the organism and results in comprehensive modulations of the blood metabolome. Fluctuations in blood nutrient levels are sensed by endothelial cells as the cellular lining of the vascular wall. One of the main physiological features of endothelial cells is to form new blood vessels (neoangiogenesis) under low nutrient supply.

We therefore studied the dynamic metabolic activity of human umbilical vein endothelial cells (HUVECs) under basal conditions and after serum starvation. We used a pulse-stable-isotope-resolved metabolic analysis (pSIRM) by applying labeled 13C-glucose or 13C-glutamine measured with gas chromatography-mass spectrometry (GC-MS).

Metabolites of glycolysis, the tricarboxylic acid (TCA) cycle, and the glycerol pathway showed lower levels after serum starvation, whereas amino acids had increased levels. The pSIRM analysis using 13C-glucose or 13C-glutamine for different time points reached a plateau phase of label incorporation after 30 hours for 13C-glucose and after 8 hours for 13C-glutamine under both basal and serum starved conditions. We observed a faster label incorporation for both 13C-glucose and 13C-glutamine after serum starvation. In the linear range of label incorporation after 3 hours, we found a significantly faster incorporation of central carbon metabolites after serum starvation compared to the basal state.

The faster incorporation of both 13C-glucose and 13C-glutamine after serum starvation was an unexpected finding, since it can be anticipated that cells would rather diminish their metabolic activity under conditions of impaired nutrient supply. However, the physiological characteristic for endothelial cells to form new blood vessels under unfavorable conditions in vivo provides a biological rationale for their need to increase rather than to reduce their metabolic activity.

2077 B — Spatial mapping of 15N-labeled ammonia with MALDI-FTICR imaging in regenerating liver tissue: Re-directing of toxic byproduct into anabolic pathways

PRESENTING AUTHOR: *Lukas Kucera, Institute Of Molecular Genetics Of CAS, Czech Republic*
CO-AUTHORS: *Endaya B. Berwini, Dominika Luptakova, Vladimir Havlicek, Radislav Sedlacek, Jan Prochazka, Michael Berridge V., Jiri Neuzil*
SUB THEME: *Cellular metabolism*

Liver is endowed with high regenerative activity, such that the tissue will regrow in the mouse after partial hepatectomy within days. We reasoned that this requires de novo pyrimidine synthesis to support rapid progression via the cell cycle. With MALDI imaging technique we revealed that suppression of de novo pyrimidine synthesis prevents proliferation in regenerating liver, suppressing liver regrowth. Tracing studies and spatial metabolomics revealed a metabolic shift such that ammonia, normally detoxified to urea in the periportal region under homeostasis, is redirected for generating aspartate and carbamoyl phosphate periportally, and glutamine pericentrally, and these products are utilized as precursors by the de novo pyrimidine synthesis pathway. This study uncovers a metabolic reprogramming leading to utilization of a toxic byproduct for anabolic pathways that are essential for liver regeneration.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2078 B — Shotgun lipidomics shows clear lipid specificity and plasticity between organelles across tissues

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SUB THEME: ***Cellular metabolism***

Mitochondria and endoplasmic reticulum (ER) are essential organelles that play key roles in a variety of cellular physiological and pathological processes. Recent studies have revealed that approximately 5%–20% of the mitochondrial outer membrane forms a dynamic physical connection with the ER, maintained at 10–30 nm. These connections, referred to as mitochondrial-associated membranes (MAMs), are a conserved feature in eukaryotic cells and serve as a crucial platform for material exchange between the mitochondria and ER, helping to regulate multiple aspects of cellular homeostasis. Lipids, as essential membranes constituents, play a key role in that matter.

By shotgun lipidomics, we show that mitochondria, ER and MAMs exhibit specific lipidome and lipid plasticity, especially across tissues, underlying the fact that those “compartments” must have different roles and ways of action depending on the tissue. Indeed, principal component analysis show clear and distinct clusters of every sample type. As expected, pure mitochondria fraction exhibits a very low diversity and concentration for neutral lipid species, especially triacylglycerols, as opposition to the well-known cardiolipin class. Lipid plasticity, which refers to the capacity of a lipid class/lipidome to experience alterations in the composition of its molecular lipid specie, also reveals a strong organelle- and organ- specificity. Generally, for every lipid class, organ type explains the highest separation, followed by the nature of the organelles. As unexpected, LPS shows the highest plasticity with 14.5-22 carbons in the fatty acid moiety and 0.5-6 double bonds, with longest and most unsaturated lipids in brain, and shortest and less unsaturated lipids in heart.

We present here a shotgun lipidomic method that allows the fast, robust and accurate analysis of 21 lipid classes in a various number of organelles and organs. This methodology allows the investigation of organelle-based research, especially mitochondria and MAMs, known to be involved in numerous diseases/disorders.

2079 B — An LC-MS/MS workflow to study the NAD+ metabolome across biological samples

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SUB THEME: ***Cellular metabolism***

Nicotinamide adenine dinucleotide (NAD+) and its reduced form (NADH) are essential coenzymes involved in cellular energy metabolism and redox balance. They function as hydride carriers in redox reactions, supporting cellular energy production and catabolic pathways. Due to its crucial biological role, the study of NAD+ metabolism has become a key focus in understanding cellular metabolic states and their links to disease. In fact, altered NAD+ levels are correlated with ageing, obesity, neurodegenerative diseases and cancer.

The goal of this study is to develop a comprehensive metabolomics workflow for the analysis of the NAD+ metabolome across biological matrices using liquid chromatography coupled with mass spectrometry (LC-MS). The high polarity of these metabolites, their pH sensitivity and the rapid interconversion between the oxidized and reduced forms still represent a challenge in sample preparation and chromatographic separation.

Here, we present an approach based on hydrophilic interaction chromatography (HILIC) coupled with high resolution multiple reaction monitoring (MRMHR) MS. Different chromatographic gradients and mobile phase conditions (e.g. pH) were used to optimize peak shape and intensity, as well as chromatographic resolution. Additionally, different extraction protocols were tested to assess metabolite recovery and matrix composition. Our optimized method includes NAD+ metabolic intermediates, precursors and catabolites. Using this approach, we measured the NAD+ metabolome of human hepatoma cell lines, which are widely used in drug metabolism and hepatotoxicity testing. To ensure a wider applicability, the method was extended to other sample types, including urine, serum, plasma, whole blood, and cerebrospinal fluid (CSF).

By leveraging the sensitivity and specificity of LC-MS, this workflow provides a detailed and comprehensive assessment of NAD+ metabolic pathways across different biological matrices. Such approaches will assist the development of therapeutic strategies around NAD+, by enabling systems-wide investigations of its role in health and disease.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2080 B — Characterizing poorly controlled type 2 diabetes using 1H-NMR metabolomics

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SUB THEME: ***Endocrine and metabolic diseases***

The global prevalence of type 2 diabetes has surged over the last two decades, reinforcing its pre-existing classification as an epidemic. Preventing disease progression requires the correct treatment and strict adherence to it. However, despite these efforts, some individuals with type 2 diabetes still progress to a poorly controlled state, characterized by hyperglycemia, which can lead to a range of serious health consequences and eventually even death. Although numerous studies in metabolomics related to type 2 diabetes have explained some of the metabolic changes associated with various physiological and immunological changes, fully understanding the metabolic alterations associated with poorly controlled type 2 diabetes remains a critical research gap. This investigation aimed to characterize the urine metabolome of poorly controlled type 2 diabetes in a South African cohort. Using an untargeted proton nuclear magnetic resonance metabolomics approach, urine samples from 15 poorly controlled type 2 diabetes patients and 25 healthy controls were analyzed and statistically compared to identify differentiating metabolites. The poorly controlled type 2 diabetes patients were characterized by elevated concentrations of various metabolites associated with changes to the macro-fuel pathways (including carbohydrate metabolism, ketogenesis, proteolysis, and the tricarboxylic acid cycle), autophagy and/or apoptosis, an uncontrolled diet, and kidney and liver damage. These results indicate that inhibited cellular glucose uptake in poorly controlled type 2 diabetes significantly affects all energy-producing pathways, leading to apoptosis and/or autophagy, ultimately contributing to kidney and mild liver damage. The study also shows poor dietary compliance as a cause of the patient's uncontrolled glycemic state. Collectively these findings offer a first-time comprehensive overview of urine metabolic changes in poorly controlled type 2 diabetes and its association with secondary diseases, offering potential insights for more targeted treatment strategies to prevent disease progression, treatment efficacy, and markers for monitoring diet/treatment compliance.

2081 B — Role of lipid mediators in cell-cell communication in the context of MASLD-associated fibrosis

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SUB THEME: ***Endocrine and metabolic diseases***

Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most prevalent liver disease worldwide. MASLD comprises a spectrum of pathologies including steatosis, fibrosis, cirrhosis and hepatocellular carcinoma. Fibrosis is a major determinant of liver-related mortality in MASLD patients. Genetic and environmental factors significantly influence MASLD risk. MBOAT7 rs641738T risk variant, associated with fibrosis development, leads to reduced levels of acyltransferase MBOAT7, a key enzyme involved in phospholipid remodeling. Loss of MBOAT7 in hepatocyte results in accumulation of bioactive lipid mediators, including lysophosphatidylinositol (LPI) and lysophosphatidic acid (LPA), both correlated with fibrosis progression. However, the molecular details on the role of bioactive lipids in hepatocytes and HSC cross-talk are still subject to investigation.

Quantitative analysis of lipid mediators and their role in cell-cell communications are analytically challenging due to their low concentration in biological matrixes. Here, we present the development of an assay for accurate quantification of lysophospholipids in the cellular secretome based on tailored enrichment and targeted MS detection. To ensure complete coverage of low abundant lipids, we optimized an enrichment strategy by testing various solid phase extraction protocols. Targeted LC-MS/MS method based on the multiple reaction monitoring (MRM) allowed for selective and sensitive detection of a wide range of bioactive lipid mediators. To support absolute quantification, method was validated using referenced standards following guidelines of European Medicines Agency (EMA). Optimized enrichment and quantification protocols were applied for the analysis of secretome of Huh7 human hepatocytes with and without MBOAT7 silencing. Bioactive lipids at detected concentrations were used to validate their role in HSC activation in hepatocyte conditioning medium transfer experiments. Overall, we developed an assay that allows for accurate quantification of bioactive lipid mediators and is used for the identification druggable molecular targets to halt hepatic fibrosis development in MASLD.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2082 B — A Robust Sphingolipid Profiling Approach for Deciphering Tissue-Specific Roles of Sphingolipids in Obesity-Associated Insulin Resistance

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CO-AUTHORS: *Tova Eurén, Leah She Jacobsson, Helena Edlund, Pär Steneberg, Elin Chorell*

SUB THEME: *Endocrine and metabolic diseases*

Sphingolipids (SL) are bioactive lipids implicated in the pathogenesis of insulin resistance and type 2 diabetes. However, SL analysis presents challenges due to their low abundance, structural diversity and interference from more abundant lipids in biological matrices, all of which hinder SL ionization in mass spectrometry-based lipidomics.

Methods: We present a robust sphingolipidomics platform optimized for plasma, liver, skeletal muscle and adipose tissue. By integrating a hydrolysis step into the Folch extraction procedure [1], we minimized the interference from triacylglycerols and phospholipids while preserving SL integrity. SL profiling was performed using a LC-MS-Q-TOF, and targeted quantification was validated using a tandem LC-MSMS analysis with triple quadrupole to confirm key species and avoid co-eluting artifacts.

Application in Insulin Resistance Study: We applied this method to a mouse model of obesity-induced insulin resistance. Tissues were collected from (a) mice on high fat diet (HFD) with insulin resistance (N=5), (b) HFD mice treated with the small molecule drug ATX-304 [2] (N=6), which preserves the obese phenotype and prevents insulin resistance, and (c) control littermates (N=5).

Results and conclusions: Our method enabled comprehensive profiling of sphingolipid species, including sphingomyelins, ceramides and ceramide precursors. Notably, we identified a tissue-specific association between ceramides and insulin resistance. Moreover, concentrations of deoxysphingolipids, a class of atypical SL, were altered in insulin-resistant tissues, highlighting the potential pathophysiological relevance. This study underscores the importance of combining global lipid screening with targeted LC-MSMS analysis to manage co-elution of isobaric interferences, with both methods being critical for a comprehensive and accurate interpretation of lipidomics data. Our approach provides a reliable platform for mechanistic studies and biomarker discovery in metabolic diseases.

[1] Sullards et al., Biochimica et Biophysica Acta 1811, 838 (2011)

[2] Steneberg et al., JCI Insight 3(12), e99114 (2018)

2083 B — Cardiolipin is differentially remodeled in oxidative and glycolytic skeletal muscle tissue during high-fat-diet-induced insulin resistance

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CO-AUTHORS: *Timotej Strmen, Tova Eurén, Madelene Ericsson, Pär Steneberg, Helena Edlund, Elin Chorell*

SUB THEME: *Endocrine and metabolic diseases*

Cardiolipin (CL) is a phospholipid most abundant in the inner mitochondrial membrane where it is the bounding site for the complexes in the electron transfer system and contributes to the folding of the crista, thereby acting as a regulator of mitochondrial oxidative capacity and function. Altered CL composition has been linked to loss of mitochondrial function and insulin resistance through a selective remodeling with reduced levels of tetra-linoleoyl CL(18:2)4, and increased incorporation of palmitoleic acid (16:1). Skeletal muscle insulin resistance strongly contributes to the development of type 2 diabetes. However, the impact of muscle typology on CL remodeling during insulin resistance remains poorly understood. To investigate this, we applied targeted liquid chromatography quadrupole time-of-flight mass-spectrometry to quantify CL species in muscles categorized as oxidative (soleus) and glycolytic (vastus lateralis) from high-fat diet (HFD)-induced insulin resistance mice and their control littermates fed a low-energy diet (CON). HFD did not alter total CL and CL72:8 levels in either muscle type. However, the CL72:8 to total ratio was lower in glycolytic muscle, indicating selective remodeling. In oxidative muscle, several shorter-chain CL species (<C72) were reduced, while in glycolytic muscle, species with longer acyl chain compositions (>C72) were increased following HFD. Correlation analyses revealed a significant association between CL72:8/CLtotal ratio and estimate of insulin resistance in glycolytic, but not oxidative muscle. We conclude that CL is differentially remodeled in oxidative and glycolytic skeletal muscle during HFD-induced insulin resistance and that changes in glycolytic muscle tissue appear to be of certain importance during the development of insulin resistance.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2084 B — Sex-specific ceramide accumulation and mitochondrial alterations in obese liver

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SUB THEME: ***Endocrine and metabolic diseases***

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the hepatic manifestation of obesity, affecting roughly one-third of the worldwide population. Female sex has been found to decrease the likelihood of onset, severity and mortality associated with MASLD. We hypothesised that during MASLD development, hepatic lipid influx alters lipid metabolism and mitochondrial respiration in a sex-dependent manner. In this study, mice were fed a high-fat high sucrose (HFHS) diet for 14 weeks, inducing early stage MASLD in males and females.

Changes in metabolism and lipid accumulation were measured with respirometry and lipidomics, respectively. MASLD status was confirmed by histology using H&E staining with fibrosis quantified using picrosirius red. A respirometry protocol, optimised for frozen liver tissue, revealed a potentially compensatory increase in mitochondrial respiratory capacity in female mice fed a HFHS diet that was absent in male HFHS mice and was not related to mitochondrial content. OPLS-DA of the lipidome revealed greater separation by sex in HDHS-fed mice than in chow-fed mice. Lipids identified by VIP score included ceramides which were greater in obese compared with lean groups, and especially so in male mice (80% $p<0.0001$) compared with females (52% $p=0.29$). Increasing abundance of ceramides correlated negatively with mitochondrial respiration ($p=0.001$), reaching statistical significance in males but not females. One specific ceramide, Cer(d18:1/18:0), previously negatively associated with insulin resistance in the liver was higher in females than males (70% $p=0.015$) with a significant interaction ($p=0.0035$), but with abundance being lower in female HFHS mice than male HFHS-fed mice but greater in HFHS-fed mice. In conclusion, although MASLD status was comparable in males and females, a potentially compensatory mechanism in mitochondrial function was linked to a lesser increase in ceramide accumulation in females than males, potentially providing mechanistic insight into MASLD protection in females.

2085 B — Untargeted Metabolomics for differentiation of NASH, Non-NASH, and borderline NASH Patients

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SUB THEME: ***Endocrine and metabolic diseases***

Introduction: Non-alcoholic steatohepatitis (NASH) is a progressive form of non-alcoholic fatty liver disease (NAFLD) associated with increased risk of liver-related morbidity and mortality. Its diagnosis currently relies mainly on histopathological evaluation, which is invasive, expensive, and often yields inconclusive results. There's referred to as "borderline NASH." Present a combination of clinical and histological features, that make accurate classification particularly difficult. Additionally, the absence of reliable, non-invasive biomarkers complicate patient stratification and clinical management. These challenges underscore the need for novel approaches to enhance diagnostic accuracy across the entire spectrum of NASH.

Aim: To investigate plasma metabolic profiles through untargeted metabolomics to detect endogenous biomarkers that can effectively differentiate between non-NASH, uncertain NASH, and NASH patients.

Methods: 405 plasma samples from human volunteers were analysed using high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-ESI-QTOF-MS). Participants were evenly diagnosed into three groups (n=135 each): non-NASH, borderline NASH, and NASH, based on combined clinical and histopathological evaluations. Data preprocessing was done using MZmine 3, and Notame R package. Multivariate modeling was performed using MUVR, and univariate analysis was conducted with MetaboAnalyst. Significant metabolites were annotated comparing MS/MS fragmentation patters with those available in metabolomic databases or predicted by in-silico software like Sirius.

Results: Although a completely unique metabolic fingerprint is not observed for each group, significant differences were detected among various metabolite classes. Notably, changes in phospholipids, lysophospholipids, triglycerides, and acylcarnitines contribute to the differentiation between study groups. These metabolic changes are especially valuable for the identification of borderline NASH patients, a subgroup often misclassified by current diagnostic techniques.

Conclusions: Untargeted metabolomics reveals metabolic differences across the NASH spectrum. Lipid-related metabolites show promise as non-invasive biomarkers, potentially improving diagnostic accuracy and patient stratification, particularly in borderline cases.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2086 B — Metabolic and Lipidomic Consequences of MTARC1 and MTARC2 Deficiency In Mice Under Normal and GAN Diets

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SUB THEME: ***Endocrine and metabolic diseases***

Background

Mitochondrial amidoxime reducing components 1 and 2 (Mtarc1/2) are molybdenum cofactor–containing enzymes that catalyse the reduction of N-hydroxylated compounds and have recently been linked to liver lipid metabolism. Human genetic studies reveal that Mtarc1 loss-of-function alleles protect against non alcoholic steatohepatitis (NASH) and cirrhosis. Despite these observations, the precise contribution of Mtarc1/2 to metabolic reprogramming during diet-induced steatohepatitis remains unclear, and resolving this gap could uncover new checkpoints and biomarkers for liver disease.

Methods

Wild-type (WT), Mtarc1-knockout (KO) and Mtarc2-KO C57BL/6N mice (n = 100) were fed either a normal chow diet (ND) or a GAN (Gubra-Amylin NASH) diet for 24 weeks. Polar and lipid extracts from liver tissue were analysed by untargeted ^1H NMR spectroscopy and LC-QTOF-MS/MS. More than 200 metabolites and 500 lipid species were annotated using MS-DIAL and examined through univariate and multivariate statistics together with lipid ontology enrichment analysis.

Results

Diet was the dominant driver of metabolic variance, but genotype introduced distinct signatures. Under GAN feeding, both KO mouse strains displayed lower saturated fatty acids and marked shifts in phosphatidylcholine composition. Mtarc2-KO livers exhibited more profound lipidomic alterations: polyunsaturated long-chain fatty acids increased, triacylglycerol pools expanded, and phosphatidylcholines were selectively remodelled. Several sulphated, microbiota-derived metabolites trended upward in Mtarc2-KO mice. A modest increase in sulphonolipid-like features was also observed, particularly in Mtarc2-KO mice under ND, suggesting an extension of previously reported GAN diet effects and a potential link between Mtarc2 deficiency and gut-derived sulphur-lipid accumulation. These findings suggest a genotype-specific interaction with the gut–liver axis.

Conclusions

Our findings indicate that Mtarc1 and Mtarc2 modulate hepatic lipid partitioning and gut–liver metabolic communication during dietary stress. The quantitative lipidomic shifts and emergence of sulphur-containing metabolites identified here nominate new biomarkers and pathways relevant to the progression of non-alcoholic steatohepatitis.

2087 B — Body composition subphenotypes, derived from distributions of n = 29 adipose tissue depots measured by magnetic resonance imaging, show distinct patterns of targeted serum metabolites: the population-based KORA-MRI study

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SUB THEME: ***Epidemiology***

Intro: Location and distribution of adipose tissue (AT) has more impact on cardiometabolic risk than overall body size. We have previously established subphenotypes of body composition based on AT distribution. Serum metabolite patterns of AT distribution could characterize potential pathophysiological pathways.

Aim: To identify metabolite signatures of single AT depots and body composition subphenotypes.

Methods: Targeted metabolites (Biocrates p180 kit) were measured in fasted serum for N=390 individuals from the population-based KORA-FF4 cohort (42% women, average age 56y). AT was measured by magnetic resonance imaging. Association of n=29 AT depots (visceral (VAT), subcutaneous (SAT), liver, pancreas, bone marrow, skeletal muscle, heart, kidney) and five subphenotypes with 146 metabolites and 40 derived indicators were investigated by linear regressions with confounder adjustment and multiple testing correction.

Results: Subphenoytpes were associated with 59, and single ATs with 275 metabolites/indicators, with VAT and SAT showing most associations. Compared to subphenotype I (low overall ATs), subphenotype II (average ATs) showed positive associations with sphingomyelin C20.2, hydroxysphingomyelin C22.1 and diacylPC with differently saturated C32 fatty acid. Subphenotype III (high muscle and bone marrow fat) was negatively associated with total lysophosphatidylcholines (lysoPCs) and total monounsaturated lysoPCs, while showing a positive association with total longchain acylcarnitines (C14–C18). Subphenotype IV (high SAT/VAT and liver fat) exhibited positive associations with short-chain acylcarnitines, alanine and aromatic amino acids. Subphenotype V (high pancreatic fat) was related to arginine and the ratio of ornithine and arginine as surrogate for ornithine synthesis. Three metabolites/indicators (lysoPC C 18.2, total polyunsaturated lysoPC, phospholipase A2 as ratio of lysoPC/diacyl and acylalkyl PC) were associated with all subphenotypes. These results were supported by the associations of individual ATs with metabolites.

Conclusions: ATs and subphenotypes of body composition show distinct serum metabolite patterns, which can serve as a first step to characterize potential pathophysiological pathways.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2088 B — Revealing the urinary exposome fingerprint of paediatric obesity: The EXPOSOME project

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SUB THEME: *Exposome research*

Childhood obesity is a growing global health emergency linked to an increased risk of metabolic, cardiovascular, and neurodevelopmental disorders later in life. Beyond diet and physical inactivity, recent research highlights the exposome—the totality of non-genetic environmental exposures across the lifespan—as a key contributor to obesity development. During early life, children are especially vulnerable to environmental pollutants that can disrupt endocrine and metabolic systems in lasting ways, inducing epigenetic changes, chronic inflammation, and metabolic dysfunctions that may promote weight gain. Yet, most studies focus on a limited number of compounds, overlooking the complexity of real-world chemical exposure. To better understand how these mixtures influence paediatric obesity, broad and high-resolution analytical approaches are needed to capture the full chemical fingerprint present in biological samples like urine.

The main goal of this study is to unravel the complex relationship between children's exposure to environmental pollutants and the biochemical markers that signal obesity. We have applied a wide-scope target workflow to determine exposure biomarkers in urine samples from 275 Spanish children with obesity (8-14 years old) enrolled in the EXPOSOME project. The method targeted 688 exposome metabolites, including pesticides, plasticisers, pharmaceuticals, and other contaminants of emerging concern.

Urine samples were treated using clean-up Captiva filters and subsequently analysed by reverse-phase ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS). Raw spectral data were processed using MS-DIAL. Compound annotation and identification were performed by comparing the experimental data with retention time, MS1, and MS/MS data from our in-house library. The resulting, rigorously curated abundance matrix provided the foundation for subsequent multivariate and statistical analyses aimed at delineating the urinary exposomic "fingerprint" associated with paediatric obesity.

Ultimately, these findings are intended to shed light on how environmental chemicals contribute to obesity development and to pinpoint early targets for public health prevention and intervention strategies.

2089 B — Non-Targeted Screening of Urinary Biomarkers Identifies Endocrine Disruptors Linked to Ovarian Malignancies

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SUB THEME: *Exposome research*

Endocrine-disrupting compounds (EDCs) are widespread environmental contaminants linked to various adverse health effects, including hormone-related cancers. However, their specific roles in many cancers remain unclear, hindering a comprehensive evaluation of their impact on human health. High-resolution mass spectrometry (HRMS)-based non-targeted screening (NTS) has become essential for detecting biomarkers of exposure (BoEs) in biological samples, enabling broader assessment of chemical burden. However, challenges persist in identifying low-abundance and highly polar contaminants, limiting comprehensive exposure assessment.

This study compared urinary BoEs of women with ovarian malignancies including borderline ovarian tumors and invasive ovarian cancer to healthy controls. A HRMS-based NTS approach was employed, integrating hydrophilic interaction liquid chromatography (HILIC) and reversed-phase (RP) separations to detect a broader range of biomarkers. A fractionation-based solid-phase extraction protocol was developed yielding two complementary extracts: a polar analyte-rich fraction for HILIC-based and a semi/nonpolar analyte-rich fraction for RP-based instrumental analysis. This approach expanded chemical space coverage and improved detection rate. Data analysis used open-access software, integrating public and in-house MS2 spectral libraries and an in-house retention time prediction model to enhance annotation accuracy.

Among 106 identified BoEs, six exhibited significantly higher normalized intensities in ovarian cancer patients' urine, including metabolites of phthalates (Diethylhexyl phthalate, Diethyl phthalate), pesticides (Metolachlor TP NOA 413173, 4-Nitrophenol), a UV filter (Benzophenone-1), and an industrial byproduct (4-Methyl-2-nitrophenol). Notably, one Diethylhexyl phthalate metabolite showed a dose-dependent increase from borderline tumors to invasive cancer. These EDCs may contribute to cancer development through hormonal dysregulation, oxidative stress, and chronic inflammation.

This study highlights the potential of urinary NTS in assessing chemical exposures linked to ovarian malignancies. Combined HILIC and RP analysis expanded chemical coverage, facilitating comprehensive detection of urinary BoEs. Future research should validate these findings in larger cohorts and explore links between EDCs and ovarian cancer progression to inform early detection and prevention strategies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2090 B — Enhancing Chemical Coverage in Urinary Exposome Profiling: Compared Chemical Spaces Captured by SPE and SUPRAS Extraction

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SUB THEME: *Exposome research*

Widespread human exposure to organic chemical contaminants is associated with numerous adverse health effects. Traditional human biomonitoring (HBM) methods focus on tracking specific biomarkers of exposure (BoE) in a targeted way, potentially overlooking crucial contaminants, which are essential for understanding holistic exposure profiles and related mixture effects. To address this limitation, HRMS-based suspect and non-targeted screening (SS/NTS) has gained prominence in exposome research.

Despite their broad scope, non-targeted analysis methods often face limitations in chemical space coverage. These limitations arise partly from the inefficiencies of single sample preparation techniques in extracting a diverse range of chemical entities and from significant matrix effects—such as ion suppression—that can substantially mask important signals. To address these challenges, we developed the supramolecular solvent (SUPRAS)-based extraction method and compared its chemical coverage with that of the conventional solid-phase extraction (SPE)-based sample preparation. Our analytical workflow integrated gas and liquid chromatography, the latter employing both the reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC) separations.

The comparison was based on the extraction efficiency from urine samples fortified with over 80 analytical standards possessing diverse physicochemical properties. The results indicated that the majority of tested compounds exhibited higher recoveries with SPE, while others—generally less polar ones—were more efficiently extracted using SUPRAS. These findings position SUPRAS as a viable complement to SPE, particularly for less polar compounds. By combining both methods, the overall chemical space coverage is significantly enhanced, enabling more comprehensive detection of environmental contaminants and providing a deeper understanding of human exposure profiles.

2091 B — Metabolic Disruptions Induced by PFAS Exposure: Insights from Human Studies and Models in Cells, Avian Species, and Mice

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SUB THEME: *Exposome research*

Per- and polyfluoroalkyl substances (PFAS) are widespread environmental contaminants known to disrupt metabolic processes and pose adverse health effects. PFAS exposure often leads to non-monotonic dose-response curves, where both low and high doses can have opposite or differing effects. In this study, we examined aviation firefighters (N=37) with high PFAS exposure, and two general population group with typical exposure levels (n=300+1500), alongside in vitro and in vivo models.

In the firefighters, metabolomic analysis revealed changes in free fatty acids, bile acids, amino acids, lipids, and gut microbial metabolites, correlating with firefighting experience and PFAS levels. Notably, a non-monotonic response was observed in saturated fatty acids, with low-exposed firefighters showing a positive association with PFAS, while highly exposed firefighters showed an inverse relationship. The metabolic alterations in low-exposed firefighters resembled those seen in the general population, suggesting a complex metabolic response disrupted by long-term high-level PFAS exposure.

In vitro studies using three hepatocyte models across a wide exposure concentration range showed dose-dependent disruptions in lipid, steroid, amino acid, and carbohydrate metabolism which were linked with structural changes in mitochondria and cell membranes. Key mechanisms were oxidative stress and inflammation. These findings were consistent with the human data, but specific differences were noted due to the absence of secondary effects of exposure via gut microbiota modulation in in vitro models.

Further in vitro experiments involved extracting gut-derived metabolites from a PFAS-exposed colon model and exposing hepatocytes to these extracts. Changes in steroid hormone, bile acid, and fatty acid metabolism were observed. These findings were compared with data from in-ovo exposure in chicken embryos and PFOA-exposed mice. The results showed dose-dependent, non-monotonic perturbations in lipid, bile acid, steroid and amino acid metabolism. These findings highlight the need for comprehensive metabolic profiling in evaluating PFAS-related health risks.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2092 B — Reflections on scaling gas chromatography–mass spectrometry for profiling environmental chemicals in human cohorts

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CO-AUTHORS: ***Kateřina Coufalíková, Akrem Jbebli, Romana Ševčíková, Elliott James Price, EIRENE-CZ***
SUB THEME: ***Exposome research***

Maintaining measurement consistency of small molecule profiling is challenging, and few academic settings conduct routine analysis of studies comprising > 1000 samples.

We aimed to scale an established gas chromatography – mass spectrometry (GC-MS) method for profiling environmental chemicals in human blood plasma, making it suitable for routine application to population cohorts.

During implementation, several limitations of the prior established methods were identified and addressed. Key optimizations included transitioning from off-line to automated on-line sample preparation, refining analytical batch design, and modifying interventional maintenance procedures and schedules.

We shall present six months monitoring data acquired for certified reference materials (CRM) used for quality control, including system suitability mixture, retention-indexing mixture and numerous matrix materials incorporated into each analytical batch. Additionally, we will report our systematically recorded errors, such as collision errors during sample extraction and injection failures, providing a critical reflection of our implementation.

Our transparent reporting and critical appraisal will transfer knowledge that enables others to scale GC-MS based small molecule profiling methods more efficiently.

2093 B — Effects of 7-ketolithocholic Acid on Intestinal Epithelial Barrier Integrity, Inflammation, and Homeostasis

PRESENTING AUTHOR: ***Justina Guzauskiene, Lithuanian University of Health Sciences, Lithuania***
CO-AUTHORS: ***Ruta Inciuraite, Violeta Salteniene, Ugne Kulokiene, Deimante Tilinde, Vytautas Kiudelis, Juozas Kupcinskas, Jurgita Skieceviciene***
SUB THEME: ***Gastrointestinal diseases***

Introduction The integrity of the gut epithelial barrier and its modulation of inflammation is crucial for overall health. These factors play a significant role in maintaining intestinal homeostasis and preventing diseases like inflammatory bowel disease (IBD). A bile acid metabolite, derivative of lithocholic acid, 7-ketolithocholic acid (7-keto-LCA), has been identified as potential therapeutic agent due to its ability to act as a farnesoid X receptor (FXR) antagonist. Because of this action, 7-keto-LCA may promote intestinal epithelial repair and enhance Wnt signalling, thereby facilitating the self-renewal of intestinal stem cells (Li et al., 2023).

Methods Primary screening of 7-keto-LCA (5μM, 10μM, 50μM, and 100μM) was done in Caco-2 cells. Metabolite was further tested on inflamed 3D intestinal epithelial organoids derived from non-IBD individual and ulcerative colitis patient. Inflammation was induced by cultivation of cells with cytokine mix of IFN-γ and TNF-α for 24 h. The expression of key genes TNF-α, CXCL9, OCLN, TJP1, FGF19 and NR0B2 were analysed using qRT-PCR. Student's t-test was used to assess group differences. Results were considered statistically significant when $p \leq 0.05$.

Results Primary screening on Caco-2 cells revealed that 7-keto-LCA downregulated the expression of genes encoding inflammatory cytokines (TNF-α and CXCL9; $p < 0.05$) and upregulated the expression of tight junction proteins (OCLN and TPJ1; $p < 0.05$) and FXR downstream marker (FGF19, $p < 0.05$). Selected 10μM concentration then was further analysed in inflamed 3D intestinal organoids. 7-keto-LCA showed a tendency to reduce the expression of TNF-α in patient organoids. In organoids of non-IBD 7-keto-LCA significantly upregulated the expression of NR0B2 ($p < 0.05$).

Conclusion 7-keto-LCA showed a trend to reduce inflammation and restore homeostasis in intestinal organoid model.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2094 B — METABOLOMIC PROFILING OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

PRESENTING AUTHOR: ***Wioleta Gosk, Medical University of Bialystok, Poland***
CO-AUTHORS: ***Rafal Kucharski, Karolina Pietrowska Pietrowska, Joanna Godzien, Jaroslaw Daniluk, Adam Kretowski, Michal Ciborowski***
SUB THEME: ***Gastrointestinal diseases***

Background: Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic gastrointestinal disease which diagnosis is mainly based on invasive endoscopic examinations. Due to the lack of unequivocal laboratory markers, there is a need to develop less invasive methods to support diagnosis, but this requires a profound understanding of metabolic alterations underlying the development of IBD. The study aimed to analyze the plasma metabolomic profile of patients with IBD compared to healthy individuals and identify metabolites differentiating CD and UC.

Methods: Peripheral blood plasma was collected from 38 patients with CD, 39 with UC, and 30 controls. This resulted in the collection of 107 plasma samples. The collected material was used to determine morphological and inflammatory parameters, along with LC/MS untargeted metabolic profiling. Metabolites were separated employing reversed-phase chromatography and detected in both MS polarity modes. The obtained data were statistically analyzed in three comparisons: CD vs. controls, UC vs. controls, and CD vs. UC.

Results: A total of 45 statistically significant metabolites were identified. Of these, 6 metabolites (ceramide and its derivatives, cortisol, SM 32:2, hexacosanedioic acid, and DHA) were significant in CD vs. controls, 32 metabolites (including ceramide and its derivatives, phenol and its derivatives, and phospholipids) differentiated UC patients from controls; and finally, 11 metabolites (including glutamine and its derivatives, phenol and indoxyl sulphates and selected phospholipids) distinguished CD from UC.

Conclusion: The results obtained confirm that metabolic abnormalities may reflect clinical status and pathophysiological differences between IBD subtypes. The identified metabolites have the potential to become noninvasive diagnostic markers and, after validation, may support disease diagnosis and monitoring in clinical practice.

2095 B — Metabolic Adaptations of Candida albicans in Ulcerative Colitis Environment

PRESENTING AUTHOR: ***Daehwan Kim, Seoul National University, South Korea***
CO-AUTHORS: ***Sohyeong Choi, Won Hee Jung, Do Yup Lee***
SUB THEME: ***Gastrointestinal diseases***

The human gut hosts diverse microbial communities. Candida albicans is a common yeast normally regulated by gut bacteria. In ulcerative colitis (UC) patients, this balance can be disrupted by antibiotics or immune dysfunction. This disruption often leads to C. albicans overgrowth. The UC environment features lower pH, reduced oxygen, and altered nutrient availability. These conditions may trigger metabolic changes in C. albicans. Higher C. albicans levels occur in UC patients. However, specific metabolic adaptations remain poorly characterized.

We isolated C. albicans strains from blood, mucosa, and feces of UC patients and healthy controls. Each strain was cultured for LC-MS-based metabolic profiling of culture supernatants. MS-DIAL5 was used for peak detection and alignment. Pathway enrichment analysis was performed using MetaboAnalyst 6.0.

Metabolic profiles showed subtle differences between groups. Pathway analysis revealed significant changes in purine metabolism, pyrimidine metabolism, and arginine/proline metabolism. These findings suggest C. albicans adapts metabolically to the inflamed UC environment. Such adaptations may help manage nutrient limitation or oxidative stress.

Our results illuminate metabolic aspects of C. albicans adaptation during inflammation. This observation may provide insights into the complex host-microbe interactions in inflammatory conditions.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2097 B — Fecal Metabolomic Profiling of Very Preterm and Extremely Premature Infants with Bronchopulmonary Dysplasia Using Liquid Chromatography-Mass Spectrometry

PRESENTING AUTHOR: *Chieh-Ni Kuo, Linkou Chang Gung Memorial Hospital, Taiwan*
CO-AUTHORS: *Meng-Han Chiang, Yu-Ying Yu, Chih-Yung Chiu*
SUB THEME: *Immunometabolism*

Background:

Bronchopulmonary dysplasia (BPD) is a frequent complication in preterm infants, especially those born before 32 weeks of gestation. Several studies have shown that the fecal microbial composition and metabolites of premature infants are closely linked to their health and development. LC/MS-based metabolomics can identify BPD metabolic signatures, enabling early diagnosis and targeted interventions. Understanding disrupted pathways may also support personalized treatments and improve outcomes.

Methods:

This study enrolled 124 infants, including 45 healthy controls and 79 preterm infants (<32 weeks gestation). According to 2019 NICHD criteria, preterm infants were classified into no/mild BPD (n = 32) and moderate/severe BPD (n = 47). Fecal metabolomic analysis was performed at 6 months corrected age using the AbsoluteIDQ® p180 Kit. Partial least squares discriminant analysis (PLS-DA) was used to identify metabolites associated with gestational age (GA) and BPD severity.

Results:

Extremely preterm infants (GA < 28 weeks) had significantly lower body weight and BMI percentiles than full-term infants ($P < 0.001$). Six aqueous and nine lipid metabolites were significantly associated with GA, while seven aqueous and fifteen lipid metabolites were linked to BPD severity ($P < 0.05$). Among these, spermidine, involved in β-alanine and arginine metabolism, was strongly associated with GA and BPD development ($P < 0.01$). Six carnitine-related lipids and two sphingomyelins (36:1, 36:2) were related to both GA and BPD severity. Phosphatidylcholine, involved in linoleic acid metabolism, was specifically associated with BPD severity.

Conclusion:

LC/MS-based fecal metabolomics identified spermidine, carnitine-related lipids, and sphingomyelins as key metabolites linked to GA and BPD severity. Phosphatidylcholine may serve as a distinct biomarker of BPD severity, providing insight for early diagnosis and personalized treatment.

2098 B — Phosphatidylethanolamine Modulates Macrophage Function and Suppresses LPS-Induced Inflammatory profile

PRESENTING AUTHOR: *Tatiana Maurício, University of Aveiro, Portugal*
CO-AUTHORS: *Bruno Neves, M. Rosário Domingues, Pedro Domingues*
SUB THEME: *Immunometabolism*

Inflammation is a complex physiological process aimed at restoring homeostasis, but when uncontrolled, it can trigger or exacerbate pre-existing conditions, leading to permanent damage. Monocytes and macrophages are effector cells of the immune system, with an essential role in the activation and resolution phases of the inflammatory response. Recent evidence suggests that phospholipids act as immunoregulators, shaping the functional outcome of macrophage-mediated immune responses. Our study investigates the immunomodulatory effects of phosphatidylethanolamine (PE) species esterified with n-3 (PE 18:0/22:6) or n-6 (PE 18:0/20:4) fatty acids on RAW 264.7 macrophages using an in vitro model of lipopolysaccharide (LPS)-induced inflammation. A multi-omics approach based on mass spectrometry (C18-RP-LC-MS/MS) was employed to analyze the lipidome and the proteome of supplemented macrophages. Macrophage exposure to PE 18:0/22:6 and PE 18:0/20:4, resulted in significant reprogramming of the lipidome and proteome, not only under basal conditions but also during LPS-induced activation. DIABLO analysis revealed a strong correlation between lipidome and proteome profiles (correlation coefficient: 0.97) in PE-supplemented macrophages. This approach identified key changes associated with PE supplementation, encompassing 337 lipid species and 1488 proteins. Clustering analysis of the key discriminative variables between conditions revealed coordinated lipid-protein interactions in response to PE supplementation. Under basal conditions, PE supplementation induced distinct macrophage remodeling, characterized by specific lipid-protein profiles, including the upregulation of antioxidant defense proteins (Catalase, Transaldolase, and Glutathione reductase). Upon LPS stimulation, our analysis revealed a coordinated response in which PE supplementation attenuated LPS-induced inflammatory changes. These included a reduction in pro-inflammatory lipids, such as phosphatidylcholine species (PC and PC-O), and an upregulation of immune signaling proteins (Sequestosome-1, CD47, and PRDX5). This integrative multi-omics approach elucidates the connection between lipid composition and protein expression in macrophage immunometabolism, demonstrating the nuanced effects of PE species (PE 18:0/22:6 and PE 18:0/20:4) on inflammatory and antioxidant responses.

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2099 B — Maternal capric acid and childhood immune proteome predict progression to neurodevelopmental outcomes: a multi-omics birth cohort study.

PRESENTING AUTHOR: ★ *Aaron Barron, University of Turku, Finland*

CO-AUTHORS: *Alex Dickens, Minna Lukkarinen, Tuulia Hyötylainen, Linnea Karlsson, Matej Oresic, Hasse Karlsson*

SUB THEME: *Immunometabolism*

Introduction:

Abnormalities in peripheral immune signalling are linked to neuropsychiatric disorders and neurodegeneration, but studies on the immune proteome and metabolism, especially in children, are rare. Thus, the role of immunometabolism in early brain development remains unknown.

Aim:

To determine if the immune proteome in 5-year-olds differs based on (1) neurodevelopmental or affective disorder diagnosis and (2) multimodal brain outcomes, and whether these factors are predicted by peripheral metabolites.

Methods:

Serum immune proteomics was performed on 5-year-old children (N=779) using Olink Explore Inflammation 384. Polar/semi-polar metabolomics and lipidomics were performed on maternal gestational serum using ultra-high-performance liquid-chromatography quadrupole time-of-flight mass-spectrometry, now being applied to children's sera. Medical registry data identified individuals with ADHD, autism, depression, or anxiety by age 11. Children underwent structural and diffusion-weighted MRI to assess grey matter volume, white matter integrity, and cerebrocortical thickness and surface area.

Results:

Children who develop anxiety exhibit elevated inflammation-associated proteins; after covariate adjustment and false discovery rate (FDR) correction, IL-18, CSF-1, CCL23, CCL25, TNFRSF13B, and PTH1R were higher, a phenotype not seen for other diagnoses. Model-based clustering summarized the immune proteome into six clusters, with all above proteins belonging to the same cluster, characterized by inflammatory cytokines and chemokines, and predicting lower whole-brain cortical thickness (R^2=0.09, p=0.002). Notably, maternal capric acid strongly and negatively predicted CLEC4D, TGF-α, PLAUR, and FCAR (R^2=0.13-0.18, pFDR<0.05), all belonging to the same cluster which is elevated in anxiety. Lastly, maternal capric acid predicted a lower risk for anxiety diagnosis in the children (pseudoR^2 =0.12, p=0.01).

Conclusions:

These data demonstrate that children who develop anxiety have a pro-inflammatory immune proteome, and maternal capric acid, derived mainly from coconut and palm oil, predicts lower inflammation and reduced anxiety risk. These findings will be integrated with children's metabolomics in Spring 2025, creating a comprehensive immunometabolic landscape of human neurodevelopment.

2100 B — Advancing metabolomics with ion mobility: distinguishing isovalerylcarnitine and 2-methylbutyrylcarnitine with a novel timsTOF

PRESENTING AUTHOR: *Michel van Weeghel, Amsterdam UMC, Netherlands*

CO-AUTHORS: *Sven W. Meyer, Henk van Lenthe, Matthew R. Lewis, Frédéric M. Vaz*

SUB THEME: *Inborn errors of metabolism and neonatal screening*

In this study, we evaluated a novel Q-TOF mass spectrometer integrated with trapped ion mobility spectrometry (TIMS) for its application in metabolomics, particularly in analyzing small molecules relevant to metabolic disorders. The instrument's design incorporates hardware optimized for small molecule analysis, facilitating enhanced sensitivity and structural resolution.

We applied the system to analyze butylated standards and plasma samples from patients with isovaleric acidemia (IVA) and short-chain acyl-CoA dehydrogenase (SBCAD) deficiency. Ion mobility spectrometry enabled the separation of isovalerylcarnitine and 2-methylbutyrylcarnitine based on their distinct drift times, even in the absence of chromatographic separation. The instrument demonstrated sensitivity comparable to triple quadrupole (QQQ) mass spectrometers, while offering good mass resolution and the additional capability of ion mobility separation. This combination resulted in cleaner spectra, improved differentiation of isomers, and more accurate identification in both standard mixtures and complex biological samples.

In summary, the integration of ion mobility spectrometry with Q-TOF mass spectrometry optimized for small molecules significantly improves the characterization of polar metabolites, including isomeric and isobaric species, thereby enhancing the analytical precision in metabolomics studies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2101 B — Tackling biotransformations in the extremely rare metabolic condition alkaptonuria using nuclear magnetic resonance

PRESENTING AUTHOR: ***Andrea Bernini, University of Siena, Italy***
CO-AUTHORS: ***Daniela Grasso, Valentina Balloni, Adele Angelini, Andrea Bernini***
SUB THEME: ***Inborn errors of metabolism and neonatal screening***

Dr. Archibald Garrod changed history in the early 19th century by coining the term “inborn error of metabolism” for alkaptonuria (AKU), or “black bone” disease. He suggested that the reddish-brown tissue coloration, ochronosis, resulted from the accumulation of a phenolic compound (alkapton) due to a missing enzyme inherited as “chemical individuality.” His insights predate the gene concept, marking AKU as the first described autosomal recessive disease. Today, mutations in the HGD gene, also termed “chemical individuality,” impair homogentisate 1,2 dioxygenase, leading to homogentisic acid (HGA) build-up and ochronosis. AKU patients face severe consequences like valvular heart disease and tendon ruptures in their third to fourth decade of life. The European Medicines Agency recently approved Orfadin® (nitisinone) for adult AKU patients, with the main side effect being elevated plasma tyrosine levels, similar to tyrosinemia type 2, which may cause eye issues. Hence, alternative treatments to lower HGA without inducing tyrosinemia are urgently required, based on better understanding of biotransformation. We conducted a metabolome-wide comparison of untreated and treated AKU patients via Nuclear Magnetic Resonance. Our findings indicate that non-reactive organic acids, including 4-hydroxyphenylacetate and 4-hydroxyphenyllactate, emerge in AKU as a result of tyrosine decarboxylation to tyramine and pathway blockade by nitisinone, respectively. These mechanisms warrant further exploration from genomics and proteomics perspectives to develop alternative treatments targeting HGA accumulation.

2102 B — Tuberculosis treatment success vs failure: Insights from a multi-platform metabolomics approach

PRESENTING AUTHOR: ***★ Monique Opperman, Centre for Human Metabolomics - NWU, South Africa***
CO-AUTHORS: ***Ilse Du Preez, Du Toit Loots***
SUB THEME: ***Infectious diseases***

Tuberculosis (TB) treatment failure remains a significant challenge, influenced by both drug metabolism and host-specific factors. This study applies a multi-platform metabolomics approach to investigate host metabolic responses to standard TB therapy (DOTS) and their potential role in treatment outcomes. Urine samples were collected from 46 culture-confirmed TB patients (29 cured, 17 failed) at baseline, during (weeks 1, 2, 4), and post-treatment (week 26). Untargeted two-dimensional gas chromatography-mass spectrometry (GC-MS) was initially employed to characterise drug-induced metabolic changes in treatment-cured patients, revealing time-dependent reductions in oxidative stress, altered enzyme activity (CYP2E1, CYP3A4, ADH, ACMSDase) and upregulation of the urea cycle. To explore metabolic differences in treatment failure, targeted metabolomics assessed urinary acylcarnitines (via liquid chromatography-mass spectrometry) and amino acid profiles (via GC-MS) across both outcome groups. While both groups exhibited drug-induced metabolic shifts, these changes occurred earlier in treatment-failed patients, possibly due to differences in drug-metabolising enzyme activity or oxidative stress levels. Further, using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy TB drug metabolites were quantified, revealing elevated isoniazid (INH)-related metabolites in treatment-failed patients. Notably, these differences were independent of acetylator genotype, suggesting alternative metabolic pathways or non-genetic INH metabolism influences in this cohort. Long-term drug-induced metabolic effects were assessed using combined targeted (GC-MS) and semi-targeted (^{1}H-NMR) analyses. Treatment-cured patients exhibited increased fumarate and decreased serine, glycine, cystathionine, cysteine, and formate—metabolic patterns consistent with INH exposure. In contrast, treatment-failed patients displayed opposite trends, indicating greater metabolic disruption post-treatment. These findings highlight altered enzymatic reactions in vitamin B-dependent pathways, as well as potential gut microbiota perturbations in treatment failure cases. These results underscore the complexity of TB drug metabolism and suggest that inter-individual metabolic variability may contribute to treatment failure. A deeper understanding of these metabolic signatures could inform future personalised treatment strategies, optimising TB therapy based on individual metabolic profiles.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2103 B — Gaining Insights into the MTBVAC live-attenuated vaccine against Tuberculosis through interrogation of the exometabolome

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CO-AUTHORS: *Irene Pérez, Jesús Gonzálo-Asensio, Patricia Mena, Olga Genilloud, Francisca Vicente, Carlos Martín, Rosario Fernández-Godino*
SUB THEME: *Infectious diseases*

Tuberculosis remains the leading cause of death in the world caused by an infectious pathogen, presenting a death toll of 1.25 million people based on the WHO report. The challenge for science to put an end to this serious threat to global health raises the need to invest in new therapies that improve the efficacy of existing ones, such as the development of new vaccines. The MTBVAC vaccine was constructed at the University of Zaragoza using an attenuated strain of M. tuberculosis through the deletion of two genes (phoP and fadD26) involved in virulence. This vaccine is undergoing a phase 3 clinical trial (NCT04975178) to evaluate its efficacy, safety and immunogenicity compared to Bacillus Calmette Guerin in healthy, HIV-unexposed and HIV-exposed uninfected newborns in tuberculosis-endemic regions of sub-Saharan Africa. Molecular characterization of the vaccine may help to better understand its role in the host immune response. We have compared the exometabolome of the attenuated strain and its parental strain MT103 to decipher the role of the phoP gene in the expression of metabolites that reinforce its role in the efficacy and safety of the vaccine. For this purpose, a metabolomic analysis of the culture supernatants of the following strains: MT103, MTBVAC, H37Rv, H37RvΔphoP and phoP-complemented H37Rv was carried out using liquid chromatography coupled to high resolution mass spectrometry with four different analytical approaches. Metabolomic analysis showed significant differences between strains related to phoP gene deletion. This study highlights the alteration of molecular pathways related to lipid production and involved in signaling pathways, some of them with a role in immune response. Metabolomics proves to be a powerful tool to characterize attenuation in virulent strains for use as potential vaccines.

2104 B — Exploring untargeted metabolomics to establish mechanistic action of alternative drugs to treat neglected diseases

PRESENTING AUTHOR: *Marina Tavares, Institute of Chemistry, University of Sao Paulo, Brazil*
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SUB THEME: *Infectious diseases*

The Center for Multiplatform Metabolomics Studies (CEMM) at IQ-USP, implemented during the COVID-19 pandemics (August 2020), inaugurated a novel research front, that intends to explore the potential of targeted and untargeted multiplatform metabolomics to reveal the mechanism of action at molecular level of candidate drugs to the treatment of infectious and parasitic diseases. Among the diseases of interest there are included infectious diseases: chikungunya and yellow fever (arboviruses), both neglected in Brazil, and COVID-19, considered an emergent disease; in addition there are included several neglected parasitic diseases: leishmaniasis (visceral form is re-emergent) and Chagas disease, both caused by protozoa, and schistosomiasis, caused by helminths. There are hundreds of compounds being screened at the moment using high-throughput and/or high-content screening techniques; such compounds result from the efforts of CEMM collaborators, locally and abroad, working on the synthesis, extraction, purification, as well as theoretical calculations, and they are originated from computer simulations, natural products, synthetic chemicals libraries, and drug repositioning. Preliminary results for Leishmaniasis will be presented demonstrating the impact of the listed drug classes into the parasite metabolism. The results generated so far may contribute in a concerted manner to elucidate the broad action of several candidate drugs against infectious and parasitic diseases, paving the way into the searching of novel alternative therapies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2105 B — Characterizing metabolic capacity of ex vivo gut microbiota fermentations by merged targeted/untargeted LC-MS/MS

PRESENTING AUTHOR: *Jacob Folz, Eth Zurich, Switzerland*
CO-AUTHORS: *Raúl Fernández Cereijo, Maja Stevanoska, Georg Aichinger, Shana Sturla*
SUB THEME: *Microbiome research*

Microbiota perform many chemical reactions within our gut with direct impact on human health. Sequencing of microbiota communities is insufficient to accurately decipher the chemical reactions occurring within the gut. Thus, we use anaerobic fermentations of fecal microbiota, time-series sampling, and a merged targeted/untargeted LC-MS/MS analysis to quantify chemical transformation rates of known food, drug, and endogenous host metabolites. Metabolic profiles from 20 spiked and monitored chemicals were generated for 5 human donors. Chemical reaction rates varied dramatically by chemical structure and across donors. Conditions of fermentation were tested including multiple growth media, fresh and frozen samples, and inoculum dilutions to optimize a protocol to quantify microbiota metabolic profiles. Dilution of the inoculated microbiota influenced chemical reaction rates, while nutrient rich media increased reaction rates of most monitored chemicals compared to nutrient scarce media. Fresh compared to frozen microbiota samples did not significantly change chemical reaction rates for targeted chemicals. Non-targeted analysis annotated over 350 metabolites and revealed distinct time- and condition-dependent metabolic shifts across ex vivo microbiota fermentations. Principal component analysis highlights nutrient availability and inoculation density as factors that strongly influence temporal metabolome shifts. Nutrient rich media and high-bacteria density inoculum induced rapid and extensive metabolic shifts optimal to identify inter-donor variation. Hierarchical clustering of metabolite correlations identified five clusters of metabolites with distinct temporal trends including degradation of peptides and sugars, microbially generated metabolites such as lactic acid, and biotransformation patterns of flavonoid derivatives. A subset of metabolites exhibited strong donor specificity, highlighting interindividual variation in microbial metabolism. Correlation analysis further linked baseline stool metabolites to spiked chemical degradation rates, identifying potential functional biomarkers of microbiota metabolism. This approach provides a basis to characterize metabolism in complex microbial communities, identify personalized microbiota metabolism function profiles, and uncover chemical-microbiota interactions in the gut.

2106 B — Ex vivo cultivation of human gut microbiota - effects of media and culture conditions on metabolic activity and microbial diversity

PRESENTING AUTHOR: *Svea Gluesing, Kiel University Cau, Germany*
CO-AUTHORS: *Julia Jensen-Kroll, Tobias Demetrowitsch, Sabrina Sprotte, Erik Brinks, Charles Franz, Karin Schwarz*
SUB THEME: *Microbiome research*

The human gut microbiota (GM) plays a key role in health. Ex-vivo fermentation studies with the GM in faecal cultures have been used in nutrition and health research to overcome the limitations of in vivo studies. Polyphenols, phytochemicals with high biological activity, are part of most diets. Because of their low rate of absorption, they can reach the colon and influence the resident microbiota. Research to elucidate the direct effects of polyphenols on the microbial community and metabolism is lacking. Therefore, we established an ex-vivo cultivation method to study the effect of polyphenols on the GM under standardised conditions, allowing a stable bacterial diversity during the experiment. However, cultivation of most bacterial species is still a major challenge requiring protocols to be optimised.

Our aim was thus to evaluate relevant parameters of the cultivation experiments based on changes in the metabolome and diversity of GM.

For this, different cultivation media for ex-vivo cultivation experiments were compared. Moreover, the effects of continuous sample stirring on the microbial community and its metabolites were investigated. Fresh faecal samples of healthy donors were pooled and cultured for 48 hours under anaerobic conditions using three different media. Samples were separately enriched with two polyphenols, quercetin and chlorogenic acid. The GM and metabolites were analysed at different time points by 16S rRNA amplicon metagenomics and a non-targeted metabolomics approach based on FT-ICR-Mass spectrometry.

Different effects on the metabolic profile for the different media could be observed. 4-sulfo-ferulic acid, for instance, was detected exclusively in BHI samples. After 48 h, quercetin and chlorogenic acid were degraded, and metabolites such as propionic acid, benzoic acid and phenylacetic acid were significantly altered across all investigated media. Quercetin-enriched samples displayed altered methylkaempferol-sulfate levels after 24 h, as did 4-sulfo-ferulic acid. Furthermore, a faster degradation was observed in stirred samples.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2107 B — Metabolic Impacts of Genetic Modifications to Spermidine Biosynthesis in Escherichia coli

PRESENTING AUTHOR: ***Cécilia Berges, MetaboHUB MetaToul, France***
CO-AUTHORS: ***David Hoarau, Audrey Le Gouellec, Justine Bertrand Michel, Floriant Bellvert***
SUB THEME: ***Microbiome research***

Intestinal bacteria play a crucial role in metabolism, influencing overall health by producing various metabolites. Signals from these microbial metabolites have important and diverse effects on host physiology. One notable example is spermidine, a polyamine produced from L-arginine metabolism, which is gaining attention for its potential immunomodulatory and anti-inflammatory properties.

In the context of the holobiont, the probiotic strain Escherichia coli Nissle 1917 (E. coli) has been genetically engineered to overproduce and secrete spermidine. Previous studies have shown that enhancing the production of enzymes involved in spermidine biosynthesis and export in this strain significantly increases extracellular spermidine levels. However, in vivo experiments in mice revealed viability issues with the modified strains, indicating that these genetic modifications affect other essential cellular processes.

To investigate these effects, we perform ^{13}C-labeling experiments under microaerobic cultivation conditions. This setup aimed to more accurately mimic the oxygenation conditions of the intestinal environment, providing physiologically relevant insights into E. coli metabolism. Using a high throughput fluxomic workflow[1] composed of automated robotic platforms for cell cultivation and sample preparation, coupled with mass spectrometry and NMR analysis, we performed an exhaustive exploration of intracellular fluxes in E. coli mutants. Advanced bioinformatics tools were employed to facilitate data analysis and biological interpretation.

This study illustrates how high-throughput fluxomic experiments have enhanced our understanding of the metabolic impacts of various genetic modifications in E. coli. By performing ^{13}C labelling experiment, and analyzing extensive isotopic datasets, we can generate highly detailed maps of carbon fluxes in the central carbon metabolism. This approach enables the identification of bottlenecks that may adversely affect strain viability and facilitates the proposal of modifications to optimize spermidine production.

[1] Bergès et al. “Exploring the Glucose Fluxotype of the E. coli y-ome Using High-Resolution Fluxomics.” Metabolites vol. 11,5 271. 26 Apr. 2021, doi:10.3390/metabo11050271

2108 B — Fecal short-chain fatty acids profiles across life stages and mammalian hosts

PRESENTING AUTHOR: ***Veronika Pettersen, UiT The Arctic University of Norway, Norway***
CO-AUTHORS: ***Gaute Hovde Bø, Anna Jelinkova, Tomasso Stefani, Terje Vasskog, Zdenek Kamenik, Veronika Pettersen***
SUB THEME: ***Microbiome research***

Background

Short-chain fatty acids (SCFA) produced by gut bacteria have essential roles in mammalian physiology, including immune system modulation and protection against enteric pathogens. However, only a few studies have reported absolute levels of faecal SCFA, and a comprehensive overview of SCFA levels across different mammalian hosts is lacking. We developed a robust and inter-lab validated method for absolute quantification of SCFA and applied it to stool from gnotobiotic animals, neonates, children, adults, and the elderly.

Method

We employed a derivatisation strategy based on 3-nitrophenyl hydrazine for simultaneous analysis of SCFA and citric acid cycle intermediates. After evaluating the matrix effect through the standard addition method and confirming the method’s robustness by an interlaboratory study, we applied it to fecal samples from germ-free and mono-colonised mice and pigs, as well as to human samples, including neonates (n=41), children (n=130), adults (n=20), and elderly (n=30).

Results

The results show SCFA profiles across different life stages and mammalian hosts, reflecting the colonisation status of the host and age-associated microbial loads. Our findings show, among others, that SCFA profiles gradually increase in humans from birth, with neonates less than 48 old having similar profiles to germ-free mammals. Further, known factors affecting the gut microbiome, such as diet and medication, are associated with SCFA levels. For accurate and reproducible analytical runs, a simple vortexing step has the highest efficiency for reproducible extraction of SCFA, but it is necessary to quench the derivatisation reaction and clean the sample with 10 kDa cut-off spin to reduce the matrix effect.

Conclusion

This work answers the current need for a fully validated and standardized protocol for the detection of SCFA absolute levels in stool. The dynamics of SCFA can serve as predictors of the presence or even abundance of specific bacterial genera in the gut microbiome.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2109 B — Investigating microbial conjugated lipids in an in-vitro gut model

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CO-AUTHORS: *Katja Salonen, Helena Mannochio-Russo, Matilda Kråkström, Paulo Wender P. Gomes, Vincent Charron Lamoureux, Ipsita Mohanty, Sofia D, Forssten, Arthur C, Ouwehand, Tuulia Hyötyläinen, Alex M. Dickens, Pieter C. Dorrestein, Matej Orešič, Santosh Lamichhane*

SUB THEME: *Microbiome research*

Current evidence suggests that gut microbiome-derived lipids are crucial in regulating host lipid metabolism (1). However, their distribution and transformation across different gut regions remains largely unknown. To address this, we applied both targeted and untargeted metabolomics to in vitro-derived intestinal chyme, examining microbial-modified lipids. Simulated intestinal chyme was collected from in vitro gut vessels (V1–V4), representing the proximal to distal colon, at 24 and 48 hours. In total, 44 simulated intestinal samples were analyzed. Factor analysis of targeted lipids revealed that both vessel location and time significantly influenced lipid profiles. Among 52 detected conjugated N-acyl lipids, four showed statistically significant variation across vessels. Specifically, arginine-C18:0, histamine-C5:0, and putrescine-C18:2 increased from V1 to V4, emerging from minimal levels in the media. In contrast, putrescine-C19:2, initially abundant in the media, remained essentially unchanged. Untargeted MS/MS analysis and using a GNPS-based framework further uncovered microbial-derived lipids with spatial variation across vessels (V1–V4). The presence or absence of specific lipids in the media and simulator regions highlighted active microbial metabolism, particularly previously uncharacterized N-acyl lipid pools (2) and other lipids and lipid-like molecules (SIRIUS based). Moreover, associations between in vitro-derived N-acyl lipids and classical lipid species (LIPID MAPS based) revealed distinct patterns: lys-conjugated N-acyl lipids correlated with triacylglycerols (TGs), while ceramides were linked to tyrosine-conjugates. Overall, this study highlights the dynamics of non-polar microbial lipids, a largely overlooked metabolite class in microbiome-focused metabolomics.

2110 B — Chemical Metabolomics – Combining Metabolomics and Chemical Biology Tools for the Discovery of Bioactive Microbiome Metabolites

PRESENTING AUTHOR: *Daniel Globisch, Uppsala University, Sweden*

CO-AUTHORS: *Alejandro Torregrosa Chinillach, Weifeng Lin, Maria Theodorou, Wawrzyniec Haberek, Ioanna Tsiara, Vladyslav Dovhalyuk, Nikolas Kessler, Sofie Weinkouff, Aiko Barsch, Cristian De Gobba, Matthew R. Lewis, Daniel Globisch*

SUB THEME: *Microbiome research*

The human body is populated by large microbial communities, called microbiota, which profoundly impact the physiology of the human host. While metagenomic analyses are rapidly identifying disease-related microbes, the key metabolites still need to be uncovered as this gut microbial ecosystem produces bioactive metabolites orthogonal to the human metabolism. These molecules can either be toxic or beneficial for the human host. To overcome the inevitable global public health burden posed by multidrug-resistant pathogens, we have investigated microbiome-pathogen co-cultures and human fecal samples using a combination of metabolomics and chemical biology-tools.[1,2]

To overcome limitations of metabolite detection in standard global metabolomics analysis, we have developed multifunctional synthetic chemoselective probes with 13C/12C isotopically labelled analogues that allows for (semi-)quantitative analysis of metabolites in human samples at low concentrations. Coupled to magnetic beads, this method allows the straightforward chemoselective extraction of metabolites from microbial and fecal samples. We have termed this unique method quantitative Quantitative Sensitive CHEmoselective MetAbolomics (quant-SCHEMA). This method has recently been developed for important compound classes of microbial origin such as carbonyls, amines and thiols.[1,2] Our mass spectrometric investigation detected a broad range of human, dietary and bacterial metabolites in fecal and bacterial samples. To directly investigate microbial interactions, we have also developed a platform for the discovery of bioactive metabolites in co-cultures of gut microbes and pathogens.[3]

Recently, we have advanced our structure elucidation approaches of the derivatized compounds through DDA-TIMS-MS/MS (Bruker) to significantly improve annotation confidence by comparison with reference CCS values and CCS-prediction in MetaboScape.[4] This additional layer of information led to the annotation of a higher number of metabolites than standard analysis to discover bioactive metabolites.

1 Lin … Globisch Angew. Chem. Int. Ed. 2024

2 Kaur …, Globisch Chem. Sci. 2023

3 Cheng … Globisch, Du Gut Microbes 2024

4 unpublished

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2111 B — Serum 4D Lipidomic signatures Enable Stratification of Clinically Isolated Syndrome and Multiple Sclerosis Patients

PRESENTING AUTHOR: *Helen Gika, Aristotle University of Thessaoniki, Greece*

CO-AUTHORS: *Olga Begou, Petros Pousinis, Thomai Mouskeftara, Christina Virgiliou, Aristidis Koitsanos, Georgios Theodoridis, Helen Gika*

SUB THEME: *Neurological disorders*

Multiple Sclerosis (MS) is a chronic, immune-mediated disorder of the central nervous system characterized by demyelination and neurodegeneration with Relapsing-Remitting MS (RRMS) being its most common form. Clinically Isolated Syndrome (CIS) often precedes MS and may represent an early manifestation of the disease. Identifying predictive biomarkers that distinguish between CIS and early MS is critical for early diagnosis and intervention. The present study aimed to uncover serum-based lipid biomarkers that can differentiate between individuals experiencing a first demyelinating episode within the clinical context of either CIS or RRMS, thereby providing molecular insight into the transition from CIS to MS. Serum samples were collected from 143 patients from multiple sites, comprising 99 diagnosed with MS, 29 with CIS and 15 controls. Untargeted lipidomic analysis was conducted using an UHPLC – TIM TOF/MS platform. Multivariate and univariate statistical analyses were employed to identify differentially expressed lipid species after adjusting for clinical information and multiple comparison testing. Methods included ordinal regression, likelihood ratio testing and partial-correlation networks. The analysis revealed five lipids that showed significant alterations between the CIS and MS groups. Our findings suggest that serum lipid profiling can uncover distinct biochemical signatures that differentiate CIS from RRMS and may serve as early indicators of disease progression. These results highlight the potential of lipidomics in identifying individuals with CIS who are at greater risk of developing MS and support the need for larger validation studies.

Acknowledgment:

This work was performed under BiACEM project (number: 101079370), HORIZON WIDERA Twinning grant". The clinical study was performed under the "National Network for Research of Neurodegenerative Diseases on the basis of Medical Precision" funded by the GGEK.

2112 B — UHPLC-MS technologies for untargeted Lipidomics and PFAS quantification in the Serum of Patients with Alzheimer's Disease, Mild Cognitive Impairment and Controls: A Preliminary Study

PRESENTING AUTHOR: *Georgios Theodoridis, Aristotle University Thessaloniki, Greece*

CO-AUTHORS: *Magda Tsolaki, Ian Wilson, Rob Plumb, Artemis Lioupi, Stuart Adams, Olga Deda, Timothy Ebbels, Lukas Kopecky, Olga Begou, Aris Koitsanos, Helen Gika*

SUB THEME: *Neurological disorders*

Blood from 137 subjects comprising healthy controls (HC), subjects with mild cognitive impairment (MCI) and those with Alzheimer's disease (AD) was screened for AD biomarkers by untargeted lipidomics (UPLC-TOF-MS) and new complimentary UHPLC-MS methods for 6 ultra short chain (polar) and 30 long chain (and relatively non-polar) PFAS compounds. The individuals were monitored and ranked clinically by expert Alzheimer neurology clinical team and extensive biochemical, clinical and neurological assessment data was obtained. For PFAS determinations two new methods were developed to be rapid with both having analysis times of ca. 5 min to enable rapid screens of samples. Both methods were sensitive enough to detect the target PFAS over the range 0.125 -12.5 ng/mL. These methods demonstrated the presence of 33 of the targeted PFAS in the tested serum samples, present over a wide concentrations range with PFPrA found at the highest maximum concentration of 707 ng/mL. Interestingly the PFAS profiles found for the HC and MCI subjects differed from that of the AD patients. The reason(s) for PFAS profiles for serum from AD patients compared to HC/MCI subjects is unclear and may be unrelated to disease but clearly warrants further investigation in order to clarify the reasons for this difference. On the other hand, lipidomics analysis has been shown in the past to provide interesting findings on AD disease biochemistry, In the present study, a number of potential biomarkers were found to differentiate the three cohorts HC, MCI and AD. The presentation discusses the revealed biochemistry and aims to link the proposed biomarkers with disease status.

The authors acknowledge funding from the Horizon Europe Project 101079370 — BiACEM WIDERA-Twinning "Biomic_AUTh, Center of Excellence in Metabolomics research".

2113 B — Lipid Profiling of Parkinson's Disease Brain Highlights Disruption in Lysophosphatidylcholines, and Triacylglycerol Metabolism

PRESENTING AUTHOR: *Nadia Ashrafi, Corewell Health, United States*

CO-AUTHORS: *Ali Yilmaz, Romana Ashrafi Mimi, Sumeyya Akyol, Nazia Nazia Saiyed, Ieva Kerševičiūtė, Migle Gabrielaite, Juozas Gordevicius, Stewart Graham*

SUB THEME: *Neurological disorders*

Parkinson's disease (PD) is the second most common neurodegenerative disorder following Alzheimer's disease, with a 1.5 times higher prevalence in males. Several lipid-related genetic risk factors for PD have been identified, and the brain lipid signature of PD patients is distinguishable from controls. To elucidate the molecular mechanisms underlying PD and its sex differences, we conducted a lipidomic analysis of postmortem brain samples from the primary motor cortex (Brodmann area 4) of 40 PD patients and 43 age- and sex-matched matched controls. Mass spectrometry based lipidomics analysis revealed notable differences in 95 lipid species, especially Triacylglycerols and Lysophosphatidylcholines. Notably, sex-stratified analysis suggested that mitochondrial dysfunction may explain the higher prevalence of PD in males. These findings highlight lipid dysregulation in PD and point to potential biomarkers for diagnosis, warranting further validation.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2114 B — Mass spectrometry imaging of free-floating sections for the study of Alzheimer's disease

PRESENTING AUTHOR: ***Stepan Strnad, IOCB Prague, Czechia***

CO-AUTHORS: ***Vladimir Vrkoslav, Veronika Strnadova, Martina Holubova, David Sykora, Josef Cvacka, Lenka Maletinska***

SUB THEME: ***Neurological disorders***

Sample collection and storage are important steps in the mass spectrometry imaging (MSI) sample preparation procedure. The recommended and most widely used procedure for MSI preparation involves freezing the sample in isopentane and storing it at -80 °C. On the other hand, the most common and general method for preserving biological samples in clinical practice is fixation in paraformaldehyde. In the present work, we investigated paraformaldehyde-fixed free-floating sections and studied their applicability for MSI analysis of lipids in the mouse brain. The developed procedure was used in a project aimed at determining lipid changes in double transgenic APP/PS1 mice, a model of Alzheimer's-like pathology. Moreover, we examined the neuroprotective properties of palm11-PrRP31, an anorexigenic and glucose-lowering analog of prolactin-releasing peptide, using the MSI method.

From the free-floating sections, we obtained lipid images without interferences or delocalization. In the APP/PS1 mouse model, we observed altered distribution of various lipids. An increased concentration of gangliosides (GM2 36:1, GM3 36:1) and phosphatidylinositols (PI 38:4, 36:4) and a decreased concentration of sulfatides (ST 18:0, 22:0, 24:0 and ST-OH 22:0, 24:0) were observed in areas where plaque accumulation occurred, compared to the surrounding tissue. In APP/PS1 mice treated with palm11-PrRP31 for 2 months, we found a reduced area of lipids associated with senile plaques. The results indicate that palm11-PrRP31 may potentially be useful in the treatment of neurodegenerative diseases. However, the mechanisms behind its neuroprotective function require further investigation.

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2115 B — Lac-Phe and Beyond: From Gut Lactate to Circulating Lac-Phe and GLP-1 Activation

PRESENTING AUTHOR: ***Emaan Ghias, Aarhus University, Denmark***

CO-AUTHORS: ***Mette Glavind Bülow Pedersen, Camilla Bak Nielsen, Louise Vase Bech, Ida Marie Modvig, Natasa Brkovic Zubanovic, Jacob Marthinsen Seefeldt, Roni R. Nielsen, Esben Søndergaard, Kim Frisch, Jakob Hansen, Jens Juul Holst, Niels Møller, Nikolaj Rittig, Mogens Johannsen***

SUB THEME: ***Nutrimetabolomics & dietary biomarkers***

Lactate, a small organic acid related to short-chain fatty acids (SFCAs), is emerging as a key energy metabolite, although much remains unknown about its actions in the gut. In this study, we investigated how oral (PO) versus intravenous (IV) lactate administration alters the human plasma metabolome, with a particular focus on lactoylated amino acids (Lac-AAs) and metabolites linked to GLP-1 secretion and metabolic health. Using targeted and untargeted metabolomics, we show that PO lactate—but not IV lactate—robustly increases circulating L-lactoyl-phenylalanine (L-Lac-Phe) and L-lactoyl-valine (L-Lac-Val), recently identified as potential signaling molecules implicated in appetite regulation. L-Lac-Phe levels after PO lactate reached concentrations comparable to those observed post-exercise and closely followed plasma lactate kinetics, suggesting a rapid, gut-specific formation pathway. Using a perfused rat intestine model, we confirmed intestinal formation of Lac-AAs, albeit at a lower magnitude than observed in vivo, suggesting possible complementary pathways or microbial contributions. Further analysis revealed that oral lactate selectively modulates metabolites associated with GLP-1 secretion, including deoxycholic acid and gut-derived SCFAs. Notably, GLP-1 was increased only after PO lactate, which may be due to microbial fermentation of lactate to butyrate/propionate or lactate-induced bile acid signaling. Collectively, these findings provide mechanistic insight into lactate's role as a gut-derived metabolic signal and may help explain the health benefits of lactate-rich fermented foods. They also suggest new strategies for modulating metabolism and satiety via targeted activation of gut lactate signaling pathways.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2116 B — Multi-Platform Metabolomic and Lipidomic Profiling in a Multi-Country European Cohort

PRESENTING AUTHOR: ***Alexandra Tiganouria, Biomic Auth, Bioanalysis and Omics Lab, Greece***
CO-AUTHORS: ***Danai Rossiou, Olga Begou, Helene Gkika, Christina Virgiliou, Georgios Theodoridis, Olga Deda***
SUB THEME: ***Nutrimetabolomics & dietary biomarkers***

Within the research project CoDiet, an observational study was conducted to investigate the complex interplay between human diet, health, and different types of omic data. The study involves comprehensive collection of biological and lifestyle data, including chemical information derived from urine and blood samples, as well as detailed nutritional/dietary information (questionnaires and food intake monitoring), activity tracking and advanced physiological assessments. Our team has undertaken extensive metabolic profiling tasks, characterizing the biological samples collected, using six distinct UPLC-MS/MS-based methods.

Urine samples were analyzed through a targeted hydrophilic interaction liquid chromatography (HILIC) LC-MS/MS method, quantifying 57 metabolites, including vitamins, organic acids, and amino acids. In addition, a new LC-MS/MS method was used to quantify 33 aromatic amino acid-related compounds, and an untargeted metabolomics approach was also applied to urine samples.

Blood serum samples were examined using two HILIC-UPLC-MS/MS methods for the quantification of 12 saccharides and aminoacids, and 13 acyl-carnitines. Untargeted lipidomics analysis was performed on UHPLC-TiMS-TOF-MS, capturing a broad spectrum of lipid species.

The analytical results from these six methodologies are summarized in our presentation. The influence of differing dietary patterns on the levels of different detected metabolites is investigated.

Acknowledgment:

This work was financial supported by the project “Combatting Diet Related Non-Communicable Disease Through Enhanced Surveillance (CoDiet)), which is co-funded by the European Union under Horizon Europe grant number 101084642 and also supported by the project “Biomic_AUTh, Center of Excellence in Metabolomics research (BiACEM)” (Project number: 101079370), which is implemented under the Action “Horizon Europe Twinning grant”.

2117 B — Longitudinal Urinary NMR-Based Metabolomics Reveals Early Metabolic Signatures of Childhood Obesity and Allergies

PRESENTING AUTHOR: ***Chih-Yung Chiu, Clinical Metabolomics Core Laboratory, Taiwan***
CO-AUTHORS: ***Meng-Han Chiang, Chieh-Ni Kuo, Kuan-Wen Su, Jing-Long Huang, Kuo-Wei Yeh***
SUB THEME: ***Nutrimetabolomics & dietary biomarkers***

Background:

During early childhood, significant changes occur, including shifts in dietary patterns, accelerated growth, and the emergence of overweight and allergic diseases. However, a longitudinal metabolomic analysis from birth to childhood for the relationships between these factors remains lacking.

Methods:

A total of 144 children, who completed a 5-year follow-up from a birth cohort, were enrolled. Time series analysis of urinary metabolites collected at ages 1 and 6 months, and at 1, 3, and 5 years was conducted using 1H nuclear magnetic resonance (NMR) spectroscopy with partial least squares discriminant analysis (PLS-DA). Identified metabolites were studied in relation to changes over time and associations with breastfeeding patterns, allergen sensitizations, overweight/obesity, and atopic diseases during childhood.

Results:

C-means clustering identified three distinct modules of metabolite abundance patterns, highlighting an increase in amino acid and a decrease in carbohydrate metabolism across ages. The Bray-Curtis dissimilarity indices of metabolic profiles increased with age, showing lower indices in formula-fed infants and children with overweight/obesity, but a higher index in atopic diseases during childhood. Metabolites differentially expressed between breastfeeding patterns were primarily identified within the first year of life, particularly in relation to milk sensitization at age 1. Venn diagrams illustrated metabolite distributions, showing an 80% overlap between breastfeeding patterns and infant overweight/obesity, and a 53% overlap between overweight/obesity and atopic diseases in childhood.

Conclusion:

Longitudinal metabolomics analysis revealed associations between gut microbiota-related metabolites and feeding patterns, linking formula feeding to infant overweight/obesity, as well as connections between overweight/obese children and atopic diseases during childhood.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2118 B — Plasma Metabolomic Effects of 8-week Red Wine Consumption in CHD Patients

PRESENTING AUTHOR: ***Pelagia Lekka, National And Kapodistrian University Of Athens, Greece***
CO-AUTHORS: ***Evangelos Gikas, Mitja Briscik, Sébastien Déjean, Anthi Panara, Nikolaos Thomaidis, Elizabeth Fragopoulou, Marilena Dasenaki***
SUB THEME: ***Nutrimetabolomics & dietary biomarkers***

Introduction & Aim: Moderate alcohol consumption has been associated with cardiovascular benefits but its long-term metabolic impact in coronary heart disease (CHD) patients remains largely unexplored. This study aimed to characterize metabolic shifts induced by chronic red wine intake and to disentangle the effects of ethanol from those of red wine using untargeted metabolomics. This is the first study so far investigating metabolic alterations over an extended period of 8 weeks of moderate red wine consumption.

Methods: A randomized, controlled, parallel-arm clinical trial was conducted with 64 male CHD patients (61–63 years old) receiving stable medication. Participants were randomized into three groups (~20 per group): (i) red wine (27 g alcohol/day), (ii) tsipouro, a Greek grape pomace distillate (27 g alcohol/day, serving as the ethanol-only control) and (iii) water (control). Daily consumption was maintained for 8 weeks. Blood samples were collected at baseline (after a 15-day washout period), 4 weeks, and 8 weeks. Plasma metabolomic profiling was performed using LC-QTOF-MS in both positive and negative ionization modes. A non-targeted screening approach was applied to data acquired in independent acquisition mode (bbCID, DIA) for metabolic fingerprinting using MS-DIAL (v4.9). Statistical analysis was performed through MetaboAnalyst.

Results: Preliminary multivariate analyses revealed time-dependent metabolic shifts across all groups. Notably, greater changes were observed at 4 weeks, with some metabolite levels stabilizing or attenuating by 8 weeks. Distinct metabolite fingerprints between the wine and ethanol groups suggest beverage-specific biochemical pathways under chronic exposure. Temporal metabolic fluctuations were also detected in the control group, underscoring the necessity of accounting for baseline variability.

Conclusion: This study provides the first 8-week metabolomic analysis of moderate red wine consumption in CHD patients, delineating the metabolic effects of ethanol versus polyphenol-rich beverages. The findings advance clinical metabolomics and may inform precision nutritional strategies for cardiovascular health.

2119 B — Comprehensive Plasma Metabolomic Analysis in Cocoa-Fed Mice Reveals Associations with Neurogenic Biomarkers

PRESENTING AUTHOR: ***Sonia Melgar Locatelli, Universidad de Granada, Spain***
CO-AUTHORS: ***Marta Palma Morales, Oscar Rangel Huerta, Estela Castilla Ortega, Celia Rodríguez Pérez***
SUB THEME: ***Nutrimetabolomics & dietary biomarkers***

Cocoa consumption is widely recognized for its health benefits, largely due to the antioxidant properties of its polyphenols, particularly flavanols. Emerging evidence suggests cocoa may positively influence brain health by promoting adult hippocampal neurogenesis (AHN)—the process of generating new neurons in the adult brain. Metabolomics enables comprehensive profiling of small molecules in biological fluids, offering valuable insight into how dietary components like cocoa affect systemic metabolism and neurobiological outcomes. This study aimed to assess the effects of cocoa on the plasma metabolomic profile of mice and explore correlations with AHN biomarkers. Seventeen young adult C57BL/6JRj mice were randomly assigned to three groups: control (CTR), high-polyphenol cocoa (HPC), or low-polyphenol cocoa (LPC) content diet for 50 days. A battery of behavioral tests assessed exploratory behavior, emotional responses, and cognitive function. After euthanasia, hippocampal tissue was collected for neurogenesis marker analysis via western blot and immunohistochemistry. Plasma samples were analyzed using High-Performance Liquid Chromatography coupled to Electrospray Ionization Quadrupole Time-of-Flight Tandem Mass Spectrometry (HPLC-ESI-QTOF-MS/MS) in both positive and negative ionization modes. Quality controls and analytical blanks ensured data reliability. Data processing was performed using MS-DIAL (v4.9). Multivariate analysis and correlation studies with neurogenic markers were performed using R. Two separate OPLS-DA models compared HPC or LPC vs. CTR. The HPC model showed $R^2X = 0.806$, $R^2Y = 0.996$, $Q^2 = 0.826$; the LPC model showed $R^2X = 0.774$, $R^2Y = 1$, $Q^2 = 0.848$, both with CV-ANOVA p-values < 0.001. Metabolites with VIP > 1.5 were selected, identifying 52 discriminant features: 24 (HPC), 16 (LPC), and 12 shared. Of these, phenylalanine and itaconic acid significantly correlated with neurogenic markers. This study highlights a potential link between cocoa-induced metabolic changes and AHN, supporting the neuroprotective role of cocoa compounds. Project PID2020-114374RB-I00 funded by MCIN/AEI/10.13039/501100011033 and Junta de Andalucía (Project P21_00777).

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2120 B — Fermented and Unfermented Whole-Grain Rye Modulate Gut Microbiota and Methylated-Quaternary Ammonium Compounds Systemically in High-Fat Diet-Fed Mice

PRESENTING AUTHOR: *Ambrin Farizah Babu, University Of Eastern Finland, Finland*

CO-AUTHORS: *Marjo Tuomainen, Iman Zarei, Ville Koistinen, Topi Meuronen, Anna Kårlund, Marko Lehtonen, Kati Hanhineva*

SUB THEME: *Nutrimetabolomics & dietary biomarkers*

Whole-grain rye (WGR), a key component of the healthy Nordic diet, is linked to reduced metabolic disease risk. Gut-microbial metabolites and methylated-quaternary ammonium compounds (mQACs), such as betaines and acylcarnitines, are associated with WGR consumption and may drive these health benefits. These metabolites influence tissue-specific metabolic pathways (i.e., each tissue responds distinctively), with fermentation potentially enhancing their effects. Therefore, a comprehensive understanding of WGR's role in whole-body metabolic response and how it modulates metabolic homeostasis is needed.

We conducted a dietary intervention in high-fat-diet (HF)-fed mice, comparing fermented (FRB) and unfermented rye bread (URB) using LC-MS-based metabolomics across key metabolically active tissues (heart, muscle, ileum, cecum, colon), along with plasma and stool.

Both WGR diets significantly ($p<0.05$) increased gut-microbial metabolites and mQACs-TMAO, 5-AVAB, trigonelline, and homostachydrine, confirming them as rye-associated biomarkers. FRB elevated TMAO—a cardiometabolic regulator—across plasma, muscle, and gut. FBR also reduced its precursor, trimethyllysine, linked to increased cardiovascular risk, suggesting microbial modulation may counterbalance TMAO production. FRB further increased 3-phenyllactate, indoles, and kynurenate (plasma, stool), hippurate (muscle, cecum), trigonelline (muscle), and long-chain acylcarnitines (gut), all associated with enhanced energy metabolism and immune function. Despite these beneficial shifts, FRB increased plasma insulin, glucose, and cholesterol and correlated positively with plasma mQACs and gut-microbial metabolites (Ex: hippurate, indolelactate), suggesting an early-stage adaptive response to dietary shifts or potential metabolic stress. Conversely, URB increased antioxidant ergothioneine and reduced pro-inflammatory p-cresol sulfate, potentially reducing oxidative stress and chronic inflammation commonly associated with metabolic diseases.

These findings highlight the role of WGR in shaping diet-microbe-host interactions that support metabolic health and gut function, with fermentation enhancing beneficial metabolic shifts, while URB reduces the levels of potentially harmful metabolites. The interplay between tissue-specific metabolic responses and gut-microbiota interactions highlights the need for integrative, whole-body approaches to improve dietary recommendations for managing metabolic diseases.

2121 B — LC–MS/MS-Based Profiling of Urinary Aromatic Amino Acids and Their Gut Microbiota-Derived Host Co-Metabolites

PRESENTING AUTHOR: *Domniki Gallou, Greece*

CO-AUTHORS: *Olga Begou, Helen Gika, Vasiliki Sarli, Ian Wilson, Georgios Theodoridis*

SUB THEME: *Other*

Aromatic Amino Acids (AAA) are vital in various biochemical pathways and are mainly metabolized by the gut microbiome through sulfation, which serves as a key conjugation route. This metabolism can be disrupted due to inflammation, metabolic diseases, dietary and pharmaceutical interventions. Comprehensive profiling of these metabolic pathways is essential in understanding the host- microbiota interactions in dysbiosis-related conditions. One of the major challenges, regarding the quantitative analysis of gut microbial derived metabolites of Aromatic Amino Acids include the availability of authentic reference standards. In the present study, standard chemical synthesis was employed to overcome the lack of reference standards. The synthesized standards were purified by preparative HPLC-UV and their structures were confirmed by 1H-NMR and LC-MS/MS analysis. Subsequently, a RPLC-MS/MS method for the quantification of 33 aromatic amino acids metabolites in human urine was developed and validated. A simple dilution step was employed as a sample preparation protocol. The method was applied in real samples, establishing relevant concentration ranges for these metabolites. The present method can find wide applicability in metabolomics, particularly within clinical and nutritional research studies.

Acknowledgement: This work was financially supported by the project "Biomic_AUTh, Center of Excellence in Metabolomics research (BiACEM)" (Project number: 101079370), which is implemented under the Action "Horizon Europe Twinning grant" and the CoDiet project ,which is funded by the European Union under Horizon Europe grant number 101084642.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2122 B — Towards a quick, robust and traceable quality control solution for trapped ion mobility spectrometry (TIMS) TOF LC-MS instrumentation

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SUB THEME: *Other*

Implementing quality control (QC) measures to ensure accuracy and reproducibility of acquired MS data is essential in metabolomics and lipidomics research. These measures should offer comprehensive insights into all aspects of data quality for the instruments used, both in their current state and over time, while being affordable in terms of time and personnel resources. Additionally, reducing operator-induced errors (e.g., dilution, batch setup, data transfer) is crucial. Therefore, semi-automated and software-driven procedures are necessary.

The poster presents the development and validation of a comprehensive quality control (QC) solution for trapped ion mobility spectrometry (TIMS) TOF LC-MS instrumentation, aimed at ensuring accuracy and reproducibility in metabolomics and lipidomics research. The solution, tested over six months across multiple labs, integrates semi-automated and software-driven procedures to minimize operator-induced errors and streamline QC processes.

QC data were acquired with defined methods for chromatography, data acquisition, and processing, employing a mixture of synthetic compounds as the test matrix. The data, collected from various UHPLC-timsTOF devices, were analyzed using a cloud-based software tool to monitor long-term performance trends. The standardized workflows and automated data evaluation ensured consistent results, independent of the operator, and facilitated the early detection of e.g. chromatography-related issues or sprayer needle contamination. The implementation of this QC solution significantly improved system robustness and data quality, providing a reliable framework for future studies.

2123 B — Effects of Apple Phenolic compounds on the Human Metabolome: Modulation of Energy and Amino Acid Metabolism

PRESENTING AUTHOR: *Niklas Roeder, Cau, Kiel, Germany*

CO-AUTHORS: *Antje Geiken, Steffan Dussling, Louise Holtmann, Tim Hollstein, Tobias Demetrowitsch, Dominik Schulte, Ralf Schweiggert, Christof Dörfer, Karin Schwarz*

SUB THEME: *Other*

Apples are known for their potential health benefits, in part due to their phenolic compound content. However, their influence on the human metabolism has not been fully investigated. In this study, the effect of phenolic compounds from apple consumption on the metabolome was investigated by analyzing blood, urine and saliva as biomatrices. Both, short-term and long-term effects were analyzed. In addition to the identification of degradation products, the focus was on the analysis of the most affected metabolic pathways.

Materials and methods: The effects of apple phenolic compounds on the metabolome were investigated in a human intervention study. 30 healthy male participants consumed either an apple juice rich in phenolic compounds or a placebo for two weeks. The study was conducted in a double-blind, randomized, cross-over design.

Subjects were sampled for blood, urine and saliva at the beginning and end of the intervention. In addition, short-term effects were recorded on the last day of the intervention by collecting blood and urine samples 1 and 2 hours after consumption of the study drink. The samples were analyzed by DI-FTICR-MS using an untargeted metabolomics approach.

Results: Several degradation products were significantly detected in blood and urine for both short- and long-term effects. The most affected pathways included amino acid pathways such as phenylalanine, valine, leucine, isoleucine and tyrosine. The corresponding metabolites were significantly regulated in blood, urine and saliva for long-term effects and in blood and urine for short-term effects.

Conclusion: Consumption of phenolic compounds from apple leads to significant metabolic changes, affecting key amino acid pathways. These findings advance the knowledge into the metabolic effects of apple polyphenols and their potential health benefits.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2124 B — Changes in the septic liver polar metabolome analyzed with HILIC-MS, GC-MS & CE-MS

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SUB THEME: *Other*

Cholestasis is a liver disease which can be triggered by inflammatory damage resulting from systemic inflammation. There is still an urgent need to fill the gaps about the molecular principles underlying the progressive deterioration of the liver.

Metabolomics can elucidate disease dynamics and identify key factors associated with liver inflammation. However, comprehensive metabolite coverage requires capturing the hepatic polar metabolome's key characteristics. Techniques like CE-MS, HILIC-MS, and GC-MS provide overlapping and complementary information, with some metabolites detected by multiple techniques and others by only one.

Our study aimed to characterize statistically relevant compounds in the metabolomic profiles of livers from mice subjected to two sepsis models: intraperitoneal inoculation of lipopolysaccharide (LPS) from E. coli and fecal suspension (FSI), compared to a control group inoculated with phosphate buffered saline (PBS). We used a sample treatment pipeline compatible with all techniques1-3, adapting established analytical conditions to provide five matrices (including ESI +/-) with 234 annotated compounds based on over 300 real standards.

The three techniques offered extensive metabolic information on amino acids, polyamines, nucleic acids-related compounds, organic acids, and carbohydrate-related compounds. They showed high complementarity, with over 50% of compounds detected exclusively by one technique, and high orthogonality, with nearly 50% detected by more than one. This multi-platform approach enhances the study's comprehensiveness and consistency, ensuring more reliable analysis.

We identified significant differences between both treatments and the control across various metabolic pathways, particularly those related to oxidative stress, amino acid metabolism, lipid metabolism, and cofactors.

References

1.https://doi.org/10.1016/j.chroma.2022.463626

2.https://doi.org/10.1016/j.aca.2021.339043

3.https://doi.org/10.1007/978-1-0716-4116-3_23

2125 B — LIPIDOME CHARACTERISATION OF EXTRACELLULAR VESICLES FOR STUDYING INTERTISSULAR COMMUNICATION IN METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE

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SUB THEME: *Other*

Extracellular vesicles (EVs) play a crucial role in intercellular communication by transporting biomolecules involved in health and disease. Studying their lipid composition is essential for understanding their signaling roles. Lipidomics by UHPLC-MS is considered the reference technique for the analysis of lipid species in biological systems. This study focuses on the comprehensive lipid characterization of EVs in metabolic dysfunction-associated steatotic liver disease (MASLD), examining intertissular lipid transportation.

We analyzed plasmatic EVs isolated from 22 male mice fed with chow diet (control) or choline-deficient, amino acid defined high-fat diet (CDAA-HFD)-induced MASLD for 3 and 9 weeks (3-week-CDAA-HFD, and 9-week-CDAA-HFD). Lipid extraction based on previous experience (DOI:10.3390/ijms241612714, DOI:10.3389/FONC.2021.788100) was performed with a two-step MeOH:MTBE extraction, followed by UHPLC-MS-QTOF analysis with ESI +/- (DOI:10.1016/j.jcoa.2021.100018, DOI:10.1016/j.jlr.2024.100671), and data treatment using our in-house lipid database (DOI:10.1016/j.jlr.2024.100671) and MS/MS annotation tools (MassHunter LipidAnnotator, MSDIAL). Sample signals were thereafter integrated, and statistical analysis was performed to evaluate the differences between groups.

A total of 346 lipid species were initially annotated with our in-house database, which was expanded to about 600 using MS/MS annotation tools, belonging to 12 different lipid classes. Triglycerides were the most abundant, followed by phospholipids, lysophospholipids, sphingomyelins, ceramides, and free fatty acids. Ether lipids were less prevalent, and phosphatidylserines were notably absent. Comparative analysis revealed that triglycerides were enriched in the 3-week-CDAA-HFD group, while sphingomyelins and ceramides were more prominent in the 9-week-CDAA-HFD group, indicating that triglyceride transportation correlates with increased fat, and longer CDAA-HFD exposure leads to the transport of more toxic lipids.

Our findings demonstrate that lipid transportation reflects metabolic changes in MASLD, emphasizing the importance of EVs lipid composition in understanding disease mechanisms and identifying altered metabolic pathways. This characterization provides valuable insights into novel biomarkers for liver diseases, offering one of the first comprehensive lipid analyses focused on this condition.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2126 B — Implementation of a Semi-automated Sample Preparation Workflow for Comprehensive Serum Metabolomics

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SUB THEME: *Other*

Metabolomics plays a pivotal role in clinical research, uncovering variations in the human metabolome and its correlation with health-related outcomes. However, the analysis of large-scale studies poses a significant challenge for many untargeted analytical methods. Consequently, the automation of sample processing and analysis has emerged as a crucial advancement to meet the demanding experimental throughput.

In this study, we established and evaluated a high-throughput metabolomics workflow for processing human serum samples. Samples underwent extraction for metabolomics analyses using a Janus G3 (Revvity) liquid handling robot, employing two different extraction methods suitable for two separate and complementary chromatographic methods (C18 and HILIC) on 96-well plates. Samples were analyzed using ultra-performance liquid chromatography coupled with HRMS (Vanquish Duo system coupled to a Thermo Scientific Orbitrap Exploris 240 mass spectrometer) in positive and negative ionization modes on the Integrative-Metabolome AnalytiCs for Translational and Precision Medicine (IMPACT-PM) platform in Lille. This workflow was developed with an analytical batch based upon a 96-well plate containing serum samples, SQCs, RQCs, dilution quality control samples and blanks. As proof-of-concept, the workflow was tested with a cohort of serum human samples.

We assessed the coefficients of variation (CVs) for both the total number of detected and annotated features across all samples and quality control samples (QCs), and analyzed intensity plots across the injection sequence. To monitor extraction efficiency and data quality, we employed extraction internal standards (eISs) and technical internal standards (tISs), all of which showed CVs < 20% in the QCs. This workflow provides expanded coverage of serum metabolites, rendering the platform suitable for conducting serum untargeted metabolomic analysis, and it will prove beneficial for applications in larger cohort studies.

2127 B — Streamlined Analysis of Polar and Non-Polar Metabolites in Plasma Using Automated Micro-SPE LC-MS

PRESENTING AUTHOR: *Hagen M. Gegner, CTC Analytics Ag, Switzerland*

CO-AUTHORS: *Laura Gisela González Iglesias, Renzo Picenoni, Guenter Boehm, Gérard Hopfgartner*

SUB THEME: *Other*

The comprehensive analysis of the metabolome and lipidome offers insights into cellular processes and biochemical pathway alterations critical in health and disease. However, untargeted analysis of these diverse compounds in biological matrices like plasma necessitates robust sample preparation. Traditional liquid-liquid extraction (LLE) methods, while common, often exhibit limitations in reproducibility, are prone to manual handling errors, and can be time-consuming. This work presents an integrated, fully automated workflow employing micro solid phase extraction (µSPE) on a robotic sample preparation system, coupled to a UHPLC-MS (Ultra-High Performance Liquid Chromatography-Mass Spectrometry) platform, for the rapid separation and analysis of polar and non-polar metabolites from human plasma. Human plasma samples, including NIST SRM 1950 were spiked with an internal standard. Automated µSPE was performed using the PAL System robotic autosampler, separating metabolites prior to analysis on a dual LC-MS system featuring Acquity UPLC BEH HILIC and Kinetex EVO C18 columns. Detection was achieved using a SCIEX ZenoTOF® 7600 mass spectrometer with ESI (Electrospray Ionization) in both positive and negative modes, employing SWATH-DIA (Sequential Window Acquisition of all Theoretical Mass Spectra - Data Independent Acquisition) and EAD MRMHR (Electron Activated Dissociation - Multiple Reaction Monitoring High Resolution). Optimization of µSPE conditions, including sorbent chemistry (Reversed-Phase C18 and Mixed-Mode Cation Exchange (MCX)) and elution parameters, demonstrated effective separation of polar and non-polar metabolites using CEC18 cartridges post-protein precipitation, with MCX cartridges fractionating total lipids. This fully automated µSPE method simplified sample preparation, reduced sample and solvent consumption, and provided high lipidome coverage comparable to traditional LLE methods, with the added benefit of optimizing dynamic range by varying injection volumes. The platform was successfully validated with NIST SRM 1950 human plasma and is planned for application to CKD patient samples, highlighting its potential in clinical metabolomic studies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2128 B — Metabolomic profiling and network analysis of ACPA-positive and ACPA-negative RA

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CO-AUTHORS: *Seungyeon Lee, Yeeun Yun, Jiyeong Lee, Hee-Gyoo Kang*
SUB THEME: *Other*

Rheumatoid arthritis (RA) is a chronic autoimmune disorder, with anti-citrullinated protein antibodies (ACPA) playing a key role in its pathogenesis. This study explores the metabolic distinctions between ACPA-positive and ACPA-negative RA patients, with a particular focus on pathway-level differences assessed through network-based analysis. To further investigate the impact of ACPA intensity, CCP titer levels were analyzed in relation to metabolite profiles. Blood samples were obtained from ACPA-positive RA patients (n=72), ACPA-negative patients (n=36), and healthy controls (n=72). Metabolites consistently detected in all samples within each group (100% detection rate) were considered representative and used for comparative analysis. Both group-specific and shared metabolites were identified, and correlations between metabolite presence and CCP titers were evaluated. Statistical methods including Fisher's exact test, network analysis, and centrality measures were applied to examine differences in metabolic pathway regulation. Notably, some metabolites were found in all ACPA-positive samples but only in approximately 70% of ACPA-negative ones, suggesting a potential role in ACPA-related mechanisms. Higher CCP titers were associated with increased detection rates of certain metabolites. Distinct patterns in metabolic network organization were observed depending on ACPA status and CCP levels, reflecting the metabolic heterogeneity of RA. These findings provide insights into ACPA-related metabolic alterations and may support the identification of novel biomarkers. Integration with proteomic data in future studies will offer a more comprehensive understanding of RA pathophysiology.

2129 B — Serum proteome and metabolome signatures associated with drug response in rheumatoid arthritis

PRESENTING AUTHOR: *Lee Jiyeong, Eulji University, South Korea*
CO-AUTHORS: *Sora Mun, Seungyeon Lee, Yeeun Yun, Hee-Gyoo Kang*
SUB THEME: *Other*

Rheumatoid arthritis (RA) is a heterogeneous autoimmune disease, and variability in treatment response remains a major clinical challenge. In this study, we explored the proteomic and metabolomic profiles of RA patients with differential responses to pharmacologic treatment, aiming to identify potential biomarkers associated with drug responsiveness. Serum samples from RA patients were processed and analyzed using liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). Proteins and metabolites consistently detected across samples within each response group were selected for comparative analysis. The identified molecules were examined in the context of previous RA studies and known biological pathways using public databases and bioinformatics tools such as STRING, OmicsNet, Metascape, and MetaboAnalyst. Based on existing literature, several of the identified candidates are hypothesized to be involved in immune regulation, inflammation, and cellular metabolism—processes known to underlie RA pathophysiology and treatment mechanisms. Differences in metabolite or protein abundance between responder and non-responder groups may reflect distinct metabolic adaptations or immune pathway activations associated with treatment efficacy. These findings support the hypothesis that specific proteomic and metabolomic signatures may be linked to therapeutic outcomes in RA. While the biological relevance of these molecules requires further validation through integrative functional and network analyses, our results provide a basis for future studies aimed at understanding the molecular basis of treatment response and identifying stratification markers for personalized RA therapy.

2130 B — Exploring serum metabolite signatures for differentiating rheumatoid arthritis from healthy controls

PRESENTING AUTHOR: *Hee-Gyoo Kang, Eulji University, South Korea*
CO-AUTHORS: *Sora Mun, Seungyeon Lee, Yeeun Yun Yun, Jiyeong Lee*
SUB THEME: *Other*

This study aimed to identify serum metabolite biomarkers with potential utility for rheumatoid arthritis (RA) screening. Serum samples from 49 RA patients and 43 healthy controls (HCs) were subjected to global metabolomic profiling using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Molecular features (MFs) were extracted and filtered based on statistical significance ($P \leq .05$), fold change (≥1.5), and detection frequency (≥80%). Feature selection was performed using volcano plot analysis, partial least squares discriminant analysis (PLS-DA), and variable importance in projection (VIP) scoring. A total of 57 candidate MFs were selected, and 18 of them were successfully annotated as metabolites through spectral matching with the METLIN database. Among these, six metabolites—dehydroepiandrosterone sulfate, androsterone sulfate, γ-linolenic acid, 9(E),11(E)-conjugated linoleic acid, docosahexaenoic acid, and docosapentaenoic acid (22n-3)—were found to be functionally relevant to RA pathophysiology and were selected as final biomarker candidates. Receiver-operating characteristic (ROC) analysis demonstrated that each of the six metabolites had an area under the curve (AUC) exceeding 0.75, and the combined AUC reached 0.89, indicating high discriminatory power. In conclusion, the selected panel of six metabolites shows strong potential for differentiating RA patients from healthy individuals and may serve as a promising biomarker set for future clinical RA screening applications.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2131 B — Evaluation of Whole Blood Microsampling Devices using LC-MS Lipidomic Profiling and CE-MS Metabolic Fingerprinting

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CO-AUTHORS: ***Daniel Marques de Sa e Silva, Sara Martínez, Glykeria Avgerinou, Vassilis Mougios, Helen Gika, Alma C. Villaseñor, Antonia García, Víctor González-Ruiz, Ana Gradillas, Coral Barbas***

SUB THEME: ***Other***

The need for accessible, standardized, and harmonized blood sample collection methods remains a critical challenge in metabolomics. Blood microsampling (BμS) offers a promising alternative to conventional venipuncture. However, rigorous qualitative and quantitative evaluations are essential to assess its metabolite coverage and analytical reliability.

This study evaluated the performance of three BμS devices—dried blood spot (DBS), quantitative DBS (qDBS), and volumetric absorptive microsampling (VAMS)—to sample whole blood from 10 healthy individuals through multiplatform metabolomics analysis, comprehensively assessing their performance in metabolite coverage, recovery, and precision compared to plasma as a reference matrix.

Lipidomics profiling was conducted using a single-phase lipid extraction method MeOH/MTBE (1:1, v/v) followed by RP-LC-ESI(+/–)-QTOF-MS (Agilent 1290 and 6545). Lipid recovery was evaluated using deuterated lipid standards from SPLASH® LIPIDOMIX®, showing higher recovery and precision for volumetric devices (qDBS and VAMS) compared to DBS. A targeted feature extraction (TFE) was performed with an in-house lipid database[1], identifying over 500 lipid species across 20 subclasses.

Polar metabolites profiling was conducted using two extraction protocols MeOH/H_2O (50:50 and 80:20, v/v) followed by CE-ESI(+/–)-TOF-MS (Agilent 7100 and 6230). Metabolite recovery was evaluated using deuterated carnitines and amino acids from MassChrom® with better recovery achieved using MeOH/H_2O (50:50, v/v). A TFE was performed with an in-house CE-MS database[2], identifying over 150 polar compounds.

PCA and OPLS-DA analyses showed the expected differences between the whole blood profile obtained with the BμS devices and that of the reference plasma.

The results demonstrate that BμS devices yield consistent metabolic/lipidomic profiles comparable to plasma, with qDBS and VAMS showing superior precision and recovery. This comprehensive evaluation highlights the potential of BμS as a standardized whole blood sampling method for large-scale metabolomics/lipidomics studies, providing a robust workflow with the potential to improve accessibility and support decision-making in future experimental design.

1.https://doi.org/10.1016/j.jlr.2024.100671

2.https://doi.org/10.1016/j.chroma.2020.461758

2132 B — Time-Resolved Untargeted Lipidomics Mass Spectrometry of Biomolecular Coronas around Nanomedicines Produced in Serum

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CO-AUTHORS: ***Sang Yun Han, Jayong Chung***

SUB THEME: ***Other***

Once injected into living organisms, nanoparticles (NPs) interact with endogenous biomolecules in the biological milieu, leading to the spontaneous formation of biomolecular coronas on their surfaces. This phenomenon alters the biological identity of NP surfaces, which ultimately determines the behaviors of NPs in the biological system such as their biodistribution, cellular uptake, and immune response. Thus, understanding of the coronas is critical for the development of nanomedicine. While protein coronas have been extensively studied over the past decades, the coronas formed by other biomolecules with distinct biological functions, such as metabolites, remain poorly understood despite their apparent involvement. In this study, we investigated the time-dependent alterations of lipid metabolites in serum coronas. The coronas formed around two model NPs with distinct surface properties were examined: lipid-based DNA-lipoplexes and IgG-immobilized PEGylated AuNPs. To monitor dynamic changes within the coronas, we employed a combined approach of nanoparticle tracking analysis, bioassays, and untargeted lipidomics using high-resolution LC-MS/MS. Our results revealed that lipids, rather than proteins, were predominant in the coronas of both NP types. Interestingly, although the overall amounts of lipids appeared relatively stable over time, detailed mass spectrometry analyses uncovered noticeable time evolution in lipid compositions, particularly depending on lipid class. Furthermore, the nature of this temporal evolution varied according to the surface characteristics of the NPs. These findings contribute to a more comprehensive understanding of biomolecular coronas, expanding the current focus beyond protein-centric perspectives and highlighting the occurrence of dynamic changes in the metabolite coronas.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2133 B — Metabolomic Profiling of Normal Human Retinal and Choroid Tissue

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CO-AUTHORS: *Krupa Sourirajan, Ines Lains, Kevin Mendez, Haemin Kang, Roshni Bhat, Augustine Bannerman, David Wu, Ivana K Kim, John B Miller, Demetrios Vavvas, Jessica Lasky -Su, Joan Miller*

SUB THEME: *Other*

Purpose: Biofluid metabolomics has been successfully used to investigate many eye diseases. It is crucial to understand how biofluid changes relate to in situ. This study aimed to characterize the normal metabolomic profile of donor human retinal tissue and compare to the donor serum profile.

Methods: This study included human donor eyes (n=14) above the age of 50 without any retinal disease that were enucleated within six hours post-mortem. The anterior segement was removed, a 6 mm macular punch, followed by a periphery punch were then obtained. The remaining retina was separated. For these samples (macula, periphery and remaining retina), the neurosensory retina was then separated from retinal pigment epithelium (RPE)/choroid.. Serum samples were collected for the same donors. All samples were snap frozen and stored at -80C degrees in sterile vials and were shipped frozen. Non-targeted mass spectrometry analysis was conducted by Metabolon Inc. Chi-squared tests were used for comparisons.

Results: A total of 659 metabolites were identified in the retina/choroid tissue samples. Most of them were lipids (53%) and amino acids (21%). The remaining metabolites were distributed across nucleotide (7%), carbohydrate (6%), cofactors and vitamins (6%), xenobiotics (3%), peptide (2%), and energy (2%) pathways. The same type of tissue (neurosensory vs RPE/choroid) had a similar metabolomic profile irrespective of location (macula vs periphery). When looking at neurosensory retina vs RPE, most (8) of the unique metabolites to RPE were dinucleotides; for the neurosensory retina, most (5) of the unique metabolites were lyphospoholipids. There were 512 metabolites (77.8%) overlapping between the retina/choroid tissue and serum.

Conclusions: To our knowledge, this study presents the first assessment of metabolomic profiles of human retina/choroid tissue. Most metabolites identified in the retina/choroid tissue were also seen in the serum, highlighting the ability to pursue additional research to explore their potential as peripheral biomarkers.

2134 B — Identifying Lipidomic Signatures and Microbial Metabolites in Patients with Metabolic Dysfunction-Associated Steatotic Liver Disease from Serbia: A Metabolomics-Based Approach to Liver Dysfunction

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SUB THEME: *Other*

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease in the Western world, and is rapidly increasing worldwide. Serbia, along with other Balkan countries, faces particularly high rates of MASLD, yet remaining underrepresented in large-scale European research initiatives, including the MASLD registry. The absence of structured clinical and molecular datasets from this region emphasize the need for locally conducted, population-specific studies. This work presents preliminary results from the first comprehensive multi-omics study of MASLD in the population from Serbia, aiming to identify a lipidomic signature of liver dysfunction and to explore microbial metabolites potentially involved in disease pathophysiology, using both untargeted and targeted metabolomics approaches.

Plasma and fecal samples were collected from 122 participants, including MASLD patients and sex-, age-, and BMI-matched healthy controls. Lipids from plasma, as well as semi-polar and polar metabolites, were extracted using a monophasic extraction solution (MeOH:MTBE:IPA (20:15:15)) containing internal standards. The samples were analyzed using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS, Agilent Technologies, Santa Clara, CA, USA). The analysis was performed on an ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, particle size 1.7 µm; Waters, Milford, USA). Data were processed and analyzed using MZmine 3 software, with lipid and metabolite identification based on an in-house library.

A total of 1751 lipids and 1315 semi-polar/polar metabolites were detected in plasma samples, with 214 lipids and 132 semi-polar/polar metabolites identified. Principal component analysis did not reveal significant separation between MASLD patients and healthy controls. At the lipid class level, however, distinct clustering patterns emerged, pinpointing alterations in lipid metabolism. Ongoing analyses aim to further refine these observations and investigate associations with clinical parameters, with the goal of uncovering meaningful patterns that could contribute to the discovery of disease biomarkers and the elucidation of pathophysiological mechanisms.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2135 B — Assessment of Perfluoroalkyl Substances (PFAS) in MASLD Patients from Serbia: Insights from a Broader Multi-Omics Study

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SUB THEME: *Other*

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants with known bioaccumulative properties and increasing recognition for their potential to disrupt metabolic pathways, including bile acid transport and lipid metabolism. Despite growing global attention, data on PFAS exposure and distribution in the Balkan region, including Serbia, remain limited. This work presents preliminary findings on PFAS presence and explores potential associations with bile acid metabolism in patients with metabolic dysfunction-associated steatotic liver disease (MASLD) and age-, BMI-, and sex-matched controls, as part of a broader multi-omics investigation.

Plasma samples were collected from 122 participants (MASLD patients and matched controls) and metabolites were extracted using a monophasic solution of methanol, methyl tert-butyl ether, and isopropanol (20:15:15) containing internal standards. Extracts were analyzed using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry on a C18 column. Data processing was performed using MZmine 3 software, and compound annotation was based on an in-house spectral library.

Among the detected environmental contaminants, three PFAS compounds—perfluorohexane sulfonic acid (PFHxS) and both linear and branched isomers of perfluorooctane sulfonic acid (PFOS)—were consistently identified across all samples, suggesting widespread low-level exposure in the cohort, with no significant differences in PFAS abundance between MASLD patients and healthy controls.

These preliminary results highlight the presence of PFAS compounds in all participants, reflecting a baseline environmental exposure within this population. Although no clear associations with MASLD status were observed, the integration of environmental contaminant data into multi-omics frameworks is critical for uncovering subtle metabolic perturbations. Ongoing analyses will focus on profiling secondary bile acids and microbiota-derived metabolites to explore potential downstream effects of PFAS exposure and their relevance to MASLD pathophysiology.

2136 B — The Path from Lab to Home: The METAspread Urine Test for Colorectal Cancer Metastasis

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CO-AUTHORS: *Maria Llambrich, Rocío Lopez, Josep Gumà, Raquel Cumeras*

SUB THEME: *Other*

Metabolomics is a powerful tool for biomarker discovery, yet its clinical translation remains challenging due to the need for rigorous validation, regulatory approval, and integration into healthcare workflows. Standardized methodologies, multi-cohort validation, and targeted quantification strategies are essential to ensure biomarker reliability and reproducibility. For clinical adoption, biomarkers must be not only diagnostically robust but also cost-effective, and practical for point-of-care applications.

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths and the third most diagnosed worldwide. Despite treatment advances, detection remains a challenge due to the low sensitivity of current methods (fecal FIT~70%, blood CEA~47%). Screening programs are often not comprehensive, leading to many cases being diagnosed at an advanced stage, and despite proven benefits only 58 of 255 countries have established national CRC screening. Furthermore, around 30–50% of patients with CRC undergoing curative resection of the primary tumor will develop metastases in the subsequent years. Therefore, early metastasis detection is crucial for timely treatment and better outcomes.

The METAspread test proposes an innovative solution to overcome current limitations in the detection of CRC metastasis using a lateral flow assay (LFA) in urine. The test is based on the quantification of three specific biomarkers that have demonstrated an overall accuracy of 86% (83% sensitivity and 87% specificity), validated with 800 samples measured with 1H-NMR. The biomarkers are under patent application (European patent EP24382061.0, filed on 24 January 2024). METAspread received the IIIVTSO-XarSMART Innovation Award and was showcased at the 2025 Mobile World Congress-Barcelona, highlighting its potential clinical impact.

The METAspread test represents a significant step in translating laboratory results into point-of-care devices for precision medicine. During the metabolomics conference we will present in detail the path we are following to achieve clinical translation of metabolomics results, including the innovation steps needed (e.g. regulatory) as a point-of-care device.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2137 B — Decoding Skin Biology Through a Multi-Matrix Omics Strategy

PRESENTING AUTHOR: *Yeray Gabaldón, IISPV, Spain*
CO-AUTHORS: *Sanae Benabou, Silvia Sodric, Sajjan Mehta, Lorenzo Torreni, Josep Gumà, Raquel Cumeras, Paolo Bonini*
SUB THEME: *Other*

The skin is a complex and dynamic organ that serves as a primary barrier, immunological interface, and site of environmental interaction. Understanding its biochemical composition is key to advancing dermatological research, improving personalized skincare, and addressing skin-related diseases. However, the layered structure and functional diversity of the skin present analytical challenges.

Advances in omics technologies are overcoming these barriers by enabling comprehensive biochemical profiling of the skin. In this study, we present a multi-matrix, untargeted omics strategy that integrates metabolomics and lipidomics to

generate a holistic map of skin composition across four biologically distinct matrices: cultured keratinocytes, non-invasive skin strips, 3D skin models, and ex vivo skin explants. Each matrix provides a distinct and complementary analytical perspective: keratinocyte cultures provide targeted cellular insight, skin strips detect exposome components and bacterial metabolites, and 3D models or explants capture the

complexity of skin architecture and immune activity.

Dual-extraction protocols enabled parallel profiling of metabolites and lipids using a fast untargeted LC-MS-based workflow (8-minute C18 chromatography) and HRMS (Sciex ZenoTOF 7600), leveraging DDA, SWATH, and Zeno trap ion accumulation to enable the detection of low-abundance compounds.

Our analysis revealed robust performance across all matrices, with a minimum of 135 annotated metabolites and 900 lipids (skin-strip matrix). Keratinocyte cultures yielded precise cellular readouts enriched in energy-metabolism intermediates. Skin strips captured exposome-related molecules and microbially derived lipids as well as barrier lipids, offering a non-invasive snapshot of the skin

surface. As expected,3D models and explants delivered the broadest coverage, unveiling deeper-layer signatures linked to barrier integrity and immune-related mediators.

Combining metabolic and lipidomic data from diverse sample types achieves a more nuanced perspective on skin physiology and pathology. The platform is extensible to studies of environmental stressors, disease phenotypes, drug permeation, and personalized skincare development, underscoring the value of complementary omics matrices for skin research.

2138 B — Exploration of SCFA in human multi-compartments using 3-nitrophenylhydrazine-LC-MS

PRESENTING AUTHOR: *Lydie Nadal-desbarats, INSERM de Nantes DR Grand Ouest, France*
CO-AUTHORS: *Antoine Lefèvre, Jérémy Monteiro, Camille Dupuy, Adeline Oury, Diane Dufour-Rainfray, Patrick Emond, Lydie Nadal-desbarats*
SUB THEME: *Other*

Short-chain fatty acids (SCFAs) are metabolites involved in many physiological processes. A disruption of their metabolism is suspected in diseases of the digestive system (inflammatory diseases) but also in neurodegenerative diseases such as Alzheimer's disease. Currently, one of the reference methodologies for the quantitative analysis of SCFA is based on the use of GC-MS due to its sensitivity and reproducibility. However, its technical implementation is delicate, limiting in particular the throughput of the analyzes carried out.

Our objective is to propose a method for absolute quantification of SCFA that is easier to implement and for future use in a clinical environment for a large number of biological sample types (stool, urine, serum, saliva, CSF, and dried blood spot).

With this objective we selected the derivatization process, with 3-nitrophenylhydrazine (3NPH), to promote the detection and the analytical separation of these metabolites. After methodological validation, either evaluation on 10 aliquots of a sample pool (formed from 10 samples of healthy male and female patients) of robustness, repeatability, matrix effect, carryover, LLOQ, its use makes it possible to propose reference values of SCFA concentrations for each type of sample. This quantitative methodology also allows longitudinal monitoring of SCFA concentrations.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2139 B — Determining optimal analytics for accurate and reproducible untargeted lipidomics analysis using UHPLC-QTOF/MS

PRESENTING AUTHOR: *JinJu Park, Kyungpook National University, South Korea*
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SUB THEME: *Other*

Lipidomics offers valuable insights into the molecular mechanisms underlying diseases such as cancer, cardiovascular disorders, and metabolic disorders. However, accurate and reproducible lipid analysis relies on the design of analytics, including the selection of chromatographic columns, mobile phase additives, and the lipid extraction method. Therefore, this study aims to optimize analytics for accurate and reproducible untargeted lipidomics analysis.

Human plasma samples were analyzed using two different columns: ethylene bridged hybrid (BEH) or charged surface hybrid (CSH). For mobile phase additives, we evaluated a mixture of 0.1% formic acid and 10 mM ammonium formate, and 10 mM ammonium formate alone in positive ionization mode, whereas a mixture of 0.1% acetic acid and 10 mM ammonium acetate, and 10 mM ammonium acetate alone were evaluated in negative ionization mode. Human plasma samples were subjected to five distinct lipid extraction methods: chloroform-based extraction with and without re-extraction, methyl tertiary-butyl ether (MTBE)-based extraction with and without re-extraction, or butanol-based extraction. Each analysis was conducted in quadruplicate using ultra-high-performance liquid chromatography-quadrupole time-of-flight/mass spectrometry. The optimal analytics were determined based on the total number of detected features, principal component analysis (PCA), lipid annotation, and relative standard deviation (RSD) values.

The BEH column detected more features than the CSH column, particularly in negative ionization mode. Mobile phase additives of 0.1% formic acid and 10 mM ammonium formate in positive ionization mode and 10 mM ammonium acetate in negative ionization mode improved precision with RSD < 30%. Chloroform and MTBE-based extraction methods demonstrated distinct clustering patterns in the PCA plot. The chloroform-based approach detected fewer features than the MTBE-based method, despite both achieving comparable lipid annotation. Accordingly, the chloroform-based extraction method is thought to be more selective and efficient.

This study compares the key factors to be considered for lipidomics analysis, presenting optimal analytics for complex biological matrices.

2140 B — Developing and optimizing single tissue sample preparation for parallel global metabolomics and lipidomics

PRESENTING AUTHOR: *Barbora Pisklakova, Oslo University Hospital, Norway*
CO-AUTHORS: *Nathalie Øvsthun, Sander Johannes Thorbjørnsen Guttorm, Ales Kvasnicka, Anja Østeby Vassli, Helge Rootwelt, Katja Benedikte Prestø Elgstøen*
SUB THEME: *Other*

Limited amount of tissue sample often represents a big challenge for multiomics approaches. This is particularly difficult with a small amount of valuable samples given the invasive nature of sample collection. A key objective of the sample preparation strategy is to enable the simultaneous application of a single tissue sample for both metabolomic and lipidomic analyses. Several procedures were tested and samples were analysed using global metabolomics and lipidomics. For metabolomics analysis, Pursuit XRs Diphenyl column (2 x 250 mm, 3 µm, Agilent Technologies) Dionex Ultimate 3000 UHPLC and Q Exactive Orbitrap mass spectrometer (Thermo Scientific) were employed (DOI:10.1021/acs.jproteome.1c00326). Lipidomic analysis was performed using Vanquish HPLC, Accucore C30 column (150 x 2.1 mm, 2.6 µm) and Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific).

Homogeneous sample of liver tissue was selected to minimize biological variability across replicates. Isotopically labelled internal standards containing both hydrophilic and hydrophobic species were used in all procedures to track phase separation behaviour and assess extraction repeatability. Procedure 1: two-phase extraction method using chloroform or MTBE (DOI:10.1194/jlr.D700041-JLR200; PMID: 13428781), procedure 2: one-phase system based on protein precipitation with methanol or isopropanol. For procedure 3, methanol was used for tissue homogenization; supernatant was collected and the remaining supernatant and protein pellet was extracted using isopropanol and both supernatants were analysed using global metabolomics and lipidomics. The evaluation of procedure 1 demonstrated that while biphasic systems provide better separation, they also introduce significant feature loss during required solvent evaporation. Procedure 2 resulted in an issue with extraction efficiency. In contrast, tissue homogenization in methanol followed by isopropanol extraction (approach 3) proved to be the best approach, minimizing sample loss while maintaining analytical repeatability. By integrating this method, both metabolomics and lipidomics analyses can be performed from a single tissue sample.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2141 B — Lipidomic analysis of patients with systemic sclerosis and idiopathic inflammatory myopathies

PRESENTING AUTHOR: *Martina Kadláčková, Palacký University Olomouc, Czech Republic, Czech Republic*
CO-AUTHORS: *Jakub Rozhon, Radana Brumarová, Blanka Stibůrková, Michal Tomčík, Sabina Oreská, Jiří Vencovský, David Friedecký*
SUB THEME: *Other*

Introduction: Systemic sclerosis (SSc) and idiopathic inflammatory myopathies (IIM) are rare autoimmune connective tissue diseases marked by chronic inflammation, immune dysregulation, and progressive tissue fibrosis. Cardiovascular involvement is a frequent and serious complication in both conditions, significantly contributing to increased morbidity and mortality. Nevertheless, cardiovascular risk is often underestimated in these patient populations. Traditional risk assessment tools such as SCORE and SCORE2 are commonly used, although their validity in autoimmune diseases remains under investigation.

Aim: This study aimed to evaluate the plasma lipid profiles of patients with SSc and IIM in comparison with healthy controls (HC). A secondary objective was to assess the applicability of the Coronary Event Risk Test (CERT) score in estimating cardiovascular risk in these populations.

Methods: Plasma lipidomic profiling was conducted on samples from patients with SSc (n = 100), IIM (n = 90), and HC (n = 200). Samples were collected from the RU biological material bank. HPLC-MS/MS was performed using an Exion LC system coupled with a QTRAP 6500+ mass analyzer (Sciex). Lipid separation was achieved on a BEH C8 column (2.1 × 100 mm, 1.7 µm; Waters).

Results: A total of 530 lipids spanning 20 lipid classes were identified. Among these, ceramides (Cer), phosphatidylcholines (PC/O), lysophosphatidylethanolamines (LPE), lysophosphatidylcholines (LPC/O), and diacylglycerols (DG) were the most significantly altered in patients with SSc and IIM compared to healthy controls. Additionally, both patient groups demonstrated significantly elevated CERT1 and CERT2 scores, indicating a higher predicted cardiovascular risk.

Conclusions: Lipidomic analysis revealed distinct alterations in lipid profiles of patients with SSc and IIM, highlighting disease-specific changes in key lipid classes. The CERT score may offer a valuable tool for cardiovascular risk stratification in patients with autoimmune connective tissue diseases.

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2142 B — Development and validation of a HILIC-MS/MS method for the quantitative analysis of Diabetes Mellitus related serum biomarkers.

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CO-AUTHORS: *Athanasios Nikolopoulos, Miroslav Kubát, Christina Virgiliou, Helen Gika, Georgios Theodoridis*
SUB THEME: *Other*

Diabetes mellitus (DM) is a progressive metabolic disorder marked by insulin resistance, impaired insulin secretion, and chronic hyperglycemia. The disease encompasses prediabetes, type1 and type2 diabetes, gestational diabetes, and other less common forms. Persistent hyperglycemia and glycemic variability damage the cardiovascular, renal, and visual systems, leading to complications, such as heart disease, nephropathy, and retinopathy. The complexity and heterogeneity of DM highlight the need for reliable biomarkers quantification to enhance early diagnosis, monitor disease progression, and guide personalized treatment. The present study aimed to compile the most pertinent DM-related biomarkers, formulate a targeted panel, develop an efficient quantitative assay and evaluate its clinical applicability. A new HILIC-MS/MS method was developed on a Bruker EVOQ Elite instrument using an Acquity UPLC BEH HILIC column and a binary solvent system of ACN vs ACN:H2O 50:50 v/v gradient (both at 10 mM HCOONH4, pH = 9.5). The method determines 1,5- Anhydroglucitol, glucose, fructose, mannose, leucine, isoleucine, glutamine, glutamate, phenylalanine, tyrosine, valine and tryptophan. Extraction recovery, accuracy, repeatability, linearity and matrix effect were evaluated in accordance with bioanalytical method guidelines, where accuracy ranged between 80.8% and 120% across all analytes. The validated method was applied to the analysis of blood samples from pre-diabetic and diabetic human cohort (n=300) recruited from multiple sites. Absolute concentration levels were reported. Our objective is to identify glycemic variability even if conventional measurements (i.e., hemoglobin or blood glucose) show normal values. Robust biomarker analysis is crucial for improving clinical decision-making and advancing translational research in diabetes care, offering a deeper understanding of the metabolic and inflammatory disturbances associated with DM.

This work was supported by projects “Combatting Diet Related Non-Communicable Disease Through Enhanced Surveillance (CoDiet)”, Horizon Europe number 101084642 and “Biomic_AUTh, Center of Excellence in Metabolomics research (BiACEM)” (Project number: 101079370) “Horizon Europe Twinning grant”.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2143 B — Towards Improving Matrix Effect Evaluation in Plasma Metabolomics: A Comparison of Protein Precipitation and Phospholipid Removal

PRESENTING AUTHOR: *Daniel Malheiro, Univerisity of Copenhagen / Cmbio, Denmark*
CO-AUTHORS: *Lea Johnson, Morten Danielsen, Nikoline Juul Nielsen, Jan. H Christensen*
SUB THEME: *Other*

Matrix effect (ME) evaluation remains a critical yet often overlooked challenge in metabolomics, significantly impacting accuracy and reliability of analyses. While endogenous phospholipids are a well-known source of ion suppression, exogenous contaminants can also contribute to ME, influencing data interpretation. In this study, we used post-column infusion to assess the effects of phospholipids and contaminants introduced during sample preparation on ME by comparing two common sample preparation techniques: protein precipitation and phospholipid removal (PLR) filters with different bed volume washes. We demonstrate how phospholipids contribute to ME and how interfering ions and contaminants can distort conclusions. Specifically, we:

(1)Emphasize the importance of solvent blanks in ME assessment. We show that solvent blanks have distinct chemical fingerprints, making their selection critical for determining whether suppression or enhancement is observed. This highlights the need for careful blank monitoring and selection to ensure accurate ME interpretation.

(2)Investigate various sources of interference affecting ME:

a. System contaminants that compromise analytical repeatability.

b . Co-occurring contaminants, presumably from the same source e.g. PLR filters, were identified by screening for homologous series and cluster analysis grouping.

c. The role of mass resolution in distinguishing interferences from analytes and internal standards, emphasizing its often-overlooked importance for ME assessments.

By addressing these factors, we provide a comprehensive approach to improving ME evaluation in metabolomics.

2144 B — Non-Invasive Fecal Metabolomics for Comparative Profiling of Primates Using a Field-Friendly Protocol

PRESENTING AUTHOR: *Claudia Wiesner, Universitaet Leipzig, Germany*
CO-AUTHORS: *Anna Mascellani Bergo, Ruben Holland, Anja Widdig, Anna Mae Vorwerk, Susan Billig, Jaroslav Havlik, Claudia Wiesner*
SUB THEME: *Other*

Anthropogenic pressures such as deforestation, fragmentation, and hunting have significantly affected wildlife, reducing biodiversity and altering physiological health in many endangered species. In this context, non-invasive sampling methods like collection of fecal specimen are increasingly valuable for conservation and ecological studies. Fecal metabolomics offers a powerful tool to investigate species-specific metabolic responses to environmental and physiological stressors. This study aimed to compare fecal metabolomic profiles across five primate species (chimpanzee, bonobo, orangutan, gorilla, and lion-tailed macaque) kept under similar conditions at Leipzig Zoo (Germany). Samples were collected over five days, with five replicates per species and day, extracted on-site with methanol. Samples were analyzed with different GC-MS methods to acquire the profile of volatile and semi-volatile metabolites. Principal component and partial least squares discriminant analyses revealed clear species-specific metabolic fingerprints. GC-MS data showed strong separation, with orangutan and gorilla profiles notably distinct from bonobo and macaque. Headspace analysis complemented these findings, highlighting differences in volatile metabolites and showing that dried methanolic extracts of fecal samples were particularly rich in fatty acids, while short-chain fatty acids were lost with drying. This protocol demonstrates a practical approach for metabolomic screening in field-like conditions. Future work will incorporate LC-MS and NMR to broaden metabolite coverage and further evaluate the method's analytical robustness.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2145 B — PLASMA AND URINE METABOLIC AND LIPIDOMIC PROFILINGS FOR PATIENTS WITH MUCOPOLYSACCHARIDOSIS TYPE II (HUNTER SYNDROME)

PRESENTING AUTHOR: ***Asma Farjallah, Université de Sherbrooke, Canada***

CO-AUTHORS: ***Bruno Maranda, Roberto Giugliani, Christiane Auray-Blais***

SUB THEME: ***Other***

Mucopolysaccharidosis type II (MPS II, Hunter syndrome) is an X-linked lysosomal storage disorder caused by iduronate-2-sulfatase (I2S) deficiency, leading to glycosaminoglycan (GAG) accumulation in tissues and organs. This accumulation contributes to clinical manifestations such as skeletal abnormalities, organ enlargement, developmental delays, and in the neuronopathic form, neurodegeneration.

This study aimed to identify neuronopathic MPS II-specific biomarkers and explore lipid and metabolite dysregulation using untargeted lipidomic and metabolomic approaches. Plasma and urine samples from 21 untreated neuronopathic MPS II patients with severe symptoms were compared to those from 23 age- and gender-matched healthy controls. The use of hydrophilic interaction liquid chromatography (HILIC) andgan reverse-phase liquid chromatography (RPLC) coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF MS) enabled the identification of significantly elevated gangliosides, ceramides, acylaminosugars, dipeptides, and specific amino acids in MPS II patients.

More specifically, impaired degradation of heparan sulfate (HS), a hallmark of MPS II, reflects altered protein metabolism. Additionally, disruptions in the glycerophospholipid metabolism and fatty acid oxidation pathways underscore the pivotal role of lipid dysregulation in disease progression.

Future work will focus on developing quantitative tandem mass spectrometry methods of targeted metabolites to establish reference values and expand comparisons between neuronopathic and non-neuronopathic forms. Early detection of neuronopathic biomarkers could facilitate timely therapeutic intervention and enhance the monitoring and follow up of MPS II patients.

2146 B — Towards defining the chemical signature of inflammatory skin conditions: an in vitro model

PRESENTING AUTHOR: ***Flavio Francina, University Of Ferrara, Italy***

CO-AUTHORS: ***Riccardo Di Stefano, Marta Manfredini, Annalisa Marcuzzi, Elisabetta Melloni, Erika Rimondi, Paola Secchiero, Giada Lodi, Sergio Crovella, Allan Polidoro, Alberto Cavazzini, Flavio Francina***

SUB THEME: ***Other***

Human skin, a metabolically active tissue, releases numerous volatile organic compounds (VOCs) from the stratum corneum, skin glands, and resident microbiota, reflecting underlying cellular processes and offering insights into skin physiology.

In this study, we present a new bioanalytical workflow designed to accurately quantify volatile and semi-volatile compounds in an in vitro model of inflammation. Specifically, normal human dermal fibroblasts (NHDF) were treated with the following inflammatory stimuli: lipopolysaccharide (LPS), polyinosinic-polycytidylic acid (POLY I:C), and tumor necrosis factor (TNF). Following 3- and 6-hour treatments, cell supernatants were collected and analyzed via headspace solid-phase microextraction (SPME) coupled with comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS). An internal standard preloading strategy was implemented to ensure analytical robustness. The observed variation over the current data set resulted with a CV of 10%.

The analysis of the aggregated samples revealed over 1900 volatile chemical features, with approximately 500 features characterizing the volatile composition of the treated cell cultures after data curation. The volatilome of fibroblasts exposed to different inflammatory agents revealed a rich and diverse chemical profile, enabling correlations with the specific treatments. Integrating cell-based immunometabolic screening with this detailed chemical profiling holds great potential for predicting therapeutic responses at molecular and systemic levels. The ultimate goal is to identify novel drug targets based on the detected immunometabolic signatures, thereby paving the way for personalized medicine approaches.. This research is currently focused on patients with chronic graft-versus-host disease (cGVHD) and inflammatory skin diseases which are resistant to conventional therapies aiming to provide new avenues for targeted treatment strategies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2147 B — Untargeted metabolomics approaches in acute and longitudinal human interventional studies to investigate the bioactive properties of olive leaf extract

PRESENTING AUTHOR: *María de la Luz Cádiz-Gurrea, University Of Granada, Spain*

CO-AUTHORS: *Patricia Fernández-Moreno, María del Carmen Villegas-Aguilar, Andrea Erdociain-Jiménez, Enrique Barrajón-Catalán, María Herránz-López, David Arráez-Román, Antonio Segura-Carretero, Álvaro Fernández-Ochoa*

SUB THEME: *Other*

The bioavailability and metabolism of bioactive compounds from plant sources, such as Olea europaea L. leaves, are crucial for a better understanding of the reported health effects. To address this point, untargeted metabolomics strategies represent a valuable tool, as they enable the detection of both endogenous and exogenous metabolites, offering a comprehensive understanding of metabolite bioavailability and transformation, as well as their biological mechanisms. To investigate these properties through untargeted approaches, this study proposes two interventional designs in healthy volunteers, one acute and one longitudinal, both based on olive leaf extract administration.

The acute study involved 17 participants, divided into two groups: one receiving 500 mg of olive leaf (n=8) and placebo (n=9). The longitudinal study lasted two months with 128 participants divided into the same two groups. Blood and urine samples were collected at baseline (t=0) and at different times post-consumption (acute) or after two months (longitudinal). After pretreatment, all samples were analyzed by HPLC-ESI-QTOF-MS/MS. Pooled-QC samples were analysed multiple times throughout the sequences for both matrices. Data processing was performed using MZmine software and notame R package. Univariate and multivariate statistical analyses were employed to detect significant exogenous and endogenous metabolites. Metabolites were annotated by comparing MS/MS spectra with databases.

49 exogenous metabolites (e.g. hydroxyoleuropein, oleuropein aglycone glucuronide, hydroxy-methoxyoleuropein,...) were annotated in the samples collected in the acute study. Regarding the effect on endogenous metabolism, the results of the longitudinal study suggest a potential influence on lipid metabolism, as evidenced by the presence of various phospholipids derivatives, such as phosphatidylcholine, diacylglycerols, and triglycerides. These changes may reflect the ability of olive polyphenols to modulate plasma lipid composition, supporting lipid metabolism regulation and fatty acid homeostasis. In conclusion, this study highlights the importance of untargeted metabolomics in intervention studies to enhance understanding of the bioactive effects of plant extracts.

2148 B — Citrus depressa Hayata alleviates pulmonary fibrosis via modulation of tryptophan metabolism

PRESENTING AUTHOR: *Ta-wei Liu, University Of Graz, Taiwan*

CO-AUTHORS: *Man-Hsiu Chu, Yun-Han Wang, Ching-Kuo Lee*

SUB THEME: *Other*

Citrus depressa Hayata, a citrus species native to Taiwan, has long been used locally as a source of Chenpi, a traditional herbal remedy for respiratory conditions. Our previous research demonstrated that C. depressa extract suppresses SARS-CoV-2 infection. Given that pulmonary fibrosis is a common complication in severe COVID-19 patients, this study further explored the potential of C. depressa in treating fibrosis. Using bleomycin-induced BEAS-2B cells and a mouse model, we confirmed that crude extract of C. depressa effectively reduced TGF-β1 elevation, epithelial–mesenchymal transition (EMT), and extracellular matrix (ECM) accumulation. While network pharmacology analysis suggested that the extract may act through multiple pathways relevant to fibrosis regulation. Metabolomic profiling of serum, urine, feces, and lung tissue from treated mice, followed by pathway analysis, revealed that the most affected metabolites were linked to tryptophan metabolism. In parallel, serum pharmacology identified eight flavonoids as potential active compounds, and subsequent cell-based screening narrowed these to two candidates. Finally, we evaluated these two flavonoids and aromatic amino acids, identified through metabolomics, as therapeutic agents in fibrotic mice. Among them, aromatic amino acid supplementation showed the greatest promise in slowing the progression of fibrosis.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2149 B — Multi-organ and plasma metabolomics comparison of the biological effects of gadodiamide (Omniscan) and gadoteridol (ProHance)

PRESENTING AUTHOR: *Francesca Romano, University of Naples Federico II, Italy*

CO-AUTHORS: *Enza Di Gregorio, Gelsomina Riccardi, Chiara Furlan, Nicola Cavallini, Francesco Savorani, Anna Di Porzio, Antonio Randazzo, Eliana Gianolio, Nunzia Iaccarino*

SUB THEME: *Other*

Gadolinium-based contrast agents (GBCAs) are the most widely employed in radiology to enhance the sensitivity of magnetic resonance imaging (MRI), thanks to the strong paramagnetic properties of gadolinium ions. However, growing concerns remain about gadolinium (Gd) retention and its potential biological effects, particularly following repeated administrations of less stable linear GBCAs. To date, systematic studies investigating global metabolic alterations induced by GBCA exposure are still limited.

In this study, we applied multi-organ and plasma metabolomics to evaluate the systemic effects of repeated administrations of a linear GBCA (gadodiamide, Omniscan) versus a macrocyclic GBCA (gadoteridol, ProHance) in healthy mice. Animals received 20 intravenous injections over five weeks, followed by a one-month washout. Plasma and tissues (brain, cerebellum, liver, kidney, spleen) were analysed by 1H-NMR and GC-MS.

Multivariate analysis on the collected data revealed that, while plasma metabolomic profiles showed no significant differences between groups, multi-organ analysis displayed marked metabolic alterations in Omniscan-treated mice compared to control and the ProHance groups. These perturbations were organ-specific, with brain, cerebellum, and liver showing the most pronounced effects, including upregulation of glycolysis, the tricarboxylic acid cycle, and gluconeogenesis, along with dysregulation of amino acid and nucleotide metabolism. Notably, increased brain urea levels were detected in Omniscan-treated animals, a feature associated with neurodegenerative processes.

This study provides the first comprehensive evidence of systemic metabolic disruption induced by repeated administration of a linear GBCA, likely linked to free Gd release and tissue retention. In contrast, ProHance caused minimal metabolic alterations, supporting the safer profile of macrocyclic GBCAs. These findings, published in Analyst (2023) [1], emphasize the need for cautious use of linear GBCAs and reinforce the preferential adoption of macrocyclic agents, while demonstrating the value of metabolomics for detecting subtle biochemical changes related to Gd retention.

[1] F. Romano et al., Analyst, 2023, 148, 2415; DOI: 10.1039/d3an00353a

2150 B — Exploring the Nasal Metabolome and Exposome by LC-HRMS in the NOSE infant cohort

PRESENTING AUTHOR: *Sabrina Berger, University Of Vienna, Austria*

CO-AUTHORS: *Jesse T. Peach, Anke Martens, Lukas Wisgrill, Benedikt Warth*

SUB THEME: *Other*

Preterm infants are known to experience respiratory infections more frequently and with greater severity compared to full-term infants. This increased vulnerability is attributed to factors such as immature immune responses, underdeveloped lungs, and altered microbiota. Most respiratory pathogens are airborne, making the nose a primary entry point and a promising site for studying early host-microbe-environment interactions. While individual components of the nasal environment, such as the microbiome and metabolome, have been studied, comprehensive, integrative analyses encompassing microbiome, metabolome, and exposome interactions in preterm infants remain limited. The NOSE (Nasal Cross-Omics Signatures to Decipher Viral Susceptibility in Early Childhood) study aims to longitudinally characterize the nasal metabolome and exposome of preterm and full-term infants using a minimally invasive sampling approach. Nasal epithelial lining fluid (NELF) samples were collected at multiple time points throughout the first year of life, as well as during respiratory infections. Sample preparation followed a standardized protocol, including cell lysis through freeze–thaw cycles in liquid nitrogen, protein precipitation, and a subsequent drying and reconstitution step, optimized to preserve a broad range of analytes. We established a robust analytical platform that combines ultra-high-performance liquid chromatography using both reversed-phase and hydrophilic interaction liquid chromatography with high-resolution mass spectrometry for untargeted metabolite analysis. In addition, exposomics data was extracted by a targeted data evaluation approach taking advantage of >200 known chemical exposure compounds. Preliminary observations suggest differences in inflammation- and immune-related metabolites between full-term and preterm infants during viral infections. The established workflow offers a basis for future investigations into early-life respiratory health.

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2151 B — Metabolomic study of dry blood spots in horses suffering from exertional rhabdomyolysis

PRESENTING AUTHOR: ***Radana Brumarová, University Palackeho, Czechia***

CO-AUTHORS: ***Martina Kadláčková, Hana Janečková, Dana Dobešová, Eliška Ivanovová, Tereza Novotná, Eliška Šamonilová, David Friedecký, Petr Jahn***

SUB THEME: ***Other***

Introduction

Equine myopathies are a range of muscle disorders characterized by pain, stiffness, weakness and exercise intolerance, often due to rhabdomyolysis. Atypical myopathy (AM), a severe and often fatal non-exertional form, is associated with hypoglycin A poisoning. This study investigates the metabolomic profiles of horses affected by AM, other non-toxic-related myopathies (ntM) and healthy controls to improve diagnostic accuracy.

Aims

To characterise and compare the metabolic signatures of AM, ntM and control horses using dried blood spots (DBS)

Methods

DBS samples were collected from 15 horses with AM, 19 with ntM (including exertional, immune-mediated, post-anaesthetic, nutritional and undefined causes) and 15 healthy controls. Acylcarnitines, amino acids, organic acids and acylglycines were analysed by flow injection analysis (FIA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Data were evaluated using univariate and multivariate statistical analyses to identify key discriminating metabolites.

Results

AM horses showed significant elevations in medium and long chain acylcarnitines (e.g. C8, C10, C14:1) and specific acylglycines compared to ntM and controls. OPLS-DA and ROC analyses showed excellent group separation and classification accuracy (AUC = 1.00) with no misclassifications.

Conclusion

Metabolomic profiling of DBS allows robust discrimination between atypical and non-toxic myopathies. Elevated acylcarnitines and acylglycines are reliable biomarkers of AM. This approach shows promise for rapid, non-invasive diagnosis of equine myopathies, facilitating timely clinical intervention and improved outcomes.

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2152 B — Expanding the clinical use of DBS: through a metabolomic and a lipidomic evaluation

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CO-AUTHORS: ***Cyrielle Clement, Antoine Lefèvre, Axel Dablanc, Diane Dufour-Rainfray, Camille Dupuy, Adeline Oury, Hélène Blasco, Estelle Pujos-Guillot, Florence Castelli, Justine Bertrand-Michel, Lydie Nadal-Desbarats, Patrick Emond***

SUB THEME: ***Other***

DBS (Dried Blood Spot) are used for the screening of neonatal diseases and to monitor some adults’ diseases in clinical context. DBS is a self-sampling device which can be sent by mail allowing everyone to get access to biological analysis. Their use in new contexts has been widespread: carrying out anti-doping tests or detection of cancer.

In this work, we propose to compare quantitative data and untargeted analysis data between DSB and serum, the blood sample of reference, to assess the potential of DBS.For quantitative data, we quantified 6 short-chain fatty acids, 20 bile acids, 20 tryptophan intermediates and 8 organic acids from TCA cycle by LC-QqQ-MS and around 500 lipids by SFC-QTOF-MS. Untargeted metabolomics and lipidomics have been carried out by a LC-Q-Orbitrap-MS.

Concerning metabolomics, among the quantified compounds (MassLynx) 34 compounds are present in both samples, each one allowing to detect 5 unique metabolites. From the untargeted point-of-view, 100 level 1 compounds are presents in both samples and around 50 are specific to one sample (W4M, Xcalibur). For lipidomics, more compounds are quantifiable (MZmine) and detected (W4M, LipidSearch) in serum than in DBS (124 vs.330 and 434 vs. 608 respectively).

DBS seems suitable for the widespread use of metabolomics in a clinical context whereas more development is required in lipidomics. Still, in both, several biomarkers are presents and can be used for detection and monitoring of diseases. These results make it possible to envisage the use of DBS in both quantitative and exploratory metabolomics analyses.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2154 B — Targeted Profiling of Fatty Acids in Follicular Fluid and Seminal Plasma via GC-MS/MS for Reproductive Metabolomics

PRESENTING AUTHOR: ***Magdalena Grajzer, Wroclaw Medical University, Poland***
CO-AUTHORS: ***Kamil Rodak, Iwan Zalewski, Ewa Maria Kratz, Anna Prescha***
SUB THEME: ***Other***

Authors:
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Summary:
We developed a high-sensitivity GC-MS/MS method for profiling fatty acids (FAs) in follicular fluid (FF) and seminal plasma using low sample volumes, supporting targeted reproductive metabolomics.
Introduction:
Fatty acids, particularly polyunsaturated fatty acids (PUFAs), influence gamete quality and reproductive success. Efficient profiling of FAs in FF and seminal plasma is limited by sample volume and matrix complexity. This study aimed to optimize FA extraction and detection using GC-MS/MS.
Methods:
In FF (≥50 µL), lipids were extracted (chloroform:methanol 2:1), transesterified with sodium methoxide, and analyzed using FAST-GC-MS/MS in MRM mode. In seminal plasma (75 µL), PUFAs including LA, ALA, GLA, AA, EPA, and DHA were extracted using a modified Cabrini method and converted to FAMEs using acetyl chloride in methanol. Detection was performed on an Agilent 7890B/7000D GC-MS/MS system using FastFAME columns and MRM transitions. External calibration and matrix-based quality control ensured analytical reliability.
Results:
Both protocols allowed efficient FA and PUFA extraction and accurate detection from minimal volumes. FAST-GC reduced analysis time (~20–26 min). MRM transitions enabled specific quantification of low-abundance PUFAs. Method validation confirmed linearity, sensitivity, and reproducibility. Optimal seminal plasma volume was 75 µL.
Conclusions:
We present robust, small-volume GC-MS/MS workflows for targeted FA analysis in FF and seminal plasma. These methods enable sensitive lipidomic profiling and biomarker discovery in reproductive biology.
Funding:
Supported by Wroclaw Medical University, grant no. SUBK.D0.40.25.055.
Conflict of Interest:
The authors declare no conflicts of interest.

2155 B — Metabolic analysis of the effects of heavy metals on the antioxidant activity of vitamin D in human plasma

PRESENTING AUTHOR: ***Jiseo Park, Kyungpook National University, South Korea***
CO-AUTHORS: ***Mi-Ri Gwon, Hae Won Lee, Duk-Hee Lee, Sook Jin Seong, Young-Ran Yoon***
SUB THEME: ***Pharmacometabolomics***

Introduction: Vitamin D (VD) is metabolized in the body and is crucial in regulating the antioxidant system. While exposure to heavy metals (HMs) inhibits VD activity, HMs can also be absorbed following VD stimulation. Despite varying opinions on how exposure to HMs affects VD activity, the impact of HMs on VD activity has yet to be investigated using metabolomics.

Objectives: This study aimed to use untargeted metabolic profiling to confirm the VD levels in relation to HMs identify putative metabolites, and elucidate the metabolic pathways involved.

Methods: In total, 46 plasma samples were analyzed using ultra-high-performance liquid chromatography and quadrupole time-of-flight/mass spectrometry. Metabolic analysis was performed in a group with severe VD deficiency and low HM exposure (SVDD–LHM) and a group with severe VD deficiency and high HM exposure (VDD–HHM).

Results: As a defense mechanism against increased oxidative stress induced by HMs, VD-induced antioxidant pathways possessed increased antioxidants, including bilirubin, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Additionally, elevated levels of EPA and DHA induce alterations in lipid profiles, including diacylglycerol and phosphatidylcholine. DHA demonstrated an area under the curve of 0.850, with 95% confidence intervals of 0.651–0.990. This suggests that DHA is the most appropriate biomarker for predicting VD activity in response to HM exposure.

Conclusion: The identified metabolites and pathways in this study indicate that VD activation of the antioxidant pathway is attributed to increased exposure to HMs.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2156 B — Exploring the association between intracellular accumulation of methotrexate polyglutamates and systemic metabolic signaling in patients with Crohn's disease

PRESENTING AUTHOR: ***Hak Min Kim, Seoul National University, South Korea***

CO-AUTHORS: ***Yeonseo Jang, Kyeong-Seog Kim, Joo-Youn Cho***

SUB THEME: ***Pharmacometabolomics***

Methotrexate (MTX) is a key treatment for Crohn's disease (CD), although clinical responses have been inconsistent. However, plasma MTX levels may not accurately reflect treatment response. Therefore, quantifying intracellular methotrexate polyglutamic acid (MTX-PGs), a potential biomarker, may be critical for evaluating MTX efficacy in the absence of established monitoring markers. A comprehensive understanding of the therapeutic response is essential for the development of appropriate treatment strategies. This study aimed to determine the clinical significance of intracellular MTX-PGs accumulation and the impact of MTX administration on the metabolic profile of CD.

Patients with CD receiving low-dose MTX maintenance therapy were prospectively enrolled. Blood samples were collected prior to dosing at three visits. In Study I, the levels of red blood cell (RBC) MTX-PG_2, MTX-PG_3, and MTX-PG_4 were measured by liquid chromatography-tandem mass spectrometry and analyzed for correlation with plasma MTX. In Study II, targeted plasma metabolome analysis was performed on responders and non-responders (classified by C-reactive protein/fecal calprotectin) to examine profile changes.

Plasma MTX levels have shown a strong correlation with 7-hydroxy-MTX, but not with RBC MTX-PGs. Responders exhibited significantly higher MTX-PG levels than non-responders. Metabolomic analysis revealed alterations in responders, including reduced levels of pro-inflammatory metabolites (e.g., 4-aminobiphenyl), arginine, and polyamines in responders. The metabolic pathways associated with inflammatory/immune activity (arginine, histidine, phenylalanine) were downregulated in responders, while the CoA synthesis pathway was activated.

This study quantified RBC MTX-PG levels and demonstrated that MTX responders exhibit increased intracellular accumulation of MTX-PGs. The plasma metabolome alteration provides insights into the systemic metabolic response to effective MTX treatment. Untargeted metabolomic analysis revealed systemic metabolic reprogramming in responders, suggesting changes in amino acid and energy metabolism. The observed metabolic alterations indicate a shift toward reduced inflammatory signals and enhanced detoxification capacity. This shift may have a secondary impact on the resolution of inflammation.

2157 B — Studying dynamics of cellular metabolism by real-time NMR metabolomics

PRESENTING AUTHOR: ***Sofia Moco, VU Amsterdam, Netherlands***

CO-AUTHORS: ***Valentina Ferro, Mengqiu Zhang, Brian van der Kieft, Victoria Pozo Garcia, Sofia Moco***

SUB THEME: ***Pharmacometabolomics***

Cellular metabolism is dynamic and time dependent. Bioactive compounds, such as pharmaceutical drugs, induce cellular metabolic changes. These metabolic alterations inform about the drug's mechanism-of-action or their metabolic fate. The dynamics of metabolite concentrations are then a proxy of their metabolic status. NMR gathers several advantages in studying cellular metabolism: it allows screening many cellular metabolites in parallel, in a quantitative fashion, and it even allows monitoring kinetic changes over time [1].

In our lab, we use a combination of NMR metabolomics approaches (1D, 2D) to study xenobiotic and central metabolism in real-time. By using mammalian cellular fractions, we can monitor the metabolism of cytochrome P450, a key family of enzymes in xenobiotic metabolism. Through a 1H NMR time series, enzymatic conversion of pharmaceutical drugs into their metabolites is followed. Likewise, central metabolism is also monitored using mammalian cells in suspension, allowing for assessing uptake and secretion of metabolites in real-time.

Real-time NMR can effectively monitor metabolic shifts, offering a deeper understanding of metabolism, including the identification of metabolic intermediates or products. Gathering biochemical information of drugs and their metabolic effects is essential for their development in drug discovery programs.

[1] Moco S. Studying Metabolism by NMR-Based Metabolomics. Front Mol Biosci. 2022

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★ AWARD WINNERS

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2158 B — Newborn metabolomic perturbations associated with prenatal tobacco smoke exposure and early birth in the Atlanta African American Maternal-Child cohort

PRESENTING AUTHOR: ★ *Paula-dene Nesbeth, Emory University, United States*

CO-AUTHORS: *Xiajie Lyu, Anne Dunlop, Youran Tan, Dana Boyd Barr, Volha Yakimavets, Stephanie Eick, Blake Rushing, Susan McRitchie, Susan Sumner, P. Barry Ryan, Elizabeth Corwin, Dean Jones, Donghai Liang*

SUB THEME: *Pregnancy, maternal and neonatal health*

Objective: Tobacco exposure during pregnancy is an established risk factor for early birth including early term birth (ETB) and preterm birth (PTB). However, the underlying molecular mechanisms are not well understood. In this study, we determined the associations between metabolomic profiles of newborn dried blood spots and prenatal tobacco exposure and early birth.

Methods: Our analytic samples consisted of 269 mother-child dyads in the Atlanta African American Maternal-Child Cohort (2016-2020). Established tobacco exposure biomarkers, cotinine and trans-3′-hydroxycotinine (3HC), were measured in maternal urine collected within 8-14 gestational weeks. Gestational age at birth was determined from medical records. Newborn dried blood spots were collected within 24-48 hours after birth and analyzed using untargeted high-resolution metabolomics. Metabolome-wide association studies adjusted for covariates along with metabolic pathway enrichment analyses and chemical annotations were used to determine newborn metabolite signals and pathways associated with prenatal tobacco exposure and early birth via the meet-in-the-middle approach.

Results: There were 84 (31.2%) ETB and 33 (12.3%) PTB in the cohort. Maternal urinary cotinine and 3HC concentrations were associated with 648 and 503 metabolic signals, respectively ($p < 0.05$). ETB and PTB were associated with 648 and 1,747 metabolic signals, respectively. Biopterin metabolism was associated with both tobacco exposure biomarkers and early birth (ETB and PTB). Perturbation in riboflavin metabolism was a common pathway associated with cotinine and 3HC concentrations. Early birth was also associated with lipid and fatty acid metabolic pathways. Riboflavin was positively associated and 5-hydroxytryptophan was negatively associated with both tobacco exposure biomarkers and early birth.

Conclusion: These findings suggest that the newborn metabolome is perturbed by prenatal tobacco exposure and is associated with gestational age at birth. Perturbation in biopterin metabolism is a potential mechanism linking prenatal tobacco exposure to early birth.

2159 B — Impact of Inhaled Nitric Oxide on the Neonatal Plasma Metabolome of Infants with Persistent Pulmonary Hypertension

PRESENTING AUTHOR: *Abel Albiach-Delgado, FUNDACIÓN PARA LA INVESTIGACIÓN DEL HOSPITAL LA FE, Spain*

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SUB THEME: *Pregnancy, maternal and neonatal health*

Inhaled nitric oxide (iNO) is a selective pulmonary vasodilator used to treat persistent pulmonary hypertension of the newborn (PPHN) associated with hypoxic respiratory failure. However, the formation of reactive oxygen and nitrogen species during therapy may induce oxidative and nitrosative damage in multiple organs. This has led to increasing scientific interest in identifying metabolic pathways altered by iNO therapy.

Plasma samples (37.5 µL) were extracted with 750 µL of CH_3CN/IPA/H_2O (3:3:2, v/v) containing lauric acid-d23 as an internal standard. Extracts were split into two aliquots, dried under vacuum, and cleaned up with 338 µL of CH_3CN/H_2O (1:1, v/v). One aliquot was derivatized with 8 µL methoxyamine hydrochloride (1.5 h), followed by 68 µL MSTFA for 30 minutes.

Untargeted metabolomic profiling was performed by GC-MS employing an Agilent 7890B instrument, while the second aliquot was used to acquire an Attenuated Total Reflectance – Fourier transform infrared (ATR-FTIR) spectrum using a Bruker Alpha II instrument. The method was applied to plasma from infants with PPHN treated with iNO collected before (T0, N=9), during (T1, N=21) and after iNO treatment (T2, N=17) and a control group without iNO (N=14).

ATR-FTIR biochemical fingerprints of plasma samples provided valuable insight into the metabolome of plasma samples revealing bias between participating hospitals. A total of 117 features were annotated in the GC-MS dataset using MS-based databases, including 32 amino acids, 18 carboxylic acids, 23 sugars, 9 lipids, 8 nitrogen-containing compounds, 2 pyrimidines, 4 aromatic/heterocyclic compounds, and 3 phosphate-containing metabolites. PLS-DA analysis showed statistically significant separation between T1 and the control group (Wilcoxon test, p-value=0.05). Univariate analysis revealed five metabolites with significant differences (FDR<0.05): 2-aminoadipic acid, isoleucine, L-glutathione, uric acid, and glucose.

Results revealed alterations of the metabolome due to iNO treatment as well as disease-specific traits. Future work will include LC-HRMS analysis for enhancing metabolome coverage.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2160 B — Determinants of the urinary steroid metabolome in later pregnancy: Results from the BiSC and INMA Spanish cohorts.

PRESENTING AUTHOR: ***Emily Pilar Laveriano Santos, Isglobal, Spain***

CO-AUTHORS: ***Estelle Renard, Mariona Bustamante, Zoraida Ruiz, Marina Ruiz-Rivera, Mireia Gascón, Marta Cosín, Oscar J. Pozo, Payam Davand, Martine Vrijheid, Léa Maitre***

SUB THEME: ***Pregnancy, maternal and neonatal health***

Background: Sex steroid hormones (SSH) are crucial for a healthy pregnancy. This study aimed to explore the main determinants of SSH in pregnancy.

Methods: We analyzed urine samples from 1,228 pregnant women at 3rd trimester from the BiSC (2018-2021, n=728) and INMA-SAB (2004-2022 n=500) cohorts, using UHPLC-MS/MS to quantify metabolites of progestogens, androgens, estrogens and corticosteroids. We quantified 50 individual SSH, 13 hormone groups, 9 glucuronide/sulfate (G/S) ratios, and 17 estimated steroid enzymatic activities calculated using the ratio between metabolites. We examined 118 and 84 potential determinants from both cohorts, including physiological, sociodemographic, genetic, medical history, stress, depression, and lifestyle. An exposome-wide association was applied using linear regression models across SSH metabolic indicators, corrected by gestational age and urine-specific gravity, and adjusted by sampling-collection confounders (i.e. hospital). P<0.0004 (Bonferroni cut-off by effective number of tests) was considered significant.

Results: Maternal age, ethnicity, fat intake 2nd-trimester, and fetal weight at 3rd-trimester were associated with SSH in both cohorts. Maternal age was associated with lower 5-androsten-3α17β-diol-diSulfate (BiSC: aβ=-0.04, CI95%=-0.05,-0.03; and INMA: aβ=-1.05, CI95%=-1.45,-0.65), and global CYP17 activity (BiSC: aβ=-0.04, CI95%=-0.05,-0.03; and INMA: aβ=-0.99, CI95%=-1.40,-0.59). In BiSC, fat intake was associated with lower Δ4_17,20 lyase activity; while in INMA, it was linked to lower cortisol-S and higher G/S-corticosteroids ratio. Spanish women in BiSC presented higher values of cortolone-G and G/S-cortisol ratio compared with Latin-American women, similar in INMA-SAB with 16αOH-dehydroepiandrosterone-sulfate, cortisol-21-sulfate, 5α-pregnan-3α,20α-diol-disulfate, pregnantriol-disulfate, and higher G/S-cortisol ratio. In BiSC, women reporting middle stress had higher 5-androsten-3βb17βb-diol-diSulfate-1, and those with PCOS had higher global CYP17 activity. Fetal weight was associated with higher estriol and progesterone metabolites in INMA-SAB and BiSC. In both cohorts, fetal sex, socioeconomic status, medical complications, parity, and maternal SNPs were not associated with SSH.

Conclusion: Maternal age, lifestyle, and fetal weight are key determinants of SSH metabolism at later pregnancy.

2161 B — Placental Metabolite Profiling in Preterm Birth Reveals Insights into Lipid and Amino Acid Imbalance During Pregnancy

PRESENTING AUTHOR: ***Arpita Singh, THSTI, India***

CO-AUTHORS: ***Thirunavukkarasu Ramasamy, Yashwant Kumar, Shinjini Bhatnagar, Pallavi Kshetrapal***

SUB THEME: ***Pregnancy, maternal and neonatal health***

Intro: Preterm birth (PTB) is defined as delivery before 37 weeks of pregnancy. Globally, ~15 million babies are born preterm every year and are at risk of immediate complications like respiratory distress, neonatal sepsis, and may face long-term developmental and behavioral challenges.

Need: Despite significant research on risk factors and biomarkers, PTB rates remain high due to inadequate knowledge of the complex biological mechanisms that trigger parturition earlier than expected.

Aim: Our study seeks to uncover the mechanistic pathways contributing to early parturition and identify novel signatures for screening of high-risk women for PTB. We hypothesize that placenta being a feto-maternal organ, plays a critical role and assessing its metabolome offers valuable insight into the physiological state of pregnancy.

Methods: Untargeted metabolomics was employed to analyze the metabolite profiles of spontaneous term birth (sTB) and spontaneous preterm birth (sPTB) placentae. Placental samples were selected from the ongoing GARBH-Ini cohort. Mass spectra were acquired on Orbitrap-Tribrid Fusion MS and data was processed using MZmine. Putatively identified metabolites were verified using Parallel Reaction Monitoring (PRM). Also, metabolomics data was integrated with proteomics data, obtained on the same set of placental samples. A targeted metabolomics approach was also conducted to quantify metabolites from various classes in maternal serum samples using a nested case control study design.

Results: Metabolomics and integrated omics revealed dysregulation of fatty acid oxidation in sPTB placentae, suggesting metabolic disturbances linked to inflammation. Targeted metabolomics of maternal serum across gestation revealed dysregulation of amino acids and glycerophospholipids.

Conclusion: In conclusion, our study underscores the role of dysregulated fatty acid oxidation in the pathophysiology of the sPTB and suggests that lipids, particularly phosphatidylcholines, and amino acids play a key role in PTB. These altered metabolites may serve as potential predictors of PTB, enabling earlier identification of at-risk pregnancies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2162 B — Human milk lipidomics: From deep annotation to high-throughput lipid quantitation

PRESENTING AUTHOR: ***Magdalena Vagnerova, University of Lausanne, Faculty of Biology and Medicine, Switzerland***
CO-AUTHORS: ***Hector Gallart-Ayala, Brittany Lee, Rahul Ravi Deshpande, Susan Bird, Philip Remes, Cristina Jacob, Julijana Ivanisevic***
SUB THEME: ***Pregnancy, maternal and neonatal health***

Human breast milk is an optimal source of nutrients and bioactive molecules for newborns, including lipids, which constitute 3-5% of the total content. Lipids not only provide most of the energy to the breastfed infant but also deliver bioactive species critical for early development. The lipid composition of human milk is highly diverse, encompassing glycerolipids, cholesteryl esters, gangliosides, glycerophospholipids, sterols, sphingolipids and free fatty acids. Each class comprises hundreds of individual lipid species, making their characterization and high-throughput quantitation challenging. In addition, discrimination between structurally closely related species, including isomers, is essential owing to their distinct biological functions.

We present an analytical strategy for deep annotation of the human milk lipidome using IQ-X Orbitrap Tribrid mass spectrometer and easily transferred to a Stellar MS for targeted, high through-put quantitative analysis. Breast milk extracts were probed using two complementary reversed phase methods to englobe the wide panel of complex lipids, and cholesterol derivatives (steroids and bile acids). Lipid species were characterized in full-scan mode at 120K MS resolution combined with HCD-CID-MS3 fragmentation workflow. Following the structural characterization, the m/z list was fed into the last-generation nominal mass instrument Stellar for a streamlined data acquisition using quadrupole and dual-cell linear ion trap, enabling full MS2 spectra, capturing multiple product ions per lipid species for highly specific quantification. The ability to operate in targeted CID MS3 mode allowed for the analysis of triacylglycerols with fully resolved fatty acid composition. Overall, we were able to quantify > 1000 lipid species, with high specificity and sensitivity, among which a high diversity of FAHFAs and ether-linked lipids. The developed workflow will be applied to a cohort of breastfeeding mothers to examine the individuality of human milk lipidome, the evolution of lipid composition throughout lactation and the correlation with mothers' blood lipidome and metabolic health markers.

2163 B — Integrative Metabolomics and TCR Repertoire Profiling Reveal Molecular Signatures in Childhood Asthma and Atopic Dermatitis

PRESENTING AUTHOR: ***Meng Han Chiang, Chang Gung Memorial Hospital at Linkou, Taiwan***
CO-AUTHORS: ***Chieh-Ni Kuo, Yu-Ying Yu, Chih-Yung Chiu***
SUB THEME: ***Respiratory disease***

Background:

Atopic dermatitis (AD) is a chronic inflammatory skin disorder with early onset, often preceding childhood asthma and other allergic diseases. Both AD and asthma involve exaggerated T-cell immune responses to environmental allergens. However, the contribution of the T-cell receptor (TCR) repertoires to allergen sensitization and distinct clinical phenotypes of AD and asthma in early childhood remains unclear.

Methods:

A total of 78 children were recruited, including 26 healthy controls (HC), 26 with asthma, and 26 with AD. Targeted metabolomics profiling was conducted using the AbsoluteIDQ Q500 kit, quantifying up to 630 metabolites across 26 biochemical classes. TCR repertoire profiling was performed using next-generation sequencing with unique molecular identifiers to assess TRAV and TRBV gene usages. Integrative analyses explored relationships among metabolites, TCR repertoires, allergen-specific IgE, and disease phenotypes, focusing on enriched metabolic pathways.

Results:

Children with asthma and AD exhibited significantly elevated total serum IgE and mite-specific IgE levels compared to HC ($P < 0.001$). Five aqueous and ten lipid metabolites were significantly associated with asthma, while six aqueous and fifteen lipid metabolites were linked to AD ($P < 0.05$). Arginine and indoleacetic acid (3-IAA) were differentially expressed between asthma and AD. Diacylglycerol (DG 16:0) was unique to asthma, while several lysophosphatidylcholines (lysoPCs) were specific to AD. Glutamate-associated amino acid and phospholipid biosynthesis pathways were strongly linked to allergic phenotypes ($P < 0.01$). DGs and lysoPCs were negatively associated with IgE levels. Mite-specific IgE-related TRAV13-1 and TRAV4, elevated in both conditions, were negatively associated with DGs ($P < 0.05$), while TRBV7-9, reduced in both diseases, was positively correlated with DGs ($P < 0.01$).

Conclusion:

Integrative metabolomics and TCR profiling uncover molecular mechanisms underlying childhood asthma and AD. DGs are key metabolic mediators linked to TCR repertoire and IgE responses, providing insights into allergy differentiation and potential therapeutic targets.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2164 B — LC-HRMS based non-targeted identification of bioactive metabolites produced by non-typeable H. influenzae

PRESENTING AUTHOR: ***Aleksandra Sarnowicz, Forschungszentrum Borstel, Germany***

SUB THEME: ***Respiratory disease***

Research question: Asthma is a common, heterogeneous disease resulting from chronic inflammation of the lower airways for which there is currently no cure. Its diagnosis relies primarily on patients' clinical symptoms, as non-invasive diagnostic approaches remain limited. Patients with asthma exhibit a unique metabolome that distinguishes them from healthy individuals. Purine metabolites are considered as potential diagnostic biomarkers for asthma, as they are highly elevated in patient serum, and may be distinct and involved in the pathogenesis. The pathogenic bacterium non-typeable Haemophilus influenzae (NTHi) is frequently detected in the lung microbiome of patients with severe asthma. Since NTHi has been shown to alter its metabolic profile as an adaptation to its niche, we hypothesize that it produces bioactive metabolites that contribute to disease pathogenesis and/or persistence. The discovery of these compounds could also be important in the search for new treatments as they provide novel therapeutic targets.

Objective: We perform LC-HRMS-based non-targeted metabolomics to identify bioactive compounds secreted by NTHi that will help to elucidate the physiology of the disease.

Methods: A clinical isolate, NTHi strain H-189 was cultured in a chemically defined medium. An n-butanol extract of the culture supernatant was purified by solid phase extraction, and subsequent fractions were measured by LC-HRMS and analyzed by MetaboScape.

Results and Discussion: We identified 62 metabolites exclusively produced by NTHi as being absent in the media control. Interestingly, we detected hypoxanthine, so far known as a biomarker of asthma. Since the activity of major NTHi metabolites including hypoxanthine is not known, we plan to elucidate its bioactivity in primary dendritic cells and primary epithelial cells and so to elucidate the role of hypoxanthine in the pathogenesis of asthma. In addition, the metabolic pathways of H-189 will be studied to dissect the influence of growth factors on the production of metabolites.

2165 B — Metabolomic signatures of two major respiratory pathogens in the reconstituted human airway epithelium model

PRESENTING AUTHOR: ***Charles Terra, TIMC, France***

CO-AUTHORS: ***Aurélien Gibeaud, Valérie Cunin, Elisabeth Errazuriz-Cerda, Caroline Plazy, Olivier Terrier, Audrey Le Gouellec***

SUB THEME: ***Respiratory disease***

Introduction

SARS-CoV-2 and influenza A viruses (IAV) are major pathogens for human respiratory infections. Several studies describe the impact of both viruses on host's metabolism (Ayres et al. 2020, Bahadoran et al. 2020, Rubayet Hasan et al. 2021). Nevertheless, none of those studies have exhaustively compared both viruses in a similar experimental set-up. Our study examines the impact of these viruses on the metabolism of human lung epithelium with an untargeted metabolomics approach.

Materials-and-methods

Reconstituted human airway epithelium (HAE) model was infected with SARS-CoV-2 (Wuhan strain) or IAV (H1N1 2009 pandemic strain) during 24 to 96 hours, and treated 48 hours after infections start or not with Pseudomonas aeruginosa flagellin. Infections were characterised by measuring TEER, inflammatory statement (Il-6), viral replication and transcriptomics profiles. Following cells collection, endometabolome was measured with an orbitrap LC-HRMS. Metabolomics data were processed with MZmine and MetaboAnalyst, then annotated with GNPS and Compound Discoverer in association with a homemade spectral database.

Results

Phenotype acquisition (TEER, Il-6, viral replication) and a K-means clusterisation method enabled us to define severity groups. LC-HRMS/MS analysis resulted on thousands of variables that were filtered (CV QC pool <30%, r2>0,7 for each variable). Non-supervised (PCA) or supervised (PLS-DA) multivariate statistical analysis leaded to the identification of a VIP list associated to SARS-CoV-2 and/or IAV.

Discussion

We have identified metabolic disruptions distinctive for each virus and associate to a VIP list. Disrupted metabolic pathways may lead to the identification of new biomarkers specific to each infection types or severity levels.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2166 B — A Clinical Chemical Atlas of Xenobiotic Toxicity for the Sprague-Dawley Rat

PRESENTING AUTHOR: ***Janonna Kadyrov, Australian National Phenome Centre, Australia***
SUB THEME: ***Toxicology***

Drug safety issues are a major cause of drug attrition. In silico screening tools have increasingly contributed to reducing drug induced toxicity by early elimination of products associated with toxicity or adverse reactions. The Consortium for Metabonomic Toxicology (COMET) studies were designed to model metabolic responses to organ- and mechanism-specific toxins to predict acute drug toxicity in rats. A range of clinical chemical parameters were measured in seven-day toxicology studies for 86 toxins eliciting a range of organ and mechanism specific effects. Additionally, 21 surgical or physiological stressors were evaluated to identify physiological or metabolic responses that might confound the interpretation of observed toxicity profiles. From these studies on a total of 3473 rats measured at six pharmaceutical companies, we provide a set of 12 serum and five urine physical and clinical chemistry parameters. Samples were collected at 24 h, 48 h and 168 h post-dose for each animal and are presented as a downloadable database file. We also summarise the main observations based on the group response at the individual toxin level. We demonstrate that correlations between parameters, such as serum bilirubin and aspartate aminotransferase (AST) provide a more nuanced profile of organ-specific toxicity than consideration of individual parameters. In addition, we highlight the variability in the measured parameters across the dataset attributable to inter-laboratory differences, and the heterogeneity of metabolic responses to particular compounds or differences in temporal patterns of response. This is one of the largest collections of rodent toxicology studies ever conducted under identical protocols across multiple laboratories, which we are now making publicly available to provide reference ranges for the various data modalities. Thus, we provide a clinical chemical atlas of toxicity which serves as a valuable reference tool for evaluating the potential toxicity of novel drug candidates based on standard laboratory parameters.

2167 B — Integrated analysis of behavior, pharmacokinetics, and metabolomics following co-administration of methamphetamine and ethanol in rats

PRESENTING AUTHOR: ***Mingyu Kim, Keimyung University, South Korea***
CO-AUTHORS: ***Sang-Hoon Song, Suji Kim, Ye Jin Jung, Sooyeun Lee***
SUB THEME: ***Toxicology***

Methamphetamine (MA) is a potent psychostimulant with a high potential for abuse and neurotoxicity, and its concurrent use with ethanol (EtOH) has recently been linked to significantly increased rates of hospitalization and mortality. In this study, we established rat models of MA and EtOH co-intoxication, using MA doses of 1, 4, and 10 mg/kg (i.p.) to induce dose-dependent behavioral responses, in combination with EtOH (2 g/kg, 30% v/v, i.p.). We investigated the pharmacokinetic and metabolic interactions between these substances. Pharmacokinetic analysis revealed that co-administration of MA and EtOH led to increased Cmax, t1/2, and AUC of MA, while decreasing Vz/F and CL/F. The plasma metabolite-to-parent drug AUC and urinary metabolic formation ratios indicated that EtOH decreased MA hydroxylation and enhanced its demethylation. Time-course metabolic profiling showed that the potentiation of stereotypic behaviors caused by EtOH co-exposure correlated with a significant increase in plasma spermidine and spermine levels, suggesting a temporal link between behavioral and metabolomic changes. At the time of peak behavioral abnormalities, extensive metabolic alterations were observed due to MA and EtOH co-administration. Notably, ROC curve and network analyses identified two polyamines (spermidine and spermine) and two bile acids (glycocholic acid and taurocholic acid) as key metabolites associated with co-exposure, suggesting their potential roles in the enhanced toxicity and neurotoxic effects of MA-EtOH intoxication. These findings highlight the critical pharmacokinetic and metabolic interactions between MA and EtOH and provide insights into the toxicological mechanisms underlying their synergistic neurotoxic effects.

2168 B — Identification of Etodolac Metabolite-Protein Adducts using LC-MS/MS

PRESENTING AUTHOR: ***Eui-Hyeon Kim, Kyungpook National University, South Korea***
CO-AUTHORS: ***Hye Suk Lee, Taeho Lee, Kyung-Sik Song, Kwang-Hyeon Liu***
SUB THEME: ***Toxicology***

The occurrence of drug-induced liver injury (DILI) is associated with the covalent binding of drugs or their metabolites to proteins. When drugs are metabolized to electrophilic, reactive metabolites, they bind to nucleophilic proteins and cause problems. Cases of liver damage caused by etodolac, one of the non-steroidal anti-inflammatory drugs that selectively inhibits cyclooxygenase-2 (COX-2), have been reported. In a previous study, we detected three types of etodolac reactive metabolites in in vitro human liver microsomal incubations by GSH trapping using liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS). Based on these results, we performed reactive metabolite-standard peptide and target protein adduct studies. Among the three, two reactive metabolite adducts were detected and formed covalent bonds with cysteine, which is a strong nucleophilic site within proteins. The generation and characterization of etodolac-protein adducts may help in understanding drug-induced liver injury and serve as a potential tool to assess the risk of covalent binding of drugs to proteins.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

2169 B — Biocompatibility by Design: A Multilayered Assessment of Neural Interface Materials via IN3-BIO

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CO-AUTHORS: ***Theodora Katsila, Nafsika Rossopoulou, Bianca Mihaela Boga, Ruxandra Costea, Octavian Ionescu, Carmen Moldovan, Eduard Franti, Marian Ion, Silviu Dinulescu, David Catalin Dragomir, Carmen Mihailescu, Monica Dascalu, Ioan Lascar, Ana Maria Oproiu, Walid Bahaa-Eddin, Adrian Barbilian, Cristian Stoica, Cecilia Ioana Tihuan, Teodora Vladescu, Bogdan Socea***

SUB THEME: ***Toxicology***

The safe and effective deployment of neural interfaces remains constrained by the complex biological responses elicited by implantable biomaterials. In this study, we introduce IN3-BIO, a comprehensive and scalable platform for evaluating material biocompatibility through harmonized in silico, in vitro, and in vivo pipelines.

Through AI-driven data extraction and semantic analytics, our in silico module identified inconsistencies, redundancies, and underexplored niches in existing biomaterial data. These insights directly informed our experimental hypotheses. We then conducted in vitro validation using ISO-10993-compliant protocols, including the OECD 487 micronucleus assay, confirming the low cytotoxic and genotoxic profile of candidate materials. Our in vivo investigations in Ovis aries integrated untargeted and targeted LC-MS-based metabolomics with histological and immunohistochemical tissue analysis. This revealed distinct temporal metabolic signatures, particularly within steroid biosynthesis and inflammatory lipid pathways, correlating with localized immune modulation and minimal fibrosis.

Collectively, the IN3-BIO strategy bridges predictive modeling with physiological relevance, supporting the safe translation of novel biomaterials toward neural interface systems. Our framework holds broad applicability across the neurotechnological pipeline, from early-stage biomaterial screening to preclinical validation in regenerative and restorative therapies.

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2170 B — Metabolic changes in human plasma samples after occupational exposure to diisocyanates

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CO-AUTHORS: ***Tiina Santonen, Jelle Verdonck, Katrien Poels, Lode Godderis, Simo Porras, Marko Lehtonen, Jaana Rysä, Olli Kärkkäinen***

SUB THEME: ***Toxicology***

Background: Diisocyanates are highly reactive chemicals, containing two isocyanate functional groups (R-N=C=O). In the European Union (EU), the total used amount of diisocyanates is 2.5 million tons per year. Additionally, diisocyanates are the main cause of occupational asthma in the EU and they are skin sensitizers and irritants. The aim of this study is to assess metabolic changes in human plasma samples after occupational exposure to diisocyanates. Another goal is to identify new effect biomarkers, which are relevant for the diisocyanate adverse outcome pathways. Methods: This study has used samples and data provided by previous carried out project of HBM4EU. The study population was recruited from Finland and Belgium, including 81 diisocyanate (4,4'-Methylenediphenyl diisocyanate or hexamethylene diisocyanate) exposed workers and 40 non-exposed controls. To assess metabolic changes in plasma samples, untargeted metabolomics pipeline was used. Samples were analyzed with two distinct chromatographic retention mechanisms in both positive and negative ionization, measured with a high-resolution mass spectrometer. Data analysis was done using MS-Dial and Notame R-package. Results: There were differences in metabolic profiles between exposed workers and controls. Additionally, variations were observed in metabolic profiles between individuals from Finland and Belgium. After adjusting for potential confounders (BMI, age, smoking, alcohol consumption, and country) changes were detected in the levels of metabolites belonging to fatty acids and conjugates, fatty acid esters, fatty amides, glycerophosphocholines, tetracarboxylic acids and derivatives, benzoic acids and derivatives, and pyridinecarboxylic acids and derivatives. Especially 5-aminovaleric acid betaine, tetradecenoylcarnitine, lysophosphocholine 18:1 and N1-Methyl-4-pyridone-3-carboxamide levels were altered between exposed workers and controls. Conclusions: The study is the first to assess metabolic changes in human plasma samples after occupational exposure to diisocyanates. Exposure to diisocyanates might have some effect on fatty acid metabolism but more research is needed. The results of this study may be utilized in search for effect biomarkers.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2183 B — Effects of Pyrethroids and Their Metabolites on Neural Conduction

PRESENTING AUTHOR: *Ching-kuo Lee, Taipei Medical University, Taiwan*
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SUB THEME: *Environmental exposures*

Pyrethroid pesticides are widely used in daily life for repelling and killing insects due to their relatively low toxicity to mammals, including humans. These compounds can be degraded by light, microorganisms, acids, and alkalis into various metabolites, such as cyclopropane and aromatic derivatives.

Enzymes play vital roles in physiological processes. For instance, acetylcholinesterase (AChE) hydrolyzes acetylcholine (Ach), thus regulating nerve signal transmission, while α-glucosidase influences glucose absorption in the intestine, which is closely linked to hyperglycemia.

The goal of our research is to investigate the effects of pyrethroids and their metabolites on key enzymes, including AChE and α-glucosidase, in order to better understand their potential impact on biological systems. In addition, we aim to explore the downstream effects of these interactions at the cellular level. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) data confirm the structures of the pyrethroids and their metabolites. Our preliminary results indicate that structural differences among these compounds result in varying levels of activity on a nervous system enzyme screening platform.

2184 B — Metabolic Fate and Implications of Polylactic Acid Nanoparticles in Human Intestinal Cells: A Preliminary Investigation

PRESENTING AUTHOR: *Martina Lombardi, Theoreo SRL - Univ. of Salerno, Italy*
CO-AUTHORS: *Raffaella D'Auria, Marianna Marino, Vera Abenante, Andrea Ingenito, Alessio Trotta, Jacopo Troisi, Antonietta Santoro*
SUB THEME: *Environmental exposures*

In recent years, bioplastics have emerged as a sustainable alternative to petroleum-based plastics, with polylactic acid (PLA) being the most widely used, particularly in food-contact applications. However, like traditional plastics, PLA fragments into micro- and nanoplastics (M/NPs), raising concerns about their potential impact on human health. As the gastrointestinal tract is the primary entry point for these particles, the intestinal epithelium becomes a key target for adverse effects. A thorough evaluation of PLA-induced cellular alterations is therefore essential.

This preliminary study explores the metabolomic perturbations triggered by exposure to PLA-NPs in two human colon adenocarcinoma cell lines, specifically Caco-2 and HT29. Cells were cultured at a density of 1×10^6 cells/mL and exposed to PLA-NPs at a concentration of 100 µg/mL. Metabolic effects were assessed at 24, 48, and 72 hours post-exposure in both cellular pellets and supernatants. Metabolic activity was arrested via cold methanol quenching. Following extraction, purification, and derivatization using the MetaboPrep-GC kit (Theoreo srl), metabolomic profiling was performed via Gas Chromatography-Mass Spectrometry (GC-MS). Partial Least Square Discriminant Analysis (PLS-DA) was used to reveal class separation. Additionally, by culturing cells with a fully ^{13}C-labeled glucose medium, GC-MS investigations were also used to perform a metabolic flux analysis enabling discrimination between endogenous metabolic fluxes and PLA-derived carbon contributions.

Untargeted metabolomics revealed extensive metabolic remodeling in response to PLA-NPs exposure, affecting carbohydrate metabolism, oxidative stress response, amino acid biosynthesis, energy homeostasis, and nitrogen balance. Furthermore, fluxomics analysis demonstrated the intracellular conversion of PLA into lactic acid at 24 hours, followed by its extracellular efflux, as evidenced by the progressive increase in unlabeled lactate at 48 and 72 hours.

Taken together, these findings suggest that exposure to PLA-NPs elicits profound metabolic alterations in intestinal epithelial cells, highlighting the necessity of further investigations to elucidate the biological implications of PLA-derived NPs.

2185 B — Evaluation of the nutritional profile and functional properties of quinoa and chestnut fermented beverages by 1H NMR

PRESENTING AUTHOR: *Federico Brigante, Institute Of Organic Chemistry And Biochemistry, Czech Republic*
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SUB THEME: *Fermented foods*

Plant-based beverages (PBBs) provide essential nutrients and functional benefits, fermentation enhances their antioxidant properties and changes their nutritional profile. NMR provides a fast, reproducible, non-destructive method to identify organic compounds. This study aimed to leverage the advantages of NMR to evaluate the functional properties of quinoa and chestnut fermented PBBs (CFPPB and QFPBB). PBBs were formulated with rice flour (RF) (2% w/v) and black carrot (BC) (1% w/v) as a base. QPBB contained 4% w/v quinoa flour (QF), while CPBB included 4% w/v chestnut flour (CF) and 3% w/v dried persimmon powder (PFi). After pasteurization, homogenization, inoculation, and fermentation (at 37°C for 48 h), they were stored at 4°C for 5 days. For ^1H NMR (600 MHz), samples were centrifuged, 500 µL of supernatant were mixed with 100 µL of D_2O buffer. Solid ingredients were dissolved in 900 µL H_2O + 100 µL D_2O buffer, centrifuged, and 600 µL of supernatant were analyzed. 48 compounds were identified, other signals were diagnostic of fatty acids, aldehydes, anthocyanins, oligosaccharides, and purines. Fermentation increased lactate, acetoin, and ethanol levels and decreased sugar levels as used by LAB. QFPBB was higher in fatty acids. Venn diagram analysis revealed that BC contained the most compounds (74), contributing with polyphenols (anthocyanins) and aldehydes. QF uniquely provided betaine and purines, while PFi contributed mostly fructose, fiber, and polyphenols. CF mainly added amino acids and organic acids, with fewer unique compounds than BC or PFi. RF had the smallest compound diversity, primarily providing carbohydrates for energy. The use of ^1H NMR was encouraged as a rapid and effective tool for assessing the nutritional and functional properties of FPBBs. QF, BC, and PFi were key sources of bioactive compounds. FPBBs showed both a richer nutritional profile and higher polyphenol and purines content due to fermentation and individual ingredient contribution.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2186 B — Study on change in the composition of Sake (Japanese rice wine) volatile component due to drinking temperatures

PRESENTING AUTHOR: *Eiichiro Fukusaki, Osaka University, Japan*
CO-AUTHORS: *Kyoka Maehara, Masahiro Furuno*
SUB THEME: *Fermented foods*

Sake (Japanese rice wine) can be enjoyed at a wide range of temperatures, which significantly affects its aroma and sensory characteristics. While previous studies have explored the relationship between drinking temperature and taste, there has been limited research on how temperature influences sake's aroma, particularly its volatile components. This study aimed to fill that gap by investigating the temperature-dependent changes in both retronasal and orthonasal aromas using metabolomic analysis via gas chromatography–mass spectrometry (GC/MS).

Retronasal aroma was analyzed by performing non-targeted GC/MS-based metabolomics on exhaled breath samples after sake consumption at four temperatures (15°C, 25°C, 40°C, and 50°C). A total of 95 volatile components were identified. Principal component analysis (PCA) showed that volatile profiles grouped into two clusters: one for 15°C to 40°C and another for 50°C, indicating a significant change in retronasal aroma at higher temperatures. Orthogonal partial least squares (OPLS) regression further confirmed a strong correlation between temperature and volatile composition.

For orthonasal aroma, headspace (HS) GC/MS was used to analyze key aroma compounds—3-Methylbutyl acetate, Ethyl hexanoate, Isoamyl alcohol, and 2-Phenylethyl alcohol. In contrast to the retronasal analysis, the HS method revealed significant changes in volatile component intensity with temperature. This suggests that orthonasal aroma is more sensitive to temperature changes than retronasal aroma.

This study is the first to use metabolomics analysis to investigate temperature-induced changes in retronasal aroma of sake using exhaled breath and provides new insights into the complex aroma dynamics of sake at varying temperatures.

2187 B — What happens to phytochemicals throughout fruit and vegetable processing and gastrointestinal digestion?

PRESENTING AUTHOR: *Millena Barros Santos, INRAE Avignon, France*
CO-AUTHORS: *Christian Ginies, Beatrice Gleize, Carine Le Bourvellec, Claire Dufour*
SUB THEME: *Foodomics and food security*

Fruits and vegetables are nutritionally valuable yet chemically complex matrices. Predominantly consumed in processed forms, they contribute not only to food security but also support territorial development, production seasonality, and, most importantly, the food transition and climate change mitigation. However, current studies often focus on characterising these matrices either in their raw state or as final products, overlooking the modifications occurring during processing and their fate throughout gastrointestinal digestion. This gap is particularly critical for underexplored microconstituents, despite the fact that many phytochemicals exhibit significant biological activities (e.g., anti-inflammatory, antioxidant, antitumoral). In this context, it is crucial to understand how agricultural practices, processing methods, and gastrointestinal digestion, including conservation, affect phytochemical integrity and profiles. Our project will apply a system-based approach to investigate the full continuum production-processing-consumption-digestion with a focus on (i) the impact on plant food nutritional quality, (ii) the stability of health-relevant compounds, (iii) interactions between micro- and macroconstituents, and (iv) the discovery of newly-formed molecules. To achieve these objectives, we propose combining multiple methods and analytical platforms to profile both hydrophilic and lipophilic phytochemicals simultaneously. A deep insight will be provided through untargeted metabolomics approaches. Untargeted profiling will be performed using UHPLC-HRMS (Orbitrap Exploris 240, Thermo Scientific), employing both ESI and APCI ionisation sources in positive and negative modes. Data processing and interpretation will involve tools such as MSDIAL, mzmine, SIRIUS, MetaboAnalyst, and GNPS, following best practices outlined by mQAQC and MSI guidelines. Combining various methods and platforms is essential to maximise metabolite coverage given the matrix complexity, molecular heterogeneity, and interactions between phytoconstituents. This system-based approach will allow us to understand the mechanisms of chemical and physical destructuring/reorganisation and to capture the dynamic evolution of the metabolite pools during processing and gastrointestinal digestion. Ultimately, this work will provide new insights into food transformation and nutrient bioaccessibility.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2188 B — Metabolomics-Based Characterization of Organic vs. Conventional Pineapples: Cultivar-Specific Responses and Quality Traits

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SUB THEME: *Foodomics and food security*

In Ishigaki Island, Okinawa, farmers have adopted compost and awamori distillation residue (moromi) as alternatives to synthetic fertilizers in pineapple cultivation. This study investigates whether organic farming alters pineapple quality at the metabolic and sensory levels, particularly in relation to cultivar differences. We compared organically and conventionally grown pineapple (Ananas comosus) Smooth Cayenne sub-cultivar—Hawaii, and two Queen sub-cultivars—Bogor and Soft Touch in Okinawa, alongside commercial Golden Pineapple Taiwan and Philippines SweetiO cultivars. Ten sensory panels evaluated pineapple attributes. Metabolic profiling was subsequently conducted using LC/MS, CE/MS, and GC/MS.

Results indicated that sensory and metabolic variations were generally more influenced by cultivar than by farming method. However, sub-cultivar-specific responses to organic farming were evident. Hawaii organic cultivation significantly reduced tingling sensation. Similarly, Bogor organic has a distinct lower tingling sensation and fermented off-flavors. Proteolytic activity assays revealed that Bogor organic and Hawaii organic had the lowest percentage of protein degradation at pH 4. Consistent with the sensory data, the low proteolytic activity of bromelain likely contributed to the reduced tingling sensations. Metabolomics data demonstrated lower levels of off-flavor carboxylic acids and stronger signals of monoterpenoids and short-chain alcohols, contributing to improved flavor quality. Likewise, organic Soft Touch exhibited enhanced pineapple aroma and a more balanced flavor profile. Its metabolite profile also showed a cultivar-specific response, with higher accumulation of esters compared to conventional Soft Touch.

Notably, Okinawa grown Bogor and Soft Touch cultivars showed distinct accumulation of peptides which were not present in commercial samples, suggesting a distinguishing trait. Our findings suggest that organic farming can selectively enhance desirable sensory traits depending on the cultivar.

In the future, we need to further confirm the reproducibility of the data by analyzing pineapples from different harvest periods to validate these cultivar-specific responses and support future applications in quality improvement.

2189 B — Comprehensive assessment of the quality and safety of carrot crisps prepared by alternative frying procedures

PRESENTING AUTHOR: *Tomáš Kouřimský, UCT Prague, Czechia*
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SUB THEME: *Foodomics and food security*

Vegetable crisps are sought as a ´healthier´ alternative to potato crisps, due to their higher fibre content, vitamins and lower calories. However, under conditions of conventional frying, extensive undesirable heat-induced changes occur. In addition to the degradation of bioactive compounds, processing contaminants are formed. In the case of carrots, a vegetable with a relatively high content of reducing sugars, the formation of acrylamide (AA) may occur. Levels of AA as high as 3,000 µg kg-1 have been reported in some studies. To prevent processing contaminants formation and preserve nutritional benefits, ´mild´ frying procedures enabling the production of ´healthy´ vegetable crisps represent an interesting option.

This study aimed to assess the quality and safety of fried carrot crisps obtained by vacuum frying (oil bath 120 °C) and hot air frying (130 °C) and to compare the outcomes with products obtained by conventional deep fat frying (165 °C). Slices of 7 carrot cultivars were fried to 5 different time endpoints by each technology. Besides AA content determination, ultra-high performance liquid chromatography (UHPLC) coupled with high-resolution tandem mass spectrometry (HRMS/MS) was employed for targeted screening of bioactive metabolites to characterize the impact of tested frying technologies on their profile. Multivariate statistical approaches and random forest machine learning algorithms were utilized for significant feature selection.

Effective mitigation of AA was achieved by optimised vacuum frying, AA concentration did not exceed 600 µg kg-1, while in conventionally fried carrot crisps, AA concentration was up to 20 times higher. Fitted models were able to discriminate individual carrot cultivars, as well as frying technologies sample groups. It was also possible to discuss changes in selected features, identified as phenolic acids, phenolic glucosides, carotenoids, sterols, and other lipid species, etc., during these processes.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2190 B — Sensomic profiling of coffee leaf infuses: unlocking the potential of a novel food

PRESENTING AUTHOR: *Davide Rovelli, Univeristy Of Parma, Italy*
CO-AUTHORS: *Francesca Trapani, Bianca Serito, Chiara Dall'Asta*
SUB THEME: *Foodomics and food security*

In recent years, the reintroduction of coffee by-products into the supply chain has proven to offer economic benefits for coffee producers, while also enhancing sustainability. In this context, coffee leaves represent an interesting resource, giving their use in folk medicine to prepare tea-like beverages since the 19th century. The recent approval of coffee-leaf infusion as a novel food in the European Union has opened the door to their commercialization. Although a substantial body of research exists on coffee beverages, comprehensive chemical and sensory profiling of this novel product is still limited.

This study aimed to investigate the volatile and sensory profiles of cold- and hot-brewed infusions prepared from six Coffea arabica varieties collected in three different countries using a sensomic approach. A non-targeted volatile analysis was carried out by headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS), followed by multivariate analysis to identify the main molecular markers that differentiate coffee-leaf infusions. In total, 108 volatile compounds were identified and their corresponding aromatic notes provided. These data, combined with descriptive sensory analysis conducted by a trained panel, revealed correlations and patterns that elucidate the positive and negative notes of this innovative traditional food.

The resulting molecular–sensory map provides quality markers for industry and a basis for tailoring preparation protocols as coffee leaves enter the European beverage market, advancing food sensomics and the sustainable valorization of coffee by-products.

2191 B — Changes in the Metabolome of the Yellow Mealworm (T. molitor) During Different Types of Technological Processing

PRESENTING AUTHOR: *Ondřej Pospíšil, University of Chemistry and Technology, Prague, Czechia*
CO-AUTHORS: *Kateřina Šebelová, František Beneš, Anežka Kopecká, Martin Kulma, Lenka Kouřimská, Jana Hajšlová*
SUB THEME: *Foodomics and food security*

Good farming practice and suitable feed composition are undeniably important factors that influence the quality of the produced edible insects. However, the quality can also be affected by the killing method. This is often underestimated as a possible variable. In the current study, the impact on a nutritional quality of the edible insects has been demonstrated. Two alternative killing approaches were compared: (i) 20 min in boiling water and (ii) freezing to -18 °C) on the spectrum of metabolites in the final product. Lyophilised samples, including insect broth obtained by boiling insects, were examined by untargeted screening (fingerprinting) using ultra-high performance liquid chromatography coupled with high-resolution tandem mass spectrometry (U-HPLC-HRMS/MS). Advanced statistical methods, including multivariate statistical analysis (PCA, PLS-DA), were included for data evaluation. The results showed that insect samples killed by freezing contained a higher representation of polar metabolites than those killed by boiling. The reason might be the transfer of some of these compounds, such as short peptides or phenolics to the broth. The loss of polar compounds led to an increase in the relative representation of triacylglycerols and other lipophilic compounds in the boiled insects. The results of the study may contribute to optimising the processing of edible insects for food purposes.

2192 B — Towards Mass Spectrometry Guided Characterisation of Marine Fucose Containing Sulfated Polysaccharides Driving Oceanic Carbon Sequestration

PRESENTING AUTHOR: *Christopher Fitzgerald, MARUM, Germany*
CO-AUTHORS: *Andreas Sichert, Kevin Becker, Jan-Hendrik Hehemann*
SUB THEME: *Marine metabolomics*

Marine algae fix ~50 gigatons of inorganic CO_2 annually via photosynthesis, with a significant fraction rapidly recycled by heterotrophs. A substantial portion of this fixed organic carbon is stored as glycans- some algae allocate up to 80% of their carbon to glycan synthesis. While most is remineralised, a fraction escapes degradation and is exported to depth, contributing to long-term carbon sequestration. Brown algae alone fix ~1 gigaton of carbon annually, with up to 15% potentially sequestered as nutrient-depleted, recalcitrant polysaccharides, equivalent to 0.55 gigatons CO_2, just below Germany's 2024 emissions.

A key mechanism in this process is the release of complex fucose-containing sulfated polysaccharides (FCSP), comprising up to one-third of the exuded carbon in marine algae. Due to their structural complexity and highly sulfated backbone, FCSPs resist microbial degradation and strongly contribute to the biological carbon pump, sinking to depths >1000 m where carbon may be stored for millennia.

Despite their importance, the molecular composition, fate, and turnover of FCSP remain poorly characterised due to analytical limitations. Here, we present a targeted LC-MS framework for environmental glycan analysis, including selective isolation from seawater, controlled hydrolysis, and quantification at the monomer level. Future development aims to extend this approach to oligomeric profiling using tandem MS and established bioinformatic tools optimised for sulfated polysaccharides.

This preliminary workflow enables high-throughput compound-level insights into marine glycan diversity, providing a path to resolve the contribution of specific polysaccharides like FCSP in carbon sequestration. While still in development, our approach aims to bridge metabolomics and marine biogeochemistry, opening new avenues for characterising blue carbon pathways relevant to climate mitigation.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2193 B — Pathogenic microbes and abiotic factors shape the metabolic landscape of bloom-forming algae

PRESENTING AUTHOR: ***Marine Vallet, Max Planck Institute For Chemical Ecology, Germany***

CO-AUTHORS: ***Ruchicka O'Niel, Marine Vallet***

SUB THEME: ***Marine metabolomics***

The algal holobiont—comprising photosynthetic hosts and their associated microbial communities—exhibits dynamic adaptations to environmental fluctuations, including the production of bioactive compounds. Understanding how abiotic factors, such as salinity, and biotic interactions, particularly microbial symbioses, shape algal physiology is critical for predicting shifts in community composition and primary production in aquatic ecosystems. This study examines the impact of two opportunistic pathogenic microbes—the bacterium Kordia algicida and the alveolate eukaryote Parvilucifera infectans—on the bloom-forming toxic dinoflagellate Alexandrium minutum. We further explore how salinity and temperature modulate these microbe-host interactions, influencing the parasite virulence and consequently harmful algal blooms. Our findings reveal that both pathogens compromise algal growth, disrupt metabolic profiles, and alter the composition of exudates. Through UHPLC-HRMS-based comparative metabolomics, we identified key metabolic shifts, including changes in zwitterions, saxitoxins, ectoines, and lipid metabolism-associated compounds such as carnitine and eicosapentaenoic acid. These results underscore how microbial interactions and environmental-driven metabolic reprogramming shape the algal holobiont and influence marine primary productivity in aquatic ecosystems.

2194 B — New bacterial DMSP utilization pathways in terrestial environments

PRESENTING AUTHOR: ***Yasmeen Yousfi, Cea, France***

CO-AUTHORS: ***Emilie Pateau, Nadia Perchat, Aline Mariage, Peggy Sirvain, Christophe Lechaplais, Veronique De Berardinis, Ekaterina Dariy, Alain Perret***

SUB THEME: ***Microbial metabolomics***

Acinetobacter baylyi ADP1 (ADP1) is an aerobic soil bacterium capable of metabolizing a wide range of sulfur-containing compounds, including dimethylsulfoniopropionate (DMSP). DMSP is a precursor of dimethylsulfide (DMS), a climatically active gas involved in cloud nucleation. While DMSP metabolism is well-characterized in marine microorganisms, with two primary pathways identified—demethylation, which generates 3-methylthiopropionate (MTPA), and cleavage, producing acrylate and DMS—ADP1 lacks the genes encoding the enzymes responsible for these canonical pathways. This suggests the existence of novel metabolic mechanisms for DMSP.

This project aims to identify the genes, metabolites, and enzymatic reactions involved in DMSP catabolism in ADP1. Transcriptomic analysis under sulfate starvation revealed the induction of over 100 sulfate-starvation-induced (SSI) genes, including a striking abundance of flavin-dependent monooxygenases and dioxygenases, suggesting oxidative metabolic reprogramming. Leveraging genomic resources, including a library of deletion mutants and a collection of ADP1 genes cloned for rapid protein expression in E. coli, we combined this transcriptomic analysis with mass spectrometry-based metabolomics to explore ADP1 sulfur metabolism.

Our findings propose two novel oxidative DMSP catabolic pathways in ADP1. We identified previously undescribed metabolites in a unique demethylation pathway and uncovered a new cleavage pathway involving dimethylsulfoxonium propionate (DMSOP), a compound so far observed in marine ecosystems. Previous studies conducted in marine environments have shown that DMSOP may play roles in oxidative stress protection and carbon-sulfur cycling. Notably, these metabolites were also detected in Pseudomonas putida KT2440, suggesting that the proposed pathways are widespread among terrestrial bacteria. Enzymatic screening of candidate monooxygenases demonstrated functional redundancy, with multiple enzymes acting on shared substrates, while deletion mutants confirmed their in vivo roles. This study provides new insights into the mechanisms by which bacteria adjust their metabolism in response to available sulfur sources, and opens new avenues for exploring the ecological roles of microbial sulfur metabolism in terrestrial environments.

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★ AWARD WINNERS

PLANTS, FOOD, ENVIRONMENT AND MICROBES

2195 B — Metabolomic profiling of marine bacteria mono- and co-cultures reveals natural product dysregulation and tailoring

PRESENTING AUTHOR: ★ *Monica Monge Loria, Georgia Institute of Technology, United States*
CO-AUTHORS: *Neha Garg*
SUB THEME: *Microbial metabolomics*

Coral reefs are hotspots for marine biodiversity, which translates into the synthesis of a wide variety of natural products that are useful for inter- and intraspecies communication, nutrient acquisition, and defense. Among these natural products, siderophores are iron chelators secreted by bacteria to circumvent low iron bioavailability in diverse environments. Siderophore secretion is further leveraged by pathogenic bacteria in order to induce iron starvation in other members of the bacterial community, and as such serve an additional role as virulence factors in multiple species. Comparative untargeted metabolomics between marine bacteria mono- and cocultures has revealed the enzymatic degradation of the siderophore amphibactin, produced by the coral pathogen Vibrio coralliilyticus, by commensal Microbulbifer spp. strains. A reduction in iron affinity is expected, as one iron-chelating moiety is lost upon degradation. This highlights the complex bacterial community dynamics at play surrounding iron acquisition in marine environments. Homology analysis of genomic data, along with untargeted metabolomics, has aided in the identification of two siderophore classes produced by Microbulbifer sp. and their dysregulation upon coculture with the Vibrio pathogen. Tandem mass spectrometry fragmentation patterns and nuclear magnetic resonance (NMR) have allowed for the elucidation, to our knowledge, of the first siderophore system in Microbulbifer. Apart from natural product tailoring, metabolite induction and suppression were observed in coculture for diverse classes of compounds, including signaling N-acyl amides and antimicrobials. Altogether, these observations evidence the microbial cross-talk between pathogenic and commensal bacteria in mixed cocultures. Moreover, the siderophore degradation observed herein can help us identify modes of virulence factor disruption that can be exploited to lower the pathogenic burden in coral reefs.

2196 B — Unveiling Bacterial Metabolic Interactions Using a Species-Specific Compartmentalized Co-Culture System

PRESENTING AUTHOR: *Beatrice Engelmann, Helmholtz Centre For Environmental Research, Germany*
CO-AUTHORS: *Zheng Chen, Nico Jehmlich, Ulrike Rolle-Kampczyk, Martin von Bergen*
SUB THEME: *Microbial metabolomics*

The gut microbiota plays a critical role in human health by modulating key physiological processes. Understanding species-specific metabolic interactions within this complex community remains a major challenge due to the vast diversity of microbial taxa. To address this, we employed a novel co-cultivation approach which utilizes an H-cell system that physically separates single species from a defined microbial consortium of 8 species (called SIHUMIx) via a permeable membrane. The chosen pore size permits metabolite diffusion while preventing bacterial crossover, allowing for analysis of metabolic interactions in a spatially controlled environment.

As a proof of concept, we examined interactions between Eggerthella lenta and the SIHUMIx community. Our data confirm that E. lenta consistently utilizes arginine for energy, independent of the consortium's presence. However, co-cultivation resulted in an altered community structure, with an increase in Blautia producta at the expense of Bacteroides thetaiotaomicron. Metabolically, E. lenta started acetate and butyrate production earlier in co-culture, although butyrate levels ultimately decreased. Additionally, we observed a co-culture specific decrease in citrulline levels in the E. lenta compartment, indicating that citrulline produced by E. lenta diffused across the membrane and was consumed by SIHUMIx members. Follow-up analyses identified Lactiplantibacillus plantarum as a main consumer of citrulline and ornithine, without producing either compound. In single cultures, the presence of these metabolites promoted growth of L. plantarum in a concentration dependent manner.

These findings demonstrate that the H-cell system enables precise investigation of species-specific metabolic dynamics within a community context. The method provides a scalable platform for dissecting microbial interactions in microbiota consortia of varying complexity, opening new avenues for targeted microbiome research.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2197 B — Advancing Gut Microbiome Metabolomics: A Derivatization-Based Approach

PRESENTING AUTHOR: ***Anna Michl, Institute of Microbiology AS CR, Czech Republic***
CO-AUTHORS: ***Tommaso Stefani, Ondrej Hrebicek, Martin Schwarzer, Zdenek Kamenik***
SUB THEME: ***Microbial metabolomics***

Microbiota-associated metabolites play a crucial role in host physiology and disease by mediating host-microbiota communication, providing essential nutrients, and regulating metabolism and immune functions. Understanding these interactions requires comprehensive chemical analysis of the metabolites. However, their low abundance, diverse physicochemical properties, and analysis in complex matrices present significant challenges, leaving us with no universal method to analyze them all. Chemical derivatization is a powerful approach particularly for polar metabolites. By modifying the analyte structure, it decreases their polarity and makes them compatible with conventional reverse-phase liquid chromatography.

3-nitrophenylhydrazine (3NPH) is a widely used derivatization agent, which was previously employed in studies targeting well-known microbial metabolites such as short chain fatty acids (SCFA) (1) and other primary metabolites (2). However, the potential of its applicability can be extended and go beyond to larger datasets, covering the majority of polar primary metabolites as well as known products of microbial metabolism.

Here, we present a large-scale analysis of nearly 600 gut microbiome-related chemical standards, derivatized using 3NPH. Standards were systematically classified based on structural similarities, biological relevance, and metabolic pathway associations using a Python-based automated categorization pipeline and public metabolomic databases. For streamlined identification, the recently introduced in-silico Derivatization Tool in MetaboScape by Bruker was employed for automated targeted search.

To validate the applicability of this derivatization strategy, we utilized the library of derivatized standards for the targeted analysis of microbiota associated metabolites in stool samples from mice with different statuses of microbial colonization, including germ-free models. Our results demonstrate the utility of this approach in providing valuable insights into host-microbiota metabolic interactions.

(1) Han J, Lin K, Sequeira C, Borchers CH. Anal Chim Acta. 2015, 854:86-94

(2) Meng X, Pang H, Sun Fei et al. Anal. Chem. 2021, 93, 29, 10075–10083

2198 B — Metabolomics-Based Classification of Food-Derived Yeasts and Its Relationship with Genetic Traits

PRESENTING AUTHOR: ***Nho-Eul Song, Korea Food Research Institute, South Korea***
CO-AUTHORS: ***Sunhee Kang, Jeonghyun Yun, Jang-Eun Lee***
SUB THEME: ***Microbial metabolomics***

The aim of this study is to propose a novel strategy for characterizing yeast strains by evaluating metabolomics profiles derived from yeast in comparison with traditional phylogenetic classification. Metabolite-based classification of 50 yeast strains (23 genus and 26 species) was performed using LC-QTOF/MS, high resolution-LC-Orbitrap-MS/MS and GC-MS/MS, followed by multivariate statistical analysis. Analysis of intracellular amino acid metabolites revealed that greatest variation by strain are glutamic acid, arginine, aspartic acid, and lysine, which can be used as chemical classification indicator components to determine differences between strains. Unsupervised multivariate analysis of intracellular untargeted metabolites in yeast cells showed that most strains had similar profiles across species, which was also similar to the phylogenetic tree of 50-strains obtained through 18s rRNA analysis, but some strains showed different patterns. Therefore, isolates were grouped according to their intracellular metabolites using hierarchical clustering analyses. Principal Component Analysis (PCA) indicated significant differences in the volatile secondary metabolites productivity of 50 yeast strains at the species level based on GC-MS/MS analysis. This result suggests that aromatic components of yeast are mainly strain-dependent rather than the genetic characteristics, and are more affected by the metabolic pathways. This study demonstrated that metabolite-based classification combined with genetic classification is an effective tool for distinguishing yeast strains and determining species-specific metabolites.

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★ AWARD WINNERS

PLANTS, FOOD, ENVIRONMENT AND MICROBES

2199 B — Adapting to anaerobic stress: the critical role of UspK in survival and metabolic reprogramming during pyruvate fermentation in Pseudomonas aeruginosa

PRESENTING AUTHOR: *Svea Holland, Leibniz Institute DSMZ GmbH, Germany*

CO-AUTHORS: *Ayten Mustafayeva, Alejandro Arce-Rodriguez, Gabriella Molinari, Kea Mucha, Dieter Jahn, José Borrero-de Acuña, Meina Neumann-Schaal*

SUB THEME: *Microbial metabolomics*

Pseudomonas aeruginosa is a versatile opportunistic human pathogen associated with acute and chronic infections, particularly affecting immunocompromised individuals, including those with cystic fibrosis (CF). The bacterium's ability to thrive under diverse environmental conditions, such as oxygen limitation in the mucus of CF patients, is central to its pathogenicity and a key contributor to persistence in hostile settings. Under anaerobic conditions and in the absence of other energy-generating systems, pyruvate fermentation serves as an alternative strategy, ensuring long-term survival and basal metabolism. In these growth-limiting, nutrient-deficient conditions, the universal stress protein UspK was found to be highly upregulated. This study investigates the role of UspK in facilitating adaptation to anaerobic stress, revealing a significantly decreased anaerobic survival rate during pyruvate fermentation in the absence of UspK. Upon switching to pyruvate fermentation, we observed alterations in LPS levels and a significant increase in AMP levels. Further analysis showed that UspK is endowed with a nucleotide-binding motif capable of binding AMP, suggesting high AMP levels may act as a signal for starvation beyond stringent response. Supported by phenotypic analyses, our results highlight the critical role of UspK in anaerobic stress adaptation via pyruvate fermentation and survival in such famine conditions. This study provides new insights into stress response and niche adaptation of P. aeruginosa, which are crucial for targeting chronic infections.

2200 B — Uncovering novel pathways of D-mannose and D-fructose degradation in thermoacidophilic archaeon Saccharolobus solfataricus

PRESENTING AUTHOR: *Kea Mucha, Leibniz Institute DSMZ GmbH, Germany*

CO-AUTHORS: *Jacqueline Wolf, Anna Ebel, Svea Holland, Christopher Bräsen, Bettina Siebers, Meina Neumann-Schaal*

SUB THEME: *Microbial metabolomics*

Archaea are often found in extreme environments, tolerating for instance high temperatures, salinity and acidic or alkaline environments making them valuable models for understanding life under such conditions. Their metabolism is often characterized by unique and unusual metabolic pathways and enzymes that differ from bacteria and eukaryotes. Within the Thermoproteota, Saccharolobus solfataricus is a key model organism for studying archaeal metabolism. It thrives under thermoacidophilic conditions with an optimum temperature of 80 °C and a pH range of 2 to 4. Additionally, S. solfataricus is characterized by its metabolic versatility, allowing growth on various carbon sources. For some hexoses like D-glucose and D-galactose the central carbohydrate metabolism has been extensively investigated, revealing a modified branched Entner-Doudoroff (ED) pathway for degradation. This pathway notably avoids phosphorylated intermediates, which are common in other organisms. However, the degradation of other hexoses, such as D-mannose and D-fructose, remain unexplored. To address these metabolic gaps, a GC-MS based stable isotope labeling experiment was optimized to enable time-resolved tracing of D-mannose and D-fructose degradation. The labeling experiments suggest degradation analogous to the modified ED pathway, but with a surprisingly high flux through intermediates like mannose-6-phosphate, fructose-6-phosphate, and trehalose, indicating a so far unknown degradation route within the S. solfataricus catabolism.

2201 B — Unlocking the postbiotic potentials of Lactobacillus plantarum BCC 47723 using metabologenomics approach

PRESENTING AUTHOR: ★ *Natnicha Charoenwong, Thammasat University, Thailand*

CO-AUTHORS: *Wonnop Visessnguan, Nipa Chokesajjawatee, Saowalak Adunphatcharaphon, Warissara Kasikonsunthonchai, Christopher Elliott, Awanwee Petchkongkaew, Umaporn Uawisetwathana*

SUB THEME: *Microbial metabolomics*

Probiotics play a crucial role in enhancing the bioactive properties of fermented food and promoting human health especially in intestinal flora. Lactobacillus plantarum strain BCC 47723 has demonstrated potential as a probiotic strain, attributed to its ability to adhere to mucin and survive under in vitro simulated gastrointestinal tract conditions. A combination between genomics and metabolomics, called metabologenomics approach was conducted to elucidate the postbiotic capabilities of Lactobacillus plantarum BCC 47723. Whole genome sequencing of this strain (3,188,446 bp) was analyzed using antiSMASH and Kyoto Encyclopedia of Genes and Genomes (KEGG) database to predict biosynthetic gene clusters and functional genomics. Results revealed that three key genomic regions encoded for the biosynthesis of three major postbiotic groups, namely terpenoids, type III polyketide synthases (PKSs), and cyclic lactone autoinducers were found to be dominant. While, results from KEGG highlighted genes normally found in lactic acid bacteria such as lactate in pyruvate metabolism, and riboflavin (vitamin B2) and coenzyme A for transfer of electrons in metabolic pathways. By contrast, metabolite profiles of this bacterial strain was positively correlated to the biosynthetic gene cluster prediction, accelerating the discovery process of potential postbiotics. The selected postbiotics generally exhibit potential bioactivities, including antioxidant, anti-oxidative stress, anti-inflammatory, and gut microbiota-modulating effects. Furthermore, the postbiotics from Lactobacillus plantarum BCC 47723 could play a significant role in modulating various biological pathways and systems within host organisms, underscoring their potential to health benefits.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2203 B — Metabolic adaptations associated with plasmid-mediated antimicrobial resistance in clinical Enterobacteriaceae isolates

PRESENTING AUTHOR: ***Breanna Dixon, University Of Manchester, United Kingdom***
CO-AUTHORS: ***Javier DelaFuente, Alvaro San Millan, Danielle Weaver, Michael Brockhurst, Waqar Ahmed, Stephen Fowler, Tim Felton***
SUB THEME: ***Microbial metabolomics***

Introduction:

Antimicrobial resistance (AMR) is a growing public health crisis, particularly in clinical settings where resistance plasmids facilitate the persistence and spread of nosocomial pathogens. While plasmid acquisition confers resistance, it also imposes a fitness cost on bacteria, driving metabolic reprogramming to maintain viability. Previous studies have largely examined transcriptional changes following de novo plasmid introduction into naïve strains, leaving a critical gap in understanding how wild-type clinical isolates adapt metabolically to plasmid acquisition. To address this, we employed a CRISPR-Cas9-mediated plasmid-curing system to eliminate carbapenemase-harbouring plasmids from wild-type Enterobacteriaceae isolates, generating isogenic pairs both with and without the plasmid for direct comparison. This study aimed to characterise the metabolic adaptations associated with plasmid acquisition, providing insights into the factors that influence plasmid stability and bacterial adaptation.

Methods:

We performed metabolomic profiling of wild-type and plasmid-cured Enterobacteriaceae isolates using HILIC LC-MS/MS on an Orbitrap QExactive Plus operated in positive ion mode. Bacteria were cultured under standard conditions for six hours before quenching and intracellular metabolite extraction. Multivariate statistical analysis and pathway enrichment analysis were conducted to identify metabolomic shifts associated with plasmid presence.

Findings & Implications:

Our results revealed that plasmid acquisition induces significant metabolic alterations, with specific pathways consistently enriched across multiple strains. While some metabolic changes were strain-specific, a core set of conserved pathways emerged, suggesting common mechanisms of metabolic adaptation to carbapenemase-harbouring plasmid carriage. These findings enhance our understanding of plasmid-mediated AMR evolution bacterial adaptation and provide a foundation for developing targeted strategies to mitigate resistance by disrupting key metabolic adaptations.

2204 B — Semi-automated extraction of cell and tissue samples for multi-omic analysis using the Biomek i7 Hybrid Workstation

PRESENTING AUTHOR: ***Chandini Kalsy, Beckman Coulter for Life Sciences, United States***
CO-AUTHORS: ***Martin Jones Ph.D, Mark Viant, Ph.D, Zach Smith***
SUB THEME: ***Model organisms***

Introduction:

The €20M EU Horizon 2020-funded project, 'PrecisionTox,' aims to revolutionize chemical hazard assessment by integrating transcriptomics, metabolomics, machine learning, and chemical exposure experiments using diverse model organisms (Daphnia magna, Danio rerio, Drosophila melanogaster, Caenorhabditis elegans, Xenopus laevis) and the HepG2 human cell line. With over 10,000 samples generated across six labs in Europe and the US, there's a critical need for automated extraction of polar and lipophilic compounds. We present advancements in developing a high-throughput, semi-automated extraction method using the Biomek i7 Hybrid Automated Workstation from Beckman Coulter Life Sciences.

Methods:

Each PrecisionTox sample undergoes comprehensive molecular characterization through transcriptomics and liquid-chromatography-mass-spectrometry-based metabolomics. The Biomek i7 workstation, equipped with cryogenic positions, supports a semi-automated extraction workflow crucial for these analyses. Up to 96 samples per batch, including quality control samples, are homogenized by bead beating and transferred to a 96-well plate. A portion of each homogenate is reserved for RNAseq analysis, while the rest undergoes a modified Bligh and Dyer biphasic extraction to produce polar and lipid metabolomic fractions for further analysis.

Preliminary Data:

Our workflow enhances throughput to 192 samples per day for multi-omic analyses (polar metabolomics, lipidomics, RNAseq), a fivefold increase over manual methods. It demonstrates excellent intra- and inter-day variability, with no significant trends related to extraction day or sample position on the plate. Diverse matrices, including crustacea, flies, fish larvae, embryos, and human cell lines, are processed effectively. Metabolomic analyses show distinct metabolic shifts in growth and development stages for organisms like Daphnia magna and Danio rerio, confirming the workflow's capability to detect molecular changes. In chemical-exposed samples, marked metabolic shifts compared to controls are evident across all organisms. We provide observations for Caenorhabditis elegans and Drosophila melanogaster, highlighting perturbed metabolites during exposure.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2205 B — Unveiling the metabolic profile of a vegetable protein diet in Caenorhabditis elegans

PRESENTING AUTHOR: *Sergio Forcada, CNTA, Spain*
CO-AUTHORS: *Cristina Matías-Sainz, Ana Lucía Rodas-Méndez, Carolina González-Ferrero, María-José Sáiz-Abajo*
SUB THEME: *Model organisms*

Vegetable alternatives to meat products are increasing in popularity because they can help consumers towards a plant-based diet. The consumption of plant-based proteins not only benefits individual health by reducing the risk of chronic diseases, but also significantly contributes to environmental sustainability by lowering the ecological footprint of food production. However, more research regarding the metabolic effect of this alternative diet on animal and human health is needed.

To this end, Caenorhabditis elegans (C. elegans) was employed as model organism due to its quick development and relative ease of culture. C. elegans is widely employed in aging and nutritional research. Here, it was employed to evaluate the metabolic effect of including diverse plant-based protein extracts on nematode's diet. Concretely, we compared the metabolic features of nematodes fed with two different vegetable protein sources: a faba bean protein concentrate and a pea protein concentrative versus a control group fed with E.coli OP50 and another group fed with E.coli OP50 and supplemented with an animal protein (casein).

Our results revealed significant changes in the metabolic profile of C.elegans in response to the different protein sources. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) showed a clear separation between treatments, suggesting that each protein source induces unique metabolic changes. Key metabolites contributing to these differences included amino acids, fatty acids and energy-related metabolites.

This study provides a foundation for future research into the molecular mechanisms by which the quality and origin of dietary proteins influence the physiology of the model organism C. elegans.

2206 B — Ethnopharmacology-guided metabolomics study of a forgotten medicinal plant from Provence (South of France)

PRESENTING AUTHOR: *Charifat Said Hassane, Aix Marseille University, France*
CO-AUTHORS: *Clémentine Achard-Baccati, Charifat Said Hassane, Sok-Siya Bun-Llopet, Béatrice Baghdikian, Elnur Garayev*
SUB THEME: *Natural products*

In Provence, Plantago sempervirens Crantz (Plantaginaceae) is a widely used medicinal plant and so-called panacea for external ailments and various skin conditions. This study aims to describe its understudied chemical composition, assess its biological properties, and annotate its bioactive metabolites through molecular networking.

The aerial parts of P. sempervirens were harvested in the Luberon Regional Natural Park in Provence, and underwent ultrasound-assisted extraction and liquid/liquid fractionation. Metabolomic analyses were performed based on UHPLC-MS/MS data and molecular networking. Multi-target pharmacological screening evaluated (i) antimicrobial activity using disk-diffusion and MIC assays against several strains (Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans), (ii) antioxidant potential via DPPH well-plates and post-column assays, (iii) anti-inflammatory activity through NO inhibition in LPS-induced macrophages and (iv) wound-healing potential through scratch-tests on human keratinocytes.

Bioactivity screening revealed that polar fractions were the most promising with strong antioxidant activity (IC50 <20 µg/mL), wound healing properties (>30% wound surface coverage) and moderate anti-inflammatory activity (IC50 between 50 and 100 µg/mL). The ethyl acetate fraction is the only one showing antimicrobial activity against Gram-positive bacteria. The metabolites responsible for these bioactivities were annotated using molecular networking and computational tools such as GNPS, SIRIUS and timaR.

These findings support P. sempervirens as a valuable source of bioactive compounds and demonstrates how ethnopharmacology-guided approaches can reconnect traditional knowledge about medicinal plants and modern biomedical research.

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2207 B — Advancing Biodiversity Research: The Metabolomics of Mexico Territory Project

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CO-AUTHORS: *Alan Hernández-Melgar, Rommel Carballo-Castañeda, Alejandra Correa-De la Torre, Eliaf Garcia-Loya, Irvin López-Nava, Mario Salazar-Ceseña, Rodrigo Méndez-Alonzo, María Arteaga-Uribe, Lorena Durán-Riveroll, Edelweiss Rangel-Montoya, Rufina Hernández-Martínez, Nahum Valente-Hernández, Rosa Mouriño-Pérez, Edna Sánchez-Castrejón, Elizabeth Ponce-Rivas, Erick Huerta-Romero, Nadia Caram-Salas, Jimena Carrillo-Tripp, Miguel Cuevas-Cruz, Laura Gómez-Consarnau, Luz Garduño-Villaseñor, Fadia Sara Ceccarelli, Olga Callejas-Negrete, Lorena Rodríguez-Orduña, Viridiana Pérez-García, Luz González-Salazar, Cuauhtémoc Licona-Cassani, Colaboradores Del-CIMMYT, Víctor Hugo-Reynoso, Magali Honey-Escandón, Esteban López-Sampedro, Diana Franco-Vázquez, Laura Pérez-Flores, Víctor Flores-Núñez, Laila Partida-Martínez, Ingrid Rodríguez-Buenfil, Daniela Trujillo-Silva, Robert Winkler, Felipe Vázquez-Flota, Juan Ramírez-Suárez, Erick Gutiérrez-Grijalva, José Basilio-Heredia, Hilda Ramos-Aboites, Francisco Barona-Gómez, Patricia Valdespino-Castillo, Anidia Blanco-Jarvio, Jaime Martínez-Castillo, María Venegas-Reza, Roberto Arreguín-Espinosa, Martha Manzano-Mora, Mario Figueroa, José Aguirre-Noyola, Esperanza Martínez-Romero, Laura Contreras-Angulo, Rafael Bello-Bedoy, Lilian Palomino-Álvarez, Nancy Suárez-Mozo, Pedro Homá-Canché, Nuno Simões, Marcela Gamboa-Angulo, Ernesto Beltrán-Partida, Hilda León-Tejera, Aldo Moreno-Ulloa*

SUB THEME: *Natural products*

Introduction: Mexico is recognized as a megadiverse country, harboring an estimated 10-12% of all documented species worldwide. In situ investigations have cataloged approximately 19,150 endemic species, including plants, beetles, spiders, reptiles, and bees. This exceptional biodiversity highlights the need for advanced methodologies to explore and illuminate its chemical space. Metabolomics —large-scale analysis of metabolites — facilitates the identification of natural products and chemotaxonomic markers and opens new avenues for therapeutic development. Aim: To establish a robust metabolomics data analysis pipeline, promote multi-institutional collaboration, advance chemotaxonomic profiling and drug discovery, and enhance public access to chemical diversity data. Methods: This study presents key aspects of the METabolomics of Mexico Territory Project (Aka “The METxico Project”), including community-driven sample collection, mass spectrometry-based untargeted metabolomic analysis, and the development of an interactive web platform for data integration, visualization, and dissemination. Results: In response to challenges and opportunities nationwide, The METxico Project was initiated in 2020. This nationwide, multidisciplinary initiative currently integrates 36 Centers, Institutes, or research groups and 64 academic collaborators, focusing on the metabolomic characterization of terrestrial and marine species of national interest. Additionally, we introduce a web-based platform designed to visualize and interconnect biological and chemical information of 134 metabolomes, part of the first collection, which is rapidly growing each year. Conclusions: Through this initiative, we have established a comprehensive framework for academic collaboration, driven methodological innovation in metabolomics, contributed to the global understanding of biodiversity, and facilitated data visualization through a web-platform.

2208 B — Innovative Development, Optimization and Stability Evaluation of Rhodiola Rosea Nanoemulsion for Topical Delivery Using Response Surface Methodology (RSM)

PRESENTING AUTHOR: *Benni Iskandar, Department of Pharmaceutical Technology, STIFAR, Indonesia*

SUB THEME: *Natural products*

Nanoemulsions represent an advanced drug delivery system designed to efficiently transport active pharmaceutical ingredients to targeted physiological sites. In this study, Rhodiola Rosea nanoemulsions were engineered using a low-energy emulsification technique, incorporating two distinct surfactant-cosurfactant combinations alongside varying oil phases (Group F and G). Critical formulation parameters, including particle size, polydispersity index (PDI), and zeta potential, were systematically evaluated to determine their influence on nanoemulsion stability and performance. Utilizing response surface methodology (RSM), optimal formulation conditions were established for each experimental group. In Group F, the ideal nanoemulsion composition was identified as 10% transcutol, 16.63% tween 80, and 1% Labrafac oil, yielding superior response values of 16.08 nm (particle size), 0.301 (PDI), and -47.69 (zeta potential). Conversely, Group G’s optimized formulation, containing 29.608% transcutol, 30% tween 80, and 1% soybean oil, demonstrated response values of 210.34 nm, 0.366, and -31.341, respectively. Post-optimization, the formulations underwent comprehensive stability assessments, including pH evaluation, viscosity measurements, and organoleptic analysis. Findings from this study highlight the successful fabrication of a stable Rhodiola Rosea nanoemulsion utilizing 10% transcutol, 16.63% tween 80, and 1% Labrafac oil, offering a robust formulation strategy for pharmaceutical and cosmetic applications. This research provides a novel framework for enhancing nanoemulsion stability and formulation efficiency through strategic surfactant-cosurfactant integration, paving the way for future advancements in drug delivery technologies.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2209 B — Integrative Analysis of Amphibian Metabolites via GC-MS and Molecular Networking

PRESENTING AUTHOR: *Fabiola Almeida Garcia, FCFRP-University of Sao Paulo, Brazil*
CO-AUTHORS: *Ana Caroline Zanatta, Andres Brunetti, Norberto Peporine Lopes*
SUB THEME: *Natural products*

The rapid decline in global biodiversity threatens ecosystems, highlighting the urgent need for effective conservation strategies. Amphibians, in particular, produce a rich array of small molecules in their skin secretions, which play key roles in ecological interactions such as defense, reproduction, and microbiota regulation. Despite their ecological relevance, these compounds remain largely understudied, especially from a metabolomic perspective. While liquid chromatography–mass spectrometry (LC-MS) is commonly used for analyzing polar metabolites, gas chromatography–mass spectrometry (GC-MS) provides a rapid, robust, and highly reproducible platform ideally suited for the analysis of small, low-polarity molecules—making it particularly useful for profiling amphibian volatile organic compounds (VOCs). The technique's speed and sensitivity facilitate high-throughput screening, and derivatization strategies further extend its applicability to polar compounds. In this study, we employ GC-MS in combination with molecular networking via the Global Natural Products Social Molecular Networking (GNPS) platform to comprehensively characterize the chemical profile of amphibian skin secretions. GC-MS benefits from standardized electron ionization and access to extensive spectral libraries. By integrating GC-MS data into GNPS workflows, we aim to uncover the chemical diversity of amphibian metabolites, advancing both methodological applications and our ecological understanding. Our analyses revealed 50 volatile compounds in amphibian skin secretions, predominantly derivatized amino acids, fatty acids, dicarboxylic acid esters, and steroids. This study not only broadens the chemical knowledge of amphibian secretions but also demonstrates the potential of GNPS-based workflows in GC-MS-driven chemical ecology and biodiversity research.

2210 B — Iron Competition As The Mediator In Probiotic Inhibition Of Chytridiomycosis In The Era Of The Holobiont

PRESENTING AUTHOR: *Elliot Murphy, Imperial College London, United Kingdom*
CO-AUTHORS: *Kieran Bates, Tom Bell, Trent Garner, Marc-Emmanuel Dumas*
SUB THEME: *Natural products*

Widespread declines in amphibian populations have been linked to a common yet fatal skin disease, chytridiomycosis. The infection is largely caused by one strain of fungus, Batrachochytrium dendrobatidis, and affects the host's ability to control homeostasis. This eventually leads to cardiac arrest due to an electrolyte imbalance. With the explosion of the global pet trade and an ever-changing climate, the disease is now affecting populations across the globe and mitigation strategies remain expensive and in their infancy. As complex biological systems move out of obscurity, an amphibian's holobiont becomes an invaluable resource to probe for disease resistance. In this context, our study targeted nutrient competition as a mechanism of inhibition against B. denrobatidis. Limitation of Iron, an essential mineral for fungal metabolism, has been employed by bacteria, plants and host immune systems to effectively inhibit fungal growth. Microorganisms produce siderophores, high-affinity iron-chelating compounds, to outcompete fungi for iron uptake, thereby limiting fungal proliferation. Here, we targeted iron competition in frogs' dermal holobiont as a mechanism for disease resistance. Bacteria isolated from frogs' dermal tissues were grown in iron depleted media stimulating their production of siderophores. Using native LC-MS we systematically identified both known and unknown siderophores using a targeted and untargeted approach. A database of iron chelating compounds was then built using MzVault for real time library searches and future dereplication. Co-culture assays are now being developed to investigate the bacteria's ability to inhibit the growth of B. denrobatidis.

2211 B — Non-targeted metabolomics reveals anorectic compounds from Acer pseudosieboldianum extracts

PRESENTING AUTHOR: *Young Jin Park, Incheon National University, South Korea*
CO-AUTHORS: *Tae Jin Kim, Seokjae Park, Jae Kwang Kim*
SUB THEME: *Natural products*

Korean maple (Acer pseudosieboldianum), a native species of Korea, is known for its rich polyphenolic content. To explore its potential in appetite regulation and metabolic modulation, we investigated two 70% ethanol extracts (from branch and leaf) provided by the Nakdonggang National Institute of Biological Resources.

To evaluate the anorectic response of the extract, the expression levels of hypothalamic neuropeptides (POMC, AgRP, and NPY) involved in appetite and energy balance were assessed. Both extracts significantly modulated gene expression, with approximately 70% downregulation of NPY and AgRP in treated groups. To identify anorectic activity compounds, we performed non-targeted metabolite profiling using UPLC-qTOFMS in both positive and negative ionization modes. The results showed the alignment of 546 metabolite features. Of these, flavonoids were identified 10 flavonols (six quercetin glycosides and four kaempferol glycosides), four phenolic acids, two flavanols (catechins), two flavanones (eriodictyols), and two coumarins. Flavonoid-targeted UPLC-MS/MS analysis was performed to ensure precise identification and quantification of bioactive compounds, with guaiaverin (Quercetin 3-O-alpha-L-arabinoside), isoquercitrin (Quercetin 3-O-beta-D-glucofuranoside) and hyperoside (Quercetin 3-O-beta-D-galactopyranoside) was highly enriched in both extracts. Further gene expression experiments demonstrated that guaiaverin and isoquercitrin showed the significantly downregulation of NPY and AgRP expression, supporting their role as a key bioactive flavonoid.

These findings highlight the utility of non-targeted metabolomics in the discovery of functional phytochemicals and support the potential of Acer pseudosieboldianum as a source of anti-obesity compounds.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2212 B — Metabolite Profiling and Anti-adipogenic Effects of Pawpaw (Asimina triloba) Fruit Using UPLC-QTOF/MS

PRESENTING AUTHOR: ***Sang Yoon Choi, Korea Food Research Institute, South Korea***
CO-AUTHORS: ***Chang Jun Lee, Guijae Yoo***
SUB THEME: ***Natural products***

To elucidate the bioactive properties of Pawpaw (Asimina triloba) fruit, metabolite profiling was conducted using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS). This analysis identified key flavonoids. In a 3T3-L1 adipocyte model, pawpaw fruit extract (AT) significantly suppressed the expression of lipogenic proteins (SREBP1, PPARγ, and FAS) while activating the AMPK signaling pathway, leading to reduced lipid accumulation. These findings suggest that A. triloba fruit, characterized through UPLC-QTOF/MS-based metabolite profiling, has potential as a functional ingredient for managing lipogenesis.

2213 B — Chemical constituents from the Korean endemic plant Pseudolysimachion pusanensis inhibit diffuse-type gastric cancer cells

PRESENTING AUTHOR: ***Wonkyu Kim, Korea Institute Of Science And Technology, South Korea***
CO-AUTHORS: ***Su-Yeon Cho, Hoseong Hwang, Hyeon-Seong Lee, Jaeyoung Kwon, Wonkyu Kim***
SUB THEME: ***Natural products***

Diffuse-type gastric cancer (GC) is closely associated with genetic abnormalities; however, its exact pathological mechanisms are still not understood. It manifests without symptoms before advanced stages and is often difficult to diagnose using routine imaging tests. Therefore, specific targeted therapies for diffuse GC are currently unavailable. In this study, the extract of Pseudolysimachion pusanensis, which is endemic to Korea, inhibited the proliferation of GC cells, MKN1 and SNU668, while the other extracts of the two endemic Pseudolysimachion species did not show any activity. This led to the molecular networking analysis of Pseudolysimachion species to identify the molecules mostly observed only in P. pusanensis. Thirteen new (1-13) and eight known (14-21) compounds were obtained and structurally characterized. Of these, cucurbitacin derivative compounds 1 and 14-16 showed activity, and particularly, the IC50 value of compound 1 was 0.65 and 0.21 μM. Ki-67 expression analysis, single-cell originated cell proliferation assay, and western blot analysis of apoptotic cell death-related molecules revealed that compound 1 mediated both cytostasis and cellular death via apoptosis. Particularly, this compound exhibited an anti-tumorigenic effect on the invasion of diffuse-type GC cells by perturbing the epithelial-to-mesenchymal transition (EMT) pathway, as demonstrated by invasion assays using both 2D models and in vivo-like 3D spheroid co-culture models, as well as western blot analysis of EMT markers.

2214 B — Development of Plant-Based Peptides and Antioxidants for Eco-Friendly Cosmetic Ingredients

PRESENTING AUTHOR: ***Jeongae Lee, Korea Institute Of Science And Technology, South Korea***
CO-AUTHORS: ***Hesson Chung, Sung Ui Lee***
SUB THEME: ***Natural products***

The cosmetic industry is increasingly interested in peptides for their anti-aging and moisturizing effects. Driven by the growing demand for vegan raw materials, peptides derived from beans and other vegetables have become essential ingredients in many cosmetic products. Since vegetable proteins must be hydrolyzed into smaller molecules, the sources of proteases are crucial for obtaining vegan certification.

In this study, bromelains extracted using a newly designed process were employed to hydrolyze selected bean and hibiscus species. Ground or juiced vegetables, including pineapple containing proteolytic enzymes, underwent dialysis to remove small molecules such as sugars and pesticides. Pineapple crowns were also ground for dialysis. The purified materials within the dialysis bags were mixed with ground beans for proteolytic hydrolysis. When fresh pineapple juice mixed with carbendazim (0.2 mM) was dialyzed for 8 1 hours, only 4 w/w% of the total mass remained in the bag, with no trace of sugar or pesticide. The yield of the soluble fraction remained unchanged before and after dialysis, indicating that enzyme molecules or activity were not lost during the process.

Multivariate analysis revealed that the small molecules in the soluble fraction fell into distinctly different categories of molecular species. While nuts contain various peptides, hibiscus generally contains quercetin and other phenolic compounds. The soluble fractions of beans and hibiscus species contained various peptides and antioxidant molecules. Notably, in hydrolyzed golden hibiscus, a 5-mer peptide was approximately 100 times more abundant than in hydrolyzed regular hibiscus.

This research demonstrates the potential for producing vegan peptides using plant-derived enzymes and highlights the potential of various vegetable and plant extracts as cosmetic ingredients. The high concentration of a specific peptide derived from golden hibiscus suggests its potential contribution to the development of novel functional cosmetics.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2215 B — Investigation of Secondary Metabolites of the Aerial Bulbils of Disocrea batatas

PRESENTING AUTHOR: *Somin Park, Kyung Hee University, South Korea*
CO-AUTHORS: *So-Ri Son, Dae Sik Jang*
SUB THEME: *Natural products*

Dioscorea batatas (Dioscorea), commonly known as Chinese yam, is a perennial climbing vine native to East Asia. Its rhizome is widely used in both medicinal and culinary applications due to its nutritional and therapeutic properties. Although the aerial bulbils of yam are also edible, they are often discarded as byproducts during cultivation. Despite being part of the same plant, the chemical composition and potential value of the bulbils have rarely been explored. In this study, LC-MS-guided isolation was employed to investigate the chemical constituents of Chinese yam bulbils. Methanol extracts of the aerial bulbils and rhizomes were compared using LC-MS to analyze differences in their chemical profiles. As a result, three new compounds (1–3) along with 23 known compounds were isolated. The structures of the new compounds were elucidated using spectroscopic techniques, including NMR and mass spectrometry, while the known compounds were identified by comparison with previously reported data. Notably, compounds 1 and 2 were identified as phenanthrenes, a class of compounds commonly found in the genus Dioscorea. This study provides new insight into the underutilized bulbils of D. batatas and highlights their potential as a valuable source of natural products.

2216 B — Anti-hyperglycemic-related and Bacteroides ovatus-modulating effects of galactomannans from Cordyceps militaris and Trigonella foenum-graecum enhanced by microbial transformation and cordycepin co-administration

PRESENTING AUTHOR: *Ching-yi Lu, National Taiwan University, Taiwan*
CO-AUTHORS: *Wen-Yu Hsieh, Hui-Kang Liu, Mei-Kuang Lu, Shoei-Sheng Lee, Chia-Chuan Chang*
SUB THEME: *Natural products*

Alterations in gut microbiota composition is closely related to metabolic disorders, indicating the relevance of microbiota regulation in metabolic disease management. Galactomannans, a kind of polysaccharide abundant in Trigonella foenum-graecum (fenugreek) and Cordyceps militaris, have been reported to exhibit anti-diabetic and immunomodulatory effects. Therefore, this study evaluated the blood-glucose-regulating effects and the growth-promoting potential toward B. ovatus of galactomannans from these herbs.

A total of twenty-three fractions were obtained in this study. Among them, TF-5 (from fenugreek), CM-1a02 (from mycelium part of C. militaris), and CF-1b03 (from fruit body part of C. militaris) demonstrated both in vitro anti-hyperglycemia-related effects and triggered the growth of Bacteroides ovatus. CM-1a02 exhibited the highest DPP-4 inhibitory activity (EC50 = 0.76 μM), CF-1b03 enhanced the highest GLP-1 secretion (2.4-fold), and TF-5 induced the highest insulin secretion (2.1-fold). Moreover, all three fractions promoted B. ovatus proliferation (1.3~1.5-fold), with CM-1a02 induced the highest short-chain fatty acid production (2~5-fold). These findings suggest a potential association between B. ovatus proliferation and anti-diabetic effects.

Furthermore, the oligosaccharide TFPS_BO, derived from microbial fermentation of TF-5 by B. ovatus, exhibited anti-hyperglycemic effects, including DPP-4 inhibition (EC50 = 100 μM), stimulation of GLP-1 (2.2-fold), as well as insulin secretion (1.7-fold). Molecular docking confirmed its interaction with the DPP-4 active site, providing structural insight into its mode of action. In addition, co-administration of cordycepin, a bioactive alkaloid from C. militaris, with CM-1a02 or CF-1b03 enhanced DPP-4 inhibition to 75.6% and 70.6%, representing 1.4- and 1.3-fold improvements relative to polysaccharide treatment alone. Together, these two components, a microbiota-derived oligosaccharide and a combinatory intervention, may offer complementary strategies for hyperglycemia management.

In summary, this study demonstrates that galactomannans can promote B. ovatus proliferation and alleviate hyperglycemia, while their structural characteristics and combinatory use with bioactive compounds further enhance therapeutic efficacy.

2217 B — A New Strategy for In-Depth MS/MS Mining Reveals Novel Sesquiterpenes in Artemisia heptapotamica

PRESENTING AUTHOR: *Chenlan Shu, Shanghai Institute Of Materia Medica, China*
CO-AUTHORS: *Yang Ye, Jia Liu, Jie Yuan*
SUB THEME: *Natural products*

Molecular networking has emerged as a powerful tool for navigating the vast chemical space of natural products. However, the targeted and efficient identification of structurally divergent analogs within complex mixtures remains a significant challenge. In this study, we present a novel MS/MS data-driven strategy that enables in-depth mining of fragmentation spectra, coupled with dereplication and visualization tools to prioritize structurally distinct candidate molecules. Applying this strategy to a previously published MS/MS dataset of Artemisia heptapotamica enabled the efficient identification of nine sesquiterpene dimers (1–9) with varying degrees of structural divergence, distributed among three molecular subclusters. Among them, six compounds (1–6) were identified as new natural products. Beyond its successful application in A. heptapotamica, this workflow offers a broadly applicable framework for accelerating the discovery of new scaffolds across diverse natural product sources, thereby expanding our understanding of chemical diversity and its potential biological relevance.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2218 B — Utilizing Multiple Extraction Techniques for an Untargeted, Global Characterization Analysis of Honey using GC×GC-TOFMS

PRESENTING AUTHOR: ***Sheri Schmidt, University Of Alberta, Canada***
CO-AUTHORS: ***Seolin Nam, James Harynuk***
SUB THEME: ***Natural products***

Honey is a complex, natural product produced by honeybees (Apis mellifera) using nectar collected from flowers. Its complexity arises from the vast amount of chemical components that are present, including organic acids, sugars, amino acids, polyphenols, etc. Due to this complex matrix, the extraction and separation of all chemical components is challenging. Currently, there are several extraction techniques being utilized, including solid phase extraction (SPE), liquid-liquid extraction (LLE), solid-phase microextraction (SPME), and liquid extraction followed by derivatization via oximation and trimethylsilylation. Gas chromatography coupled to mass spectrometry (GC-MS) is a popular tool to separate and identify the compounds present in honey. However, comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) offers superior separation, reducing coelutions and increasing the quality of mass spectra for improved compound identification, making it ideal for untargeted analysis. In this work, various extraction methods are explored to highlight the influence of extraction method on the observed metabolite profiles. The extractions performed were the following: liquid extraction followed by oximation and trimethylsilylation, liquid extraction followed by oximation and tert-butyldimethylsilylation, LLE, and SPME. All extracts were then analyzed via GC×GC-TOFMS to characterize the chemical profile associated with each method.

2219 B — Evolution of the saponin content of Agave parrasana throughout its life cycle

PRESENTING AUTHOR: ***Ana M. Simonet, University Of Cádiz, Spain***
CO-AUTHORS: ***Alexandra G. Durán, Rafael P. Alba, Ana M. Simonet***
SUB THEME: ***Natural products***

The Agavaceae family comprises more than 200 species and plants belonging to this family possess significant economic and ecological value. This family is recognized as the main source of steroidal saponins, natural products with a remarkable structure. Saponins from several Agave species show a good activity profiles against weeds and are proposed as possible leads for the development of natural herbicides (1). The saponin-rich fraction of young A. parrasana leaves was one of most active and a combination of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy enabled the dereplication of this fraction revealing a complex mixture of 20 saponins that combine three different sugar chains and eight aglycones. The identification of these saponins was possible without purification, including new combinations of aglycones and sugar chains (2).

The saponin content of A. parrasana was analyzed throughout its life cycle. Significant changes in saponin structure were observed, with new sugar chains appearing in the adult plant and decreasing to a major sugar chain in the flowering plant, along with the loss of oxidation in the aglycone. Saponin notation in the UPLC-MS spectra was possible by NMR data and HMAI method.

Finally, the phytotoxic activity of the enriched extracts was tested to assess their potential.

(1) Durán, A.G., Benito, J., Macías, F.A., Simonet, A.M., 2021. Agave Steroidal Saponins as Potential Bioherbicides. Agronomy 11, 2404.

(2) Simonet, A.M., Durán, Macías, F.A., 2024. Dereplication of Bioactive Agave Saponin Fractions: The Hidden Saponins. J. Agric. Food Chem. 72, 13740-13756.

2220 B — Spotting bioactive phyllobilins in senescent plant extracts via informed feature-based molecular networking

PRESENTING AUTHOR: ***Christian Elvert, University of Innsbruck, Austria***
CO-AUTHORS: ***Johanna Lückenbach, Simone Moser, Fabian Hammerle, Cornelia Karg***
SUB THEME: ***Natural products***

Phyllobilins are the final degradation products of chlorophyll and formed during plant senescence, fruit ripening, and post-harvest storage of plant-based foods. Although they are identified around 30 years ago and ubiquitous in nature, they have only recently emerged as a novel class of natural products with reported bioactivities, including antioxidative, anti-inflammatory, and anti-proliferative properties [1]. Traditionally, phyllobilins have been identified in plant extracts based on their characteristic UV absorption spectra. While mass spectrometry (MS) has been employed for their detection and structural elucidation, their MS^2 spectra have not yet been uploaded to publicly accessible mass spectral databases.

This study presents a comprehensive workflow for the identification of phyllobilins and related structures using bioactivity-guided fractionation and feature-based molecular networking. Plant extracts of Echinacea purpurea, for which six structurally different phyllobilins could be identified before [2], and Salvia officinalis, which has not yet been analyzed for the occurrence of phyllobilins, were fractionated and analyzed via UHPLC-HRMS/MS, followed by generating a molecular network using the GNPS feature-based molecular networking workflow. To establish a reference dataset, the known MS^2 spectra of phyllobilins from Echinacea were uploaded. Network analysis then suggested putative phyllobilin structures in Salvia officinalis extracts, which were subsequently isolated and confirmed through 1D- and 2D-NMR spectroscopy.

By contributing phyllobilin data to publicly available MS databases and performing a workflow using feature based molecular networking, this study provides a tool to facilitate the identification of novel phyllobilins in diverse plant extracts. This advancement enhances the accessibility of phyllobilin data for future research in plant metabolomics and bioactive natural products.

[1]P. Wang, C. A. Karg, N. Frey, J. Frädrich, A. M. Vollmar, S. Moser, Archiv der Pharmazie 2021, 354, 2100061.

[2]C. A. Karg, P. Wang, A. M. Vollmar, S. Moser, Phytomedicine 2019, 60, 152969.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2221 B — Supramolecular Solvent-Based Extraction of Urinary Biomarkers: A Sustainable Alternative for Target Analysis, Suspect and Non-Target Screening

PRESENTING AUTHOR: *Riccardo De Santo, Department of Chemistry, Sapienza University, Italy*
CO-AUTHORS: *Nicola Iacovella, Anna Laura Iamiceli*
SUB THEME: *Non-targeted analysis in biomonitoring and environmental monitoring*

Background: Human exposure to hazardous chemical mixtures remains a critical public health challenge, yet traditional biomonitoring approaches—focused on single chemicals—fail to capture this complexity. Suspect and non-target screening (SNTS) via high-resolution mass spectrometry (HRMS) can address this gap, but sample preparation remains a bottleneck. This study presents a novel application of supramolecular solvents (SUPRAS) as a sustainable, broad-spectrum extraction method for exposome research. A SUPRAS-based protocol was validated for the analysis of 11 representative compounds belonging to three different classes–bisphenols, phthalates, and per- and polyfluoroalkyl substances (PFAS)–in human urine, overcoming conventional limitations in exposure assessment.

Results: The performances of the SUPRAS-based protocol were evaluated against a conventional solid-phase extraction (SPE) clean-up method, achieving higher average extraction recoveries for all target analytes (75% for SUPRAS vs. 65% for SPE), great precision (RSD < 25%) and sensitivity (LOQs 0.04–1 ng mL-1). SUPRAS approach offered significant advantages, including broad-spectrum analyte coverage and single-step extraction efficiency, reducing organic solvent consumption (≤ 1 mL/sample), and making it particularly suitable for SNTS applications. The non-selective nature of SUPRAS extraction method enabled the identification of 27 additional compounds through HRMS, compared to SPE's 11 unique detections. The experimental data were cross-referenced against a custom in-house library, following a specific confidence framework for compounds identification. Chemical space evaluation demonstrated that SUPRAS extraction covered a wider LogP range (1.6–9.6) than SPE (1.6–7.4), highlighting its efficiency in detecting more hydrophobic substances than conventional SPE clean-up approaches.

Significance: This work establishes SUPRAS as a transformative tool for exposome research, offering a sustainable, efficient alternative to conventional methods, combining sustainability with wide chemical detection. SUPRAS-based approach significantly enhances our capacity to assess complex mixture exposures and identify emerging chemical risks in human populations. The method's adaptability to high-throughput workflows positions it as a critical advance for public health biomonitoring.

2223 B — UPLC-QTOF/MS-Based Metabolic Profiling of Aralia elata Extracts

PRESENTING AUTHOR: *Guijae Yoo, Korea Food Research Institute, South Korea*
CO-AUTHORS: *Yeseul Kim, Inwook Choi, Sang Yoon Choi*
SUB THEME: *Other*

Aralia elata extracts (AEE) have been investigated for their potential depigmentation effects and their bioactive compound composition. In this study, six quinic acid derivatives were identified using Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS). The detected compounds, including neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, and isochlorogenic acids A, B, and C, were characterized based on their retention times and mass spectral data. The identification was confirmed through mass fragmentation patterns, revealing the presence of characteristic ions associated with quinic and caffeic acids. These findings provide valuable insights into the phytochemical profile of AEE and highlight its potential as a functional ingredient in hyperpigmentation.

2224 B — Untargeted Metabolomic Profiling of Edible Parts of Korean wild chive by LC-QTOF-MS

PRESENTING AUTHOR: *Jinseo Lee, Kyungsung University, South Korea*
CO-AUTHORS: *Jihyeon Yu, Gajin Kim, Yoonjeong Kim, Younghwa Kim*
SUB THEME: *Other*

Korean wild chive (Allium monanthum) is a widely recognized seasonal vegetable in Korea, typically harvested in early spring. Although all parts of the plant—leaf, bulb, and root—are edible, their metabolite profiles likely differ due to their different biological functions. This study profiled metabolite variation across different plant parts using liquid chromatography quadrupole time-of-flight mass spectrometry in both positive and negative ionization modes. Four chemical classes were found in negative mode: flavonoids, phenolic acids and derivatives, isoflavonoids, and anthocyanins. Flavonoids were found to be the most prevalent in all parts. In positive mode, 10 classes were annotated, notably organic sulfur compounds and phenolic acids. Leaf and whole tissues showed higher metabolite diversity in negative mode, while leaf and bulb contained more compounds in positive mode. Multivariate statistical analyses revealed a clear separation among parts. The first two principal components explained 79.5% and 82.2% of the total variance in negative and positive modes, respectively, and groups were well separated. Partial least squares-discriminant analysis models showed high classification performance (Q^2 = 0.955 in negative, Q^2 = 0.967 in positive), with permutation tests confirming robustness. Several metabolites, such as apigenin, quercetin, and rosmarinic acid, were identified as important contributors based on variable importance in projection scores. Pathway enrichment analysis uncovered the notable participation of flavonoid, flavone, and phenylpropanoid biosynthesis pathways. Hierarchical clustering grouped bulbs and roots apart from leaves and the whole plant, with minor differences across ion modes. K-means clustering categorized samples into three chemically unique clusters, while random forest classification successfully achieved total separation with no classification errors, highlighting significant metabolites that predict plant part identity. These results provide insight into the distribution of metabolites within A. monanthum, providing a foundation for understanding its functional properties and potential applications in dietary and plant-based research.

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★ AWARD WINNERS

PLANTS, FOOD, ENVIRONMENT AND MICROBES

2225 B — Untargeted LC-QTOF-MS Profiling of Lignan Variability in Sesame Cultivars

PRESENTING AUTHOR: *Yoonjeong Kim, Kyungsung University, South Korea*
CO-AUTHORS: *Jina Lee, Gajin Kim, Jinseo Lee, Younghwa Kim*
SUB THEME: *Other*

Sesame (Sesamum indicum L.) is a nutritionally valuable oilseed crop known for its high content of lignans, a class of phenolic compounds with reported antioxidant, anti-inflammatory, and potential health-promoting effects. This study aimed to investigate cultivar-dependent differences in lignan metabolites across eight sesame cultivars (A-H) using untargeted metabolomics based on liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), combined with multivariate statistical analysis. A total of 29 lignan metabolites were detected in positive ion mode and 50 in negative mode. Principal component analysis (PCA) revealed clear metabolic differentiation among cultivars. In positive mode, PC1 accounted for 67.8% of the total variance, with cultivar H distinctly separated from the others. In negative mode, PC1 and PC2 explained 45.5% and 21.8% of the variance, respectively. The PCA models demonstrated good model fit (R^2 = 0.995 in positive mode, R^2 = 0.975 in negative mode). Further differentiation was confirmed through partial least squares discriminant analysis (PLS-DA). Models using three components yielded strong performance (positive mode: R^2 = 0.99, Q^2 = 0.95; negative mode: R^2 = 0.97, Q^2 = 0.93), with permutation testing (n = 1000) confirming statistical significance ($p < 0.001$). Key discriminant metabolites (VIP > 1) included tracheloside, sesamin, sesaminol, and matairesinol glycosides in positive mode, and hydroxysyringaresinol, demethyl-dehydroxy-matairesinol, and multiple secoisolariciresinol derivatives in negative mode. These results highlight substantial cultivar-dependent variation in lignan metabolism in sesame and underscore the utility of untargeted metabolomics in elucidating biochemical diversity. The findings provide a foundation for future studies exploring the biochemical pathways and cultivar-specific metabolic traits associated with lignan biosynthesis in sesame.

2226 B — Assessing analytical techniques for wood metabolomics through a comparison of DART-TOFMS and GC×GC-TOFMS for species-level wood identification

PRESENTING AUTHOR: *James Harynuk, University Of Alberta, Canada*
CO-AUTHORS: *Seo Lin Nam, Martin Williams, Isabelle Duchesne, Nathalie Isabel*
SUB THEME: *Other*

Illegal logging poses a significant threat to ecosystems and undermines legal timber markets. Efforts to combat the issue are often hindered by the lack of robust tools for species-level wood authentication. Traditional methods such as anatomical observations and DNA analysis frequently struggle to achieve accurate species-level identification. Chemical profiling of wood metabolites has emerged as a promising alternative. Two powerful techniques, Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (DART-TOFMS) and Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GC×GC-TOFMS) offer unique strengths for wood identification. DART-TOFMS, enables rapid chemical analysis with minimal sample preparation. GC×GC-TOFMS, known for its exceptional separation capabilities, allows for detailed chemical profiling that may enhance species-level discrimination. Despite their potential, direct comparisons of these two techniques for species-level classification have not been done. In this study, we present a comparative analysis using 60 wood core samples from two Quercus (Oak) species and 60 stemwood samples from three Picea (Spruce) species. Each sample was analyzed using both DART-TOFMS and GC×GC-TOFMS. Partial Least Squares Discriminant Analysis (PLS-DA) was employed for classification modeling, with feature selection based on VIP scores. The findings from this work will contribute to a deeper understanding of the capabilities of both techniques in wood metabolomics, with applications in forensic science and efforts to combat illegal logging.

2227 B — Integrated Metabolomics and Lipidomics Approach to Investigate Perfluorooctanesulfonate-Induced Neurotoxicity in Zebrafish Embryos

PRESENTING AUTHOR: ★ *So-Young Park, Kyungpook National University, South Korea*
CO-AUTHORS: *Eun Ki Min, Ki-Tae Kim, Kwang-Hyeon Liu*
SUB THEME: *PFAS*

Developing methodologies for performing multi-omics with one sample has been challenging in zebrafish toxicology; however, related studies are lacking. A new strategy for the simultaneous analysis of metabolomics and lipidomics in zebrafish embryos was proposed and applied to explore the neurotoxicity mechanisms of perfluorooctanesulfonate (PFOS). Metabolite and lipid profiled simultaneously with methyl tert-butyl ether (MTBE) were compared with individual results from other extraction solvents. Behavioral alterations were measured after the zebrafish embryos were exposed to 0.1–20 μM PFOS for 5 days. The metabolite-lipid profiles of the MTBE-based strategy analyzed with optimized larval pooling size of 30 were comparable to those of other extraction solvents, indicating the feasibility and efficiency of MTBE-based multi-omics analysis. Many metabolites and lipids, which were enriched more than those previously reported, completed the toxicity pathways involved in energy metabolism and sphingolipids, improving our understanding of PFOS-induced neurotoxicity mechanism manifested by increased movement under dark conditions. Our novel MTBE-based strategy enabled the multi-omics analysis of one sample with minimal use of zebrafish embryos, thereby improving data reliability on changes in multi-layered biomolecules. This study will advance multi-omics technologies that are critical to elucidating the toxicity mechanisms of toxic chemicals including per- and polyfluoroalkyl substances.

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2228 B — Metabolomics and lipidomics support plastidal acyl-ACP thioesterase as novel herbizide site-of-action

PRESENTING AUTHOR: ***Stephan Klatt, BASF Metabolome Solutions Gmbh, Germany***

CO-AUTHORS: ***Janneke Hendriks, Philipp Ternes, Stephan Klatt, Stefan Henkes, Aisha Gerhardt, Matthias Witschel, Johannes Hutzler, Michael Betz, Ruth Campe, Philipp Johnen***

SUB THEME: ***Plant defense, pathogens and interactions***

The prevalent occurrence of herbicide resistant weeds increases the necessity to identify new molecular targets for herbicides. Therefore, the mode of action of the unexploited molecule cinmethylin was reevaluated using a chemoproteomic approach, and cinmethylin was found to bind (plastidal) acyl-ACP thioesterase (fatty acid thioesterase, FAT) with high affinity. FAT plays a crucial role in plant lipid biosynthesis by mediating the cleavage of fatty acids from acyl-ACP, which is necessary for their export from the plastid to the endoplasmic reticulum. To confirm that FAT inhibition is indeed the major mode of action of cinmethylin in planta, targeted lipidomics was performed in addition to broad targeted metabolomics (M-profiling) and a set of standardized physiological assays (P-profiling). The lipidomics data show a decrease in free desaturated fatty acids, especially 18:1, as well as a strong and specific increase in di-16:0-monogalactosyldiacylglycerol (MGDG). This is consistent with an inhibition of FAT by cinmethylin. M- and P-profiling of plants treated with cinmethylin or other herbicides affecting lipid biosynthesis (acetyl-CoA-carboxylase inhibitors, very long-chain fatty acid inhibitors) showed that the plant response to cinmethylin is distinct from the response to these other herbicide classes. These results establish cinmethylin as herbicide with FAT inhibition as novel mode of action. A subsequent screen of chemically related compounds identified further molecules binding to and inhibiting FAT with high affinity in vitro, and lipidomics data revealed that the increase in di-16:0-MGDG can be used as a biomarker for FAT inhibition.

2229 B — ^{1}H NMR Metabolic Tracking of Plant-Insect Priming in the Glycine max - Rachiplusia nu System

PRESENTING AUTHOR: ***Guillermo Moyna, CENUR Litoral Norte, Universidad De La República, Uruguay***

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SUB THEME: ***Plant defense, pathogens and interactions***

Soybean (Glycine max) is the most widely cultivated summer crop in Uruguay, and one of its main pests is the soybean looper (Rachiplusia nu), whose larvae cause significant defoliation. Traditional pest management strategies have largely relied on chemical pesticides, which present numerous environmental and agronomic drawbacks. Consequently, alternative approaches that minimize pesticide use have become a research priority. Priming is a defense mechanism that places plants in a heightened state of alert following exposure to environmental elicitors, such as herbivore-induced volatiles or systemic signals from prior damage. This physiological state allows for faster and more robust responses to subsequent attacks and can be strategically integrated into pest management programs. It has been proposed that moderate herbivory may act as a natural priming stimulus. In this study, we used NMR based metabolomics to characterize metabolic changes in G. max leaves and R. nu hemolymph under varying infestation levels, considering both previously exposed (primed) and unexposed (naïve) conditions. Multivariate analyses revealed distinct metabolic signatures associated with both infestation intensity and priming. In soybean, metabolites such as 2-hydroxyisobutyrate and the carbosugar pinitol correlated with infestation density, while choline, GABA, and pinitol were associated with previous insect exposure. In larval hemolymph, significant metabolic shifts were observed in relation to both infestation levels and plant priming history. Notably, putrescine, trehalose, glycine, and histidine levels varied with infestation density, whereas phosphocholine and glycine levels were influenced by prior exposure. These findings contribute to the identification of metabolic pathways involved in herbivore-induced priming within the G. max - R. nu interaction. Combined with studies on mechanical defoliation and phytohormone treatments, this work supports the development of novel, sustainable tools for integrated pest management (IPM).

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★ AWARD WINNERS

PLANTS, FOOD, ENVIRONMENT AND MICROBES

2230 B — Stable-isotope-assisted metabolomics enables to study (a)biotic stress-related metabolism of tyrosine compared to phenylalanine in wheat

PRESENTING AUTHOR: ★ *Tomas Rypar, Mendel University In Brno, Czech Republic*

CO-AUTHORS: *Samuele Risoli, Maria Doppler, Bernhard Seidl, Christoph Bueschl, Alexandra Parich, Gerhard Adam, Rainer Schuhmacher*

SUB THEME: *Plant defense, pathogens and interactions*

To investigate the role of tyrosine (Tyr) during plant stress response, we employed a stable isotope assisted, untargeted metabolomics approach to compare its metabolism with the well studied phenylalanine (Phe) in wheat. Flowering wheat ears were treated either with 13C9-Phe or 13C9-Tyr as metabolic tracers under both control and stress conditions (mycotoxin and Fusarium spp. virulence factor deoxynivalenol; DON, 0.2 mg.ear-1). After 96 hours, samples were analyzed using LC-HRMS/MS and evaluated with MetExtract II.

Overall, 115 Phe- and Tyr-derived wheat metabolites were detected. The overall uptake and metabolization rate of Tyr was around 40% compared to 80% of Phe. Furthermore, 48 common metabolites were derived from both tracers. Approximately one-third of the detected metabolites was found to be significantly induced by DON treatment. These belonged to the classes of lignans, hydroxycinnamic acids and derivatives (including amides), which are known to be involved in fungal defense via e.g., cell wall reinforcement or direct antifungal properties. Twenty-five of the stress-related (i.e. induced/reduced) downstream metabolites were formed from both Tyr and Phe demonstrating the involvement of Tyr in the stress response. When compared to Phe, labeled Tyr showed lower incorporation into common downstream metabolites with approximately 1/4 (stress) and 1/6 (control) of the incorporation respectively. This indirectly suggests overall lower relevance of tyrosine ammonia-lyases compared to phenylalanine ammonia-lyases. However, on average, the Tyr incorporation was induced under DON stress compared to control conditions, whereas Phe incorporation rates remained relatively constant across treatments.

This study demonstrates the added value of a complementary and comparative use of labeled Tyr and Phe as tracers in combination with isotope-assisted metabolomics enhancing annotation confidence and interpretations supporting the study of molecular processes in plants during stress defense.

2231 B — BNI Potential in Wheat: Linking Root Exudates to Genotypic Variation and Soil Conditions

PRESENTING AUTHOR: *Christina Maisl, BOKU University, Austria*

CO-AUTHORS: *Bernhard Seidl, Maria Doppler, David Ruso, Alexandra Parich, Rainer Schuhmacher, Christoph Bueschl*

SUB THEME: *Plant metabolomics*

Biological nitrification inhibitors (BNIs) are released into soil by certain plant species to suppress soil nitrifier activity, thereby reducing microbial conversion from ammonium to nitrate. Since nitrogen is primarily lost to the environment in form of nitrate leaching, BNIs can help to enhance nitrogen use efficiency of agricultural fertilizers. Nitrogen losses contribute to groundwater contamination and greenhouse gas accumulation, posing significant threats to ecosystem and climate health. Wheat, one of the most important crops, has shown BNI potential, but the chemical identity of these compounds remains unknown. This study aimed to characterize the metabolic composition of wheat root exudates and identify and compare the abundance of exuded BNIs.

Eleven wheat cultivars were grown in a soil-hydroponics setup, designed to mimic field-relevant conditions while enabling efficient exudate recovery. After growing the plants in soil for two weeks, root exudates were collected by dipping roots into ultrapure water. The up-concentrated samples were analyzed using LC-HRMS/MS. Compound identification relied on spectral library matching and comparison with reference standards.

We successfully identified and quantified 6-methoxy-2-benzoxazolinone (MBOA), a known BNI compound, in all wheat cultivars. Notably, MBOA concentrations varied significantly between genotypes, with highest MBOA exudation observed for cultivar Capo, suggesting a heritable component and offering valuable insight for breeding strategies aimed at improving BNI capacity in wheat.

Further data processing will 1) employ molecular networking to search for structurally similar metabolites to known BNIs, which could potentially be novel BNIs, and 2) further characterize the metabolic composition of the exudates. Furthermore, in a follow-up experiment the influence of different soil parameters, microbial community composition, and different levels of nitrogen fertilization will be tested in respect to the exudation of MBOA. These findings will contribute to a more comprehensive understanding of BNI functionality in wheat and its potential for sustainable nitrogen management in agriculture.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2232 B — Metabolomic and transcriptomic analysis to untangle root exudation in response to iron deficiency in Capsicum spp.

PRESENTING AUTHOR: *Ana Álvarez-Fernández, EEAD, Spanish National Research Council (CSIC), Spain*
CO-AUTHORS: *Francisco José Jiménez-Pastor, Sara Ferrero de Teso, Jorge Rodríguez-Celma, Ana Álvarez-Fernández*
SUB THEME: *Plant metabolomics*

Iron (Fe) deficiency is a major abiotic stress limiting crop productivity, particularly in calcareous soils where Fe is mainly present as poorly soluble ferric oxides. While Fe acquisition in grasses via mugineic acids is well characterised, the identity and diversity of Fe-mobilising compounds secreted by non-grass species remain largely unknown. We investigated the impact of Fe deficiency on the root and root exudate metabolomes of Capsicum annuum and C. chinense. Root exudates were collected every other day over a 12-day Fe deficiency period and evaluated for their capacity to solubilise ferric oxide at physiologically relevant pHs. Exudates from days 10 and 12 showed the highest activity, independent of pH or species. Transcriptomic analysis (RNA-Seq) of C. annuum roots sampled at day 12 revealed upregulation of riboflavin and purine biosynthesis genes. Metabolomic profiling was performed exclusively on day-12 samples using RP-UHPLC-ESI(+)-QTOF-MS/MS, revealing thousands of metabolites. Of these, 140 metabolites in exudates (both species), 147 in C. chinense roots, and 1006 in C. annuum roots showed significant changes under Fe deficiency. Only 28 and 40 metabolites were shared between species in exudates and roots, respectively. Data analysis used MetaboScape, GNPS-FBMN, and Cytoscape. The annotation was based on an in-house spectral library focused on Fe-deficiency-induced specialised and public GNPS libraries. Riboflavin derivatives constituted the largest molecular cluster in exudates from both species; approximately one-third of these were also detected in root extracts, clustered primarily with nucleotide-related compounds. Phenolamides formed a smaller exudate cluster. No Fe-deficiency-induced coumarins, such as those found in Arabidopsis, were detected. Ongoing comparative analyses with other Solanaceae, including Solanum melongena, aim to expand knowledge on the metabolite diversity underlying Fe acquisition in agriculturally important species.

2233 B — Temporal Metabolomics Unveils Metabolic Reprogramming and Growth-Associated Transitions in Pea (Pisum sativum) Seedlings

PRESENTING AUTHOR: *Jongsung Lee, Incheon National University, South Korea*
CO-AUTHORS: *Yu–Mi Shin, Ye Jin Kim, HanGyeol Lee, Ji-Eun Ra, Woo Duck Seo, Jae Kwang Kim*
SUB THEME: *Plant metabolomics*

Metabolic reprogramming during early seedling development plays a crucial role in determining plant growth performance and nutritional quality. In this study, we investigated the dynamic metabolic changes in five pea (Pisum sativum) cultivars (Dacheong, Daehyeop 1ho, Sanghyeop, Sacheol, and Cheongmi) across five developmental time points (6, 9, 12, 15, and 18 days after germination) using semi targeted metabolite profiling. A total of 83 metabolites, including primary metabolites such as amino acids, organic acids, sugars, lipids, and carotenoids, as well as secondary metabolites including flavonoids, were identified.

Principal component analysis (PCA) combined with KEGG pathway annotation revealed distinct metabolic transitions corresponding to seedling growth stages. During the early stages (6 to 9 days), asparagine and raffinose accumulated prominently, indicating a reliance on seed-stored reserves to support heterotrophic metabolism. As seedlings progressed to intermediate stages (12 to 15 days), levels of branched-chain amino acids, carotenoids, and flavonoids increased, suggesting the initiation of autotrophic metabolism and activation of photosynthetic pathways. In the later stage (18 days), high concentrations of monosaccharides and chlorophylls were observed, reflecting photosynthetic maturation and a fully established autotrophic growth phase. Correlation analysis further revealed significant associations between photosynthetic pigments and secondary metabolites, particularly flavonoids, during the seedling period. Among the five cultivars, Dacheong exhibited the highest total flavonoid content (33.40 ± 1.29 mg/g) at 12 days after germination, highlighting its potential for enhanced nutritional quality.

These findings provide new insights into the metabolic strategies underlying the heterotrophic-to-autotrophic transition in pea seedlings and suggest potential approaches for improving growth characteristics and functional compound accumulation through targeted metabolic regulation.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2234 B — Metabolic profiling of pea seedlings (Pisum sativum) cultivated under various photosynthetic photon flux densities and growth periods

PRESENTING AUTHOR: *Yumi Shin, Incheon National University, South Korea*
CO-AUTHORS: *Jong Sung Lee, Ye Jin Kim, HanGyeol Lee, Ji-Eun Ra, Woo Duck Seo, Jae Kwang Kim*
SUB THEME: *Plant metabolomics*

Light intensity is a critical factor influencing plant metabolism. However, comprehensive studies on the nutritional and metabolic shifts during the pea sprouting stage are still limited, and the specific effects of light during this period remain poorly understood. Therefore, in this study, we investigated metabolic changes in pea seedlings under different photosynthetic photon flux density (PPFD) conditions (200, 400, and 800 μmol/m^2·s) and growth periods (9, 12, and 15 days).

A total of 83 metabolites, including 60 primary metabolites (organic acids, amino acids, sugars, sugar alcohols, and fatty acids) and 23 secondary metabolites (policosanols, terpenoids, carotenoids, and flavonoids), were identified in pea seedlings. Multivariate statistical analysis showed that PPFD had a greater effect on pea seedling metabolism than growth period. In addition, the contents of metabolites related to sucrose synthesis and photosynthetic pigments increased with increasing PPFD. Among the major metabolites contributing to the separation based on PPFD, photosynthetic pigments such as carotenoids and chlorophylls were more abundant under PPFD conditions of 400 and 800 μmol/m^2·s. In contrast, under PPFD level of 200 μmol/m^2·s, high contents of TCA cycle intermediates and amino acids were observed. Flavonoid content was highest at PPFD 400 μmol/m^2·s, suggesting that this condition is optimal for maximizing secondary metabolite accumulation. Additionally, the total content of flavonoids was highest in the sample collected at 9 days.

Overall, these results provide a basis for understanding the metabolism of pea sprouts under various PPFD conditions and could contribute to building a database for selecting growth conditions suitable for specific cultivation purpose.

2235 B — Analysis and annotation of phytoceramides: From apple waste to industrial applications

PRESENTING AUTHOR: *Samuel Ngure Kariithi, Institute Of Plant Biochemistry, Germany*
CO-AUTHORS: *Gregor Gottschalk, Ghislain Wabo Fotso, Henriette Uthe, Norbert Arnold, Bernhard Westermann, Jörg Ziegler*
SUB THEME: *Plant metabolomics*

Phytoceramides are plant-derived lipid compounds that mimic the function of ceramides, a class of lipids naturally found in the skin. Alterations in ceramide composition causes skin diseases such as psoriasis and atopic dermatitis. This imbalance can be compensated for by semisynthetic skin-identical ceramides in the form of semisolid creams. Ceramides are extracted from animal or plant sources with animal sources posing greater risk of diseases such as mad cow disease from bovine sources. Plants on the other hand provide safer eco-friendly alternatives. Among the plant sources is apple pomace which contains considerable levels of phytoceramides. Yearly, upto 2.7M tones of apple waste is generated worldwide which provides a cheaper alternative source of raw materials. This project was designed to i) develop an efficient eco-conscious extraction method for phytoceramides and ii) improve the annotation of existing and new phytoceramides. Extraction of apple pomace using non-chlorinated solvents resulted in crude yields ranging from 6-30% dry weight. The crude extracts were further purified first by using Diaion HP-20 followed by Sephadex LH-20 and lastly Silica gel. The fractions were then analyzed using an Agilent 1290 liquid chromatography coupled to a Bruker Daltonics timsTOFpro mass analyzer with electron spray ionization (LC-ESI-MS/MS). Separation was done using a C18 column from Bruker (Intensity Solo 2 diameter 2.1mm, particle size 2μm and length 100mm). Annotation of phytoceramides was done using Metaboscape software version 2022b from Bruker Daltonics. In total, 40 phytoceramides were detected, annotated and classified either as glycosylated or non-glycosylated phytoceramides. A commercially available ceramide together with four of the most abundant isolated phytoceramides were analyzed by the extensive use of 1D and 2D-NMR coupled with HRESIMS. The results positively correlated with the annotation results. These results highlight the underlying huge potential of apple pomace as a safer sustainable source of phytoceramides suitable for biopharmaceutical applications.

2236 B — Exploration of Metabolic Biomarkers to Assist Oil Palm Breeding and Improve Nutrient Use Efficiency

PRESENTING AUTHOR: *Rizki Anjal Puji Nugroho, Irhs, France*
CO-AUTHORS: *Guillaume Tcherkez, Jean-Pierre Caliman, Emmanuelle Lamade, Rudy Lukman, Adri Gusmar, Julie Lalande*
SUB THEME: *Plant metabolomics*

Oil palm (Elaeis guineensis) agroindustry faces several challenges, in particular for breeding. The development of elite planting materials is costly and time consuming. We have started a research program to assess the utilisation of metabolic markers to assist oil palm breeding by facilitating germplasm selection and identifying best responders to fertilizers. We took advantage of three genetic resources (populations): Deli Dura breeding population, genotypes of wild origin from Cameroon and Angola, and a group of E. oleifera x E. guineensis hybrids. For each population (cultivated in Sumatra), we applied a standard fertilization regime with different levels of N and K. Systematic metabolomics profiling via NMR were then used to document metabolic differences between, and within populations to investigate the genetic effect on metabolites and identify relationships between metabolic signatures and K and N use efficiency. Our intention is also to exploit the metabolic dataset to conduct GWAS analysis and then identify genomic regions that determine metabolites, particularly those that are responsive to N and K use efficiency. In addition, elemental and isotopic analyses have been carried out to assess changes in overall leaf composition and concurrent modification in water usage (via 13C and 2H natural abundance) or nutrient utilisation (via 15N and 34S natural abundance). Therefore, we show how metabolic methods can be used to characterize germplasms and thereby assist conventional breeding, saving time and land use.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2237 B — Metabolite-based genome-wide association studies enable the dissection of the genetic bases of bioactive metabolic compounds in Chickpea (Cicer arietinum L.)

PRESENTING AUTHOR: *Siyue Ruan, MPIMP, China*
CO-AUTHORS: *Lorenzo Rocchetti, Elena Bitocch, Regina Wendenburg, Alisdair Fernie, Roberto Papa, Saleh Alseekh*
SUB THEME: *Plant metabolomics*

Chickpea, is the second most consumed food legume, is a rich source of protein, dietary fiber, vitamins and micronutrients. Metabolites are essential for plants growth, development and influencing human nutrition and health. Secondary metabolites serve as bioactive compounds in plants and play key roles in various biological processes. During domestication, different chickpea varieties with various phenotypic changes were selected, resulting in significant metabolic variations. To address this and underlying the genetic basis of metabolic diversity in chickpea, we conducted a comprehensive metabolic profiling using UPLC-MS and analyzed both seed and leaf tissues of 450 chickpea accessions from three independent field trials. We quantified over 3000 metabolites and annotated around 700 of them. Using genome-wide association studies (GWAS) we detected numerous leading SNPs corresponding to metabolites variability, the comparison of seed and leaf GWAS datasets allowed the identification of tissue-specific QTL regulating saponins and iso-flavonoids in seeds and leaves, respectively. Through transgenic overexpression and hairy root transformation, we characterized a gene cluster of three bHLH TFs, which regulate soyasaponin biosynthesis in chickpea. In addition to support our finding, we preformed tissue-specific transcriptome profiling and conducted expression GWAS, in parallel with expression profiling through a full life-cycle of 20 chickpea accessions, to investigate the spatiotemporal distribution and biology mechanism of specific metabolites at the tissue levels. Our results reveal new insights about effect of domestication process on chickpea metabolome and origins, and provide valuable resources for genetic improving the bioactive metabolic compounds in chickpea.

2238 B — Integrated multi-omics profiling of warm-water extracts from cereal grains to identify potential bioactive compounds

PRESENTING AUTHOR: *Na Won Park, Pusan National University, South Korea*
CO-AUTHORS: *Cheol Woo Min, Ji Soo Kim, Gi Hyun Lee, Jong Ho Lee, Jeong Yoon Kim, Ravi Gupta, Sun Tae Kim*
SUB THEME: *Plant metabolomics*

Low-molecular-weight proteins have garnered significant attention due to antioxidant, anti-inflammatory, and antibacterial properties, and their enhanced ability to traverse cell membranes enables targeted health benefits. A recent study demonstrated that warm-water extraction can effectively extract proteins associated with bioactive materials synthesis in crop seeds. Therefore, in this study, we soaked barley, oat, and wheat grains at 55°C to obtain warm-water extracts (WWEs) and sequentially applied size exclusion filtration using 10KD filter unit to explore the bioactive materials. At first, we performed proteomic analysis using WWEs extracted from each grain, and this approach led to the identification of 570 differentially modulated proteins (DMPs). Further functional annotation of DMPs revealed that these are primarily associated with various cellular metabolic processes such as amino acid metabolism, stress response, and redox regulation. Subsequent metabolomic and peptidomic analyses using LMWPs fractions, enriched by 10KD filter unit from total WWE samples, were conducted to investigate potential bioactive materials in each grain. From these analyses, 747, 2,096, and 366 significant peptides, associated with antioxidant activity, stress response, and amino acid transport, were identified in barley, oat, and wheat grains, respectively. Additionally, we identified a total of 310 metabolites, of which 9 were mainly related to antioxidant, anti-inflammatory, and antibacterial activities with up-regulation exclusively in barley grain. For further verification, cell viability tests were conducted on normal human astrocytes (NHA) and bronchial epithelial cells (16HBE14o). Particularly, we observed that cell viability significantly improved after co-treatment for 72 h with barley LMWPs compared to other extracts. Furthermore, barley LMWPs enhanced the survival rate of 16HBE14o cells when co-treated with the insecticide hydramethylnon (HM). These findings strongly suggest that soaking cereal grains at 55°C results in the accumulation of diverse proteins and bioactive materials that may help prevent cell damage by regulating the cell cycle during insecticide exposure.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2239 B — Comparative acetylome analysis of two Oryza sativa subspecies japonica and indica reveals regulatory mechanisms during artificial aging

PRESENTING AUTHOR: *Ji Soo Kim, Pusan National University, South Korea*
CO-AUTHORS: *Cheol Woo Min, Ravi Gupta, Sun Tae Kim, Na Won Kim*
SUB THEME: *Plant metabolomics*

The importance of seed longevity in agricultural field is well-recognized. Various studies have successfully reported the precise genes/proteins involved in various the molecular mechanisms that lead to reduced longevity during seed aging in cereal crops. Our previous studies on rice cultivars, which included aging-sensitive and aging-tolerant types, demonstrated that the role of brassinosteroid signaling and antioxidant mechanisms for extending seed longevity during artificial aging treatment (AAT). Although post-translational modifications (PTMs) involved in various cellular metabolism are also critical for every aspect of regulation for cells and seeds viabilities, however, it still elusive. Here, we carried out enrichment of PTMs, especially acetylation, using two rice varieties (Ilmi; aging-sensitive and Dharial; aging-tolerant) after treatment of AAT for 3 and 6 days. This approach led to the identification of a total of 1,355 acetylated peptides, of which 300 and 331 acetylated peptides showed significant modulation (Benjamini-Hochberg FDR threshold <0.05) with more than 1.5-fold changes in Ilmi and Dharial varieties, respectively. Further functional classification of these using MapMan, KEGG pathway, and gene ontology (GO) enrichment analyses revealed that differentially modulated acetylome are primarily associated with various major/minor metabolisms, such as proteins and amino acid metabolism, carbon metabolism, stress responses, and various secondary metabolisms, among others, which may play a critical role in determining seed viability and longevity during AAT in rice. Taken together, our findings highlight the changes at acetylation in several key metabolic proteins provides insights into the molecular mechanisms underlying seed AAT and may contribute to the development of strategies for extending seed longevity and improving storage quality.

2240 B — Leveraging ion mobility spectrometry-mass spectrometry for the separation and structural characterization of monoterpenoid indole alkaloid isomers in Catharanthus roseus

PRESENTING AUTHOR: *Tetsuya Mori, RIKEN, Japan*
CO-AUTHORS: *Noriko Takeda-Kamiya, Takashi Nirasawa, Ryo Nakabayashi, Mai Uzaki, Yushiro Fuji, Kiminori Toyooka, Masami Hirai Yokota*
SUB THEME: *Plant metabolomics*

Plant specialized/secondary metabolites, with their structural diversity, have varying physiological activities and play a crucial role in the development of pharmaceuticals and other products. Catharanthus roseus synthesizes a range of monoterpenoid indole alkaloids (MIAs), several of which are valuable for their medicinal properties. The structural complexity of MIAs, including their stereoisomers, complicates their detailed analysis. We have been exploring the use of imaging mass spectrometry to visualize MIAs in germinating seeds of C. roseus, but differentiating between isomers has remained a challenge. To overcome this, our goal is to utilize mass spectrometry combined with ion mobility spectrometry (IMS) to analyze all MIA molecular forms, including stereoisomers, as IMS has the capability to separate these isomers. Fresh germinating seeds of C. roseus were embedded in a compound (FSC 22, Leica Microsystems) and frozen. The frozen blocks were sectioned with a cryostat (CM3050S, Leica Microsystems) and mounted on conductive copper tape (TERAOKA SEISAKUSHO) on an ITO-coated glass slide (Bruker). A matrix solution of 2,5-dihydroxybenzoic acid was sprayed onto the section using a TM Sprayer (HTX Technologies). The prepared samples were analyzed via matrix-assisted laser desorption/ionization-trapped ion mobility spectrometry-time of flight-mass spectrometry (MALDI-tims-TOF-MS, Bruker). Data processing was done with SCiLS Lab and MetaboScape software (Bruker). The combination of IMS and imaging mass spectrometry enabled us to visualize MIAs in germinating seeds and differentiate their isomers. We observed that ajmalicine and tetrahydroalstonine, which are stereoisomers, produced multiple peaks in the ion mobilograms corresponding to their m/z values. Interestingly, similar peaks appeared in the ion mobilograms of authentic standards of these compounds, suggesting the existence of other isomers and/or the protomer formation. We are also analyzing C. roseus extracts using liquid chromatography-tims-TOF-MS, with further results to be shown at the conference.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2241 B — Elucidating Zostera marina and Oryza sativa metabolomes by computational mass spectrometry techniques

PRESENTING AUTHOR: *Yoshimasa Todoroki, Tokyo University of Agriculture and Technology, Japan*

CO-AUTHORS: *Yuki Matsuzawa, Shohei Nakamukai, Keiichi Noguchi, Toshiyo Kato, Hiroyuki Kasahara, Naoko Ohkama-Ohtsu, Yu Umezawa, Hiroshi Tsugawa*

SUB THEME: *Plant metabolomics*

We compared the specialized metabolomes of the seagrass Zostera marina and the terrestrial crop Oryza sativa to investigate how secondary metabolism has adapted to contrasting marine and terrestrial environments. Z. marina, which re-adapted to the ocean from a land-based ancestor approximately 100 million years ago, has undergone major genomic changes linked to salinity tolerance, yet its capacity to produce novel specialized metabolites remains poorly understood. To address this gap, we performed untargeted metabolomics using LC-MS/MS under reverse-phase conditions, supplemented by computational tools for retention time prediction and structural annotation. A set of 599 authentic standards with measured retention time data supported the development of an XGBoost-based retention time prediction model that improved confidence in metabolite annotation from public and commercial spectral libraries.

Subsequent data analysis with MS-DIAL, incorporating molecular spectral networking, revealed substantial differences in the types of flavonoids and phenolic compounds between the two species. O. sativa showed unique tricin O-glycosides, C-linked flavonoids, and polyamine phenylpropanoids, reflecting terrestrial stress-defense mechanisms. In contrast, Z. marina contained several sulfated flavonoids, as well as distinct phenolic compounds that were particularly abundant in rhizome tissues. Among them, we isolated and structurally confirmed 7″,8″-didehydrosalvianolic acid B through NMR, demonstrating that Z. marina likely synthesizes specialized phenylpropanoid derivatives not present in terrestrial species.

Overall, our comparative approach highlighted the unique metabolic strategies each species has evolved to cope with its environment. Z. marina's distinctive sulfated flavonoids and phenolic derivatives may mitigate high salinity and microbial fouling, whereas O. sativa's specialized metabolites appear oriented toward pathogen defense and terrestrial stressors. By integrating computational mass spectrometry with advanced retention time prediction and extensive spectral libraries, we achieved a detailed picture of the metabolic divergence between these two monocots. This study expands our understanding of plant-environment interactions and underscores the evolutionary plasticity of secondary metabolism.

2242 B — Untargeted metabolomics uncovers specialized metabolites involved in Eucalyptus rooting: A foundation for enhancing vegetative propagation from mother plants in forestry.

PRESENTING AUTHOR: *Ignacio Salinas, Universidad De Concepción, Chile*

CO-AUTHORS: *Catalina Bravo, Nicolás Fernández, Javiera Nahuelcura, Alex Medina, Andy Pérez*

SUB THEME: *Plant metabolomics*

Worldwide, plantations of Eucalyptus species are widely used for paper production. E. globulus and E. nitens are the two most planted species in Chile. However, the low adaptability of E. globulus to cold climates led to hybridization breeding programs to obtain E. nitens × E. globulus, which have shown better frost tolerance and acceptable wood quality. However, such hybrid species have also shown significant variability in their rooting capacity during massive vegetative propagation from clonal mother plants. Therefore, in this work, we applied untargeted metabolomics to reveal the metabolites involved in the rooting process of small cuttings. These cuttings were obtained from mother plants of 10 different genotypes, including hybrids and parent species, with contrasting historical rooting data. After planting cuttings, they were collected on the days 7, 10, 13, 16, and 20, and processed in whole for metabolomics analysis by LC-MS. As results, the most significant metabolites in the constitutive metabolome of the high and low rooting genotypes before their propagation were identified, showing compounds as hydroxybutyl-glucosyl-butanoate, dihydro-eucaglobulin derivative and trigalloyl-glucoside derivative as up-regulated metabolites in low-rooting genotypes. While in high-rooting genotypes were identified glucogallin, 5,7-dihydroxy-2-methylpropyl-chromone-C-(galloyl)glucoside and 5,7-dihydroxy-2-methylpropyl-chromone-C-glucoside as up-regulated. It has been previously reported that tannins and monoterpenes may have an antagonistic role with some plant growth hormones such as gibberellins, which may be causing this rooting deficiency. Also, a high-rooting genotype was evaluated at different collection points in time. At the first sampling point, there is an up-regulation of mainly flavonol glycoside compunds, like quercetin-O-glucuronide and rutin. The up-regulation of these compounds from the first sampling point may mean the eventual release of the free aglycone over time, compounds that are directly related to the polar auxin transport in the plant.

Therefore, this research can help understand the biochemicals changes in plant rooting and aid forestry production.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

2243 B	**Exploring Metabolic Plasticity of Oxyria digyna for Bioactive Compound Production: Comprehensive Analysis of Natural, In Vitro, and Callus-Derived Sources**

PRESENTING AUTHOR: ***Mira Choi, University of Science and Technology (UST), South Korea***
CO-AUTHORS: ***Hyemi Jang, Moonkyo Kim, Neil Patrick Uy, Sanghyun Lee, Yoo Kyung Lee, Kyoung-Soon Jang***
SUB THEME: ***Plant metabolomics***

Oxyria digyna (mountain sorrel) is a perennial Arctic-alpine herb recognized for its adaptive metabolism and potential as a source of antioxidant and medicinal compounds. To evaluate its phytochemical plasticity across different biomass sources, we performed a comparative metabolomic analysis of ethanol extracts from natural (field-grown), in vitro-cultivated, and callus-derived tissues. Targeted analysis quantified twelve key phenolic compounds, including rutin, hirsutrin, and schaftoside. Notably, several compounds exhibited markedly higher concentrations in senescent in vitro leaves compared to their fresh counterparts, suggesting that tissue aging may enhance enzymatic conversion or promote the accumulation of specific bioactives. In parallel, untargeted metabolomic profiling using high-resolution trapped ion mobility quadrupole time-of-flight mass spectrometry (TIMS-QTOF MS) revealed distinct metabolic signatures for each tissue type. The integration of trapped ion mobility separation enabled the resolution of isomeric and low-abundance metabolites, greatly expanding the detectable chemical space. Multivariate statistical analyses further supported the differential clustering of samples, with senescent in vitro leaves displaying enriched levels of glycosylated flavonoids and phenolic derivatives. This suggests that age-related physiological shifts—such as increased oxidative stress or metabolic remodeling—may upregulate secondary metabolite biosynthesis or facilitate transformation toward bioactive end products. These findings highlight the metabolic adaptability of O. digyna and reveal the potential of senescent biomass, often overlooked in cultivation systems, as a valuable source for phytochemical production. Furthermore, the application of TIMS-QTOF MS underscores the utility of advanced analytical platforms in uncovering subtle metabolic variations and supports the development of optimized strategies for sustainable Arctic plant-based bioactive compound production.



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TECHNOLOGY ADVANCEMENTS

2252 B — Microbiopsy Sampling Coupled with Standardized Lipidomics for Assessing Skin Lipid for Dermatological Testing

PRESENTING AUTHOR: ***Alice Limonciel, biocrates life sciences ag, Austria***
CO-AUTHORS: ***Magali Sarafian, Antoine Thomas, Adrien Saliou, Florian Lapierre, Andrew Kirby, Stewart Long***
SUB THEME: ***At-home sample collection***

Introduction: The skin lipidome plays a crucial role in maintaining barrier function and hydration. However, minimally invasive and reproducible methods for assessing skin composition changes in response to topical treatments remain limited.

Aims: This study evaluates the feasibility of combining minimally invasive microbiopsy skin sampling with standardized analytical methods to robustly quantify lipid changes in small sample amounts.

Methods: Skin samples were collected in a controlled environment from 2 spots (one treated, one control) in five participants before and after a 2-week treatment with a moisturizer. Absolute lipid concentrations were measured using a broad panel including skin membrane (phospholipids, sphingolipids) and sebum lipids (glycerophospholipids).

Results: Initial findings reveal that the chosen analytical method enables the detection of 300 to 400 metabolites in pooled microbiospy samples. Upon moisturizing treatment, distinct shifts in lipid composition were identified, suggesting effects on barrier integrity and hydration.

Conclusions: This approach provides a standardized and sensitive methodology for studying skin composition changes in vivo with minimal discomfort to the study participants. The combination of microbiopsy sampling and targeted lipidomics represents a promising strategy for dermatological research, offering new insights into lipid dynamics in response to skincare interventions.

2253 B — Non-targeted metabolomics workflow for blood-microsampling devices

PRESENTING AUTHOR: ***Pauline Couacault, Helmholtz Zentrum, Germany***
CO-AUTHORS: ***Anna Artati, Michael Witting***
SUB THEME: ***At-home sample collection***

Blood and plasma are predilection matrices to monitor human health. Blood microsampling (BμS) has emerged as an alternative to these invasive sampling methods, especially for vulnerable groups such as infants or elderly. Several studies showed that BμS are suitable alternatives for analyzing endogenous metabolites and for metabolomics applications such as therapeutic drug monitoring, anti-doping, or toxicology analysis. Dried blood spots (DBS) have long been used for clinical applications, particularly for newborn screening. New quantitative BμS devices have emerged, such as volumetric absorptive microsampling (VAMS) or quantitative dried blood spots (qDBS).

Here we developed and optimized a non-targeted analytical workflow for BμS for polar metabolites with hydrophilic interaction chromatography (HILIC) and mid-polar metabolites with reversed-phase liquid chromatography (RPLC). Eight different extraction procedures were tested. The non-targeted HILIC-MS/MS method was developed with 63 standard molecules, including amino acids, co-enzyme A and derivatives, carnitines, organic acids, sugars, and biological metabolites. Analyses were performed on a Sciex ZenoTOF 7600. Data treatment and annotation were performed using mzmine 4.5 and in-house R scripts, with open and in-house libraries. Data were evaluated by the number of detected features, reproducibility and annotation rates. The workflow was tested on three commercially available BμS (DBS, VAMS, and qDBS).

*This work has been funded by the European Union under the HORIZON-MSCA-2021 project 101073062 : HUMAN – Harmonising and unifying blood metabolic analysis networks

2254 B — Optimized Methodology for Untargeted Breath Analysis by SPME-GC-MS

PRESENTING AUTHOR: ***Mª Fernanda Rey-Stolle, Cembio CEU, Spain***
CO-AUTHORS: ***Maria Bajo-Fernández, Laura Amorós, Celia Jiménes, Antonia García***
SUB THEME: ***Breath analysis/Volatolomics***

Exhaled breath comprises a wide and diverse range of volatile metabolites, offering significant insight into the biochemical processes within the human body. The development of breath analysis methodologies is encouraged due to its non-invasive nature, although it presents several challenges. The concentration levels of volatile organic compounds (VOCs) in breath are in the range of parts-per-billion (ppbs) and even parts-per-trillion (ppts). Consequently, highly sensitive analytical methods are required for their detection. Enrichment techniques are typically applied, and thermal desorption tubes (TD) are the most widely employed. However, special units are necessary for their use, entailing high acquisition and operational costs. Solid-phase microextraction (SPME) emerges as an alternative, not only because of its lower costs but also due to its reduced susceptibility to contamination, ease of transport, and reusability. This study presents the optimization of a methodology for breath analysis using SPME-GC-MS with potential applications in allergy and lung clinical research. The optimization aimed at achieving high sensitivity while covering the broadest range of VOCs. To achieve this, the GC oven program was optimized to enhance sensitivity and resolution in a short analysis time. Two sampling devices were considered, bags and vials, and their storage conditions were assessed. Additionally, three SPME fibers were evaluated, including PDMS, Car/PDMS, and DVB/Car/PDMS, along with two types of vials with different volumes (10 and 20 mL). The SPME extraction conditions of temperature and time were optimized by response surface methodology (RSM), applying a central composite design (CCD). Furthermore, three extraction approaches were evaluated: direct extraction from the two collection devices and automated extraction of samples collected in bags and transferred to vials. The comparison of these strategies aimed not only to select the most adequate method in terms of VOC detection, but also to consider reproducibility, sources of variability, contamination, and overall methodological efficiency.

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TECHNOLOGY ADVANCEMENTS

2255 B — Overcoming limitations in exhaled breath aerosol analysis – Untargeted breathomics for the identification of endogenous breath biomarkers using LC-HRMS/MS

PRESENTING AUTHOR: *Juel Maalouli Schaar, Saarland University, Germany*
CO-AUTHORS: *Selina Hemmer, Sascha K. Manier, Lea Wagmann, Markus R. Meyer*
SUB THEME: *Breath analysis/Volatolomics*

Human exhaled breath (EB) aerosol was shown to be suitable for the non-invasive bioanalysis of both volatile but also non-volatile compounds. One of the key challenges in drug monitoring studies is the lack of standardization in EB sample collection. The aim of the present study was to use untargeted breathomics for identification of potential endogenous biomarkers in EB that could address these challenges.

EB aerosol samples were collected using an impaction-based device containing three collectors. Six samples per volunteer (n=6, 3 females, 3 males) were obtained over one hour, with 12 expiration cycles collected every 10 minutes, and stored at –80 °C. For sample preparation, collectors were transferred into 5 mL tubes and extracted with 2.2 mL methanol containing internal standards. After 5 minutes of soaking, shaking, and centrifugation, 2 mL of extract was evaporated to dryness and reconstituted in 100 µL 80:20 acetonitrile (0.1% formic acid):water. Proof-of-concept extracts from 28 patient samples were processed similarly. LC-HRMS/MS analysis was performed using a TF Dionex UltiMate 3000 RS pump coupled to a Q Exactive Plus mass spectrometer with a HESI-II source. Data processing was performed in an R environment.

The results revealed a feature, that could be detected across all samples, thus representing a potential biomarker for standardizing EB aerosol sample collection. Although an annotation of this feature was not possible yet, it showed robustness and reproducibility in EB samples from healthy volunteers and could also be successfully detected in patient samples. This study represents a step forward in overcoming the current limitation of EB analysis, the lack of standardization in sampling procedure. Nevertheless, further investigation including correlation studies and more diverse populations will be essential for the confirmation of the clinical relevance and application of this potential biomarker.

2256 B — A Non-Invasive VOC-Based Diagnostic Framework for Post COVID Syndrome

PRESENTING AUTHOR: *Lea Woyciechowski, TU Braunschweig, Germany*
CO-AUTHORS: *Tushar More, Georg Behrens, Holger Volk, Karsten Hiller*
SUB THEME: *Breath analysis/Volatolomics*

Volatile organic compounds (VOCs) are small molecules (<500 Da) with high volatility, originating from exogenous sources (e.g., diet, medication) or produced endogenously as metabolic by-products. VOCs reflect cellular activity and disease processes and are excreted through sweat, breath, saliva, or urine.

Diagnosing post COVID Syndrome (PCS) is challenging due to its non-specific symptoms, which overlap with conditions such as Sjögren's Syndrome and Chronic Fatigue Syndrome (CFS), and the reliance on prior confirmed SARS-CoV-2 infection, which is now less common due to reduced testing. This highlights the urgent need for objective, non-invasive diagnostic tools. Inspired by canine olfactory detection studies, where trained dogs identified acute COVID-19 and potentially PCS through urine odor, we aim to apply VOC-based metabolomics combined with machine learning to identify disease-specific VOC signatures in immunologically characterized cohorts of PCS, Sjögren's Syndrome, and CFS patients.

We developed an optimized workflow using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) for urinary VOC analysis. Extraction parameters were refined for limited sample volumes, including adjustments to temperature, extraction time, salt concentration, and pH. A 75 µm Carboxen/PDMS fiber was selected for its broad VOC affinity. Method validation was conducted using urine from healthy volunteers, with a pooled reference sample included for normalization. Standard VOCs and mixes supported compound identification and sensitivity assessment. Data analysis included principal component analysis (PCA), heatmaps, and LASSO regression to identify discriminatory features.

This integrative approach aims to decode the VOCs detected by dogs, revealing whether viral remnants or reprogrammed metabolic pathways underlie post COVID pathogenesis.

The method may also lay the foundation for developing "electronic nose" technologies applicable to other complex, hard-to-diagnose conditions.

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TECHNOLOGY ADVANCEMENTS

2257 B — Benchmarking the ecTOF for Quantitative and Time-Resolved Cellular Metabolism Analysis

PRESENTING AUTHOR: ***Yi-Ming Denise Zhang, Max-Delbrück-Center Berlin, Germany***

CO-AUTHORS: ***Martin Forbes, Eliška Ceznerová, Steffen Bräkling, Arnd Ingendoh, Matthew Lewis, Sonja Klee, Stefan Kempa***

SUB THEME: ***Flux analysis***

The ecTOF is a novel dual-source gas chromatography-mass spectrometry (GC-MS) system that enables simultaneous acquisition of data using electron ionization (EI) and chemical ionization (CI) modes. This dual capability functionality provides the possibility to generate perfectly aligned molecular (CI) and structural (EI) information on the detected analytes within a single chromatographic run. It offers new opportunities for comprehensive metabolomic profiling, including improved sensitivity and isotopic labeling analysis.

We evaluated the ecTOF platform (Bruker Daltonics, Bremen, Germany) for biomedical metabolomics applications using MSTFA derivatization, a widely adopted method in the field. To benchmark performance, we employed in-house standard mixtures: for metabolite identification (IDENT) and for quantification (QUANT). The system offers a linear response up to 5 orders of magnitudes - we quantified metabolites in both EI and CI modes over three orders of magnitude using the QUANT mixtures, with CI mode exhibiting enhanced sensitivity achieving limits of detection (LOD) in the low picomole range – approximately 10-fold lower than those observed in EI mode.

To assess isotopomer and isotopologue analysis capability, we performed ^{13}C glucose labeling of HeLa cells for 20 minutes, followed by GC–MS analysis as described by Pietzke and Zasada et al. (2014). The ecTOF successfully detected label incorporation in central carbon metabolism, consistent with expected isotopic enrichment patterns. Among the 50 metabolites identified, 29 were quantified, and ^{13}C label incorporation was observed in four metabolites, with labeling detected in both fragment ions (EI and CI) and molecular ions (CI). Notably, the detection of label incorporation in the molecular ion provides an additional layer of structural and quantitative information, enhancing the resolution and interpretability of future metabolic flux analyses.

Our results demonstrate that the ecTOF is a powerful tool for quantitative and time-resolved metabolic flux analysis, offering robust sensitivity and dual-ionization insights for biomedical research applications.

2258 B — High-throughput ex vivo stable isotope tracing in tissue homogenates for in vivo metabolic profiling

PRESENTING AUTHOR: ***Shauni Loopmans, VIB - KU Leuven, Belgium***

CO-AUTHORS: ***Sam De Craemer, Mitsugu Shimobayashi, Steve Stegen, Bart Ghesquière***

SUB THEME: ***Flux analysis***

Stable isotope (e.g. 2H, 13C) tracing is a powerful tool for mapping metabolic activity, primarily used in vitro where systems are highly controllable, and nutrients can be completely replaced by their stable isotope counterparts. However, to fully comprehend the metabolic pathways associated with health and disease, in vivo data remain essential. Unfortunately, performing such in vivo tracing in mice or humans remains challenging for technical and ethical reasons, respectively. Moreover, in mice, studying multiple nutrients would require different animals for each stable isotope tracer, rapidly increasing the number of animals needed for experiments. Therefore, (high-throughput) ex vivo tracer metabolomics imposes itself as an attractive alternative for advancing our understanding of the metabolic pathways underlying (patho)physiology.

Here, we present a methodology for performing ex vivo stable isotope tracing on freshly collected tissue. Using gentleMACS dissociation, mice kidneys, livers and hearts were homogenized and supplemented with U-13C-glucose, U-13C-glutamine or U-13C-palmitate. A time-course analysis of glucose and glutamine incorporation showed steady-state labeling in glycolytic and TCA cycle intermediates after 8–12 hours, whereas fatty acid oxidation of U-13C-palmitate reached steady-state after 2-4 hours. Importantly, a comparison between the different organ homogenates confirmed previously described metabolic differences in nutrient usage and allocation. For example, U-13C-glucose tracing revealed higher levels of transamination to alanine in the kidney compared to liver and heart, whereas glycogen breakdown and/or gluconeogenesis were primarily observed in the liver. Likewise, U-13C-palmitate tracing showed higher fatty acid oxidation in the heart compared to liver and kidney. We also demonstrate the compatibility of our methodology with pharmacological screening, as treatment of kidney homogenates with 2-deoxy-D-glucose significantly decreased the incorporation of U-13C-glucose.

Taken together, we present a novel ex vivo tracer metabolomics method using tissue homogenates, allowing the use of multiple stable isotopes on a single tissue sample and providing compatibility with pharmacological screening.

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TECHNOLOGY ADVANCEMENTS

2259 B — 20 Key Metabolites for Unraveling Metabolic Pathway Activity via 13C-Isotopomer Analysis

PRESENTING AUTHOR: *Dries Verdegem, VIB - KU Leuven, Belgium*
CO-AUTHORS: *Bart Ghesquière*
SUB THEME: *Flux analysis*

Stable Isotope Resolved Metabolomics (SIRM) is a powerful technique in which nutrients administered to living cells, tissues, or organisms are labeled with stable, naturally low-abundant isotopes, such as 2D, 13C, or 15N. This approach has a rich history of revealing differential biochemical pathway activities in biomedical research. To ensure high sensitivity, much of this research is conducted using mass spectrometry. However, interpreting the complex labeling patterns in downstream metabolites, which often exhibit isobaric overlap, presents a significant challenge. To address this, researchers frequently employ specific singly or minimally labeled nutrient tracers, simplifying their research questions to basic presence-or-absence analyses of the labels. Unfortunately, the required tracers are often commercially unavailable or prohibitively expensive. Alternatively, labeling patterns from fully labeled nutrients can be examined through MS/MS analysis. While this isotopomer analysis shows great potential, it has thus far been limited to three metabolites: lactic acid, aspartic acid, and glutamic acid. At present, it remains unclear which metabolites are suitable for isotopomer analysis or how much additional pathway activity information can be extracted through this approach.

We conducted a theoretical study to explore the potential and limitations of isotopomer research. For each metabolite size (based on carbon content), we determined the number of orthogonal fragments necessary for a potential or guaranteed full isotopomer elucidation. Additionally, we calculated the average quantified isotopomer fraction (AQIF) as a function of the number of fragments for each metabolite size. By integrating these findings with MS/MS data and cellular abundance information from the HMDB, we compiled a unique, groundbreaking list of 20 metabolites, selected from 217,920 HMDB entries, that are well-suited for metabolic isotopomer research. Our analysis demonstrates that, given that each n-carbon metabolite has 2^n distinct 13C isotopomers, the number of theoretically distinguishable alternative biochemical pathways contributing to their abundances is remarkably vast.

2260 B — Automated High-throughput Metabolomics

PRESENTING AUTHOR: *David Wishart, University of Alberta, Canada*
SUB THEME: *High-throughput metabolomics*

In this presentation I will describe the efforts my laboratory has made towards developing fully automated, high-throughput (HT) methods for: 1) targeted NMR-based metabolomics; 2) general GC-MS metabolomics; 3) targeted LC-MS metabolomics and 4) unknown metabolite identification for untargeted LC-MS metabolomics. These efforts include the development of fast, user-friendly software, databases, kits and low-cost robotic systems. In the area of NMR-based metabolomics I will describe MagMet, a generalized spectral deconvolution program that fully automates the quantitative analysis of biofluids and beverages. When coupled with robotic NMR sample handling, the entire NMR metabolomics pipeline can be fully automated and very high throughput. In the area of GC-MS metabolomics I will report on GC-AutoFit, a program we developed that can automatically identify and quantify metabolites via targeted GC-MS studies as well as another tool called GCMS-ID, a database and program that can be used to rapidly identify metabolites and accelerate throughput for untargeted GC-MS studies. In the area of LC-MS metabolomics I will introduce the MEGA (720 metabolites) and GIGA (1780 metabolites) metabolomics kits, along with their accompanying software (LC-AutoFit) that support high throughput, semi-automated LC-MS based metabolomics. I will also describe the low-cost (modified 3D printer) robotic systems that we have developed to fully automate the operation of the GIGA and MEGA kits. Finally, I will discuss the work we have done to help automate untargeted LC-MS metabolomics, especially in the area of unknown identification. Specifically, I will introduce DeepMet and FraGNNet, two deep learning programs that show tremendous promise in being able to automate and accelerate the identification of unknowns detected via LC-MS metabolomics studies. I will conclude by describing how metabolomics researchers may freely access many of these HT tools and technologies.

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TECHNOLOGY ADVANCEMENTS

2261 B — Developing a novel MS imaging technique to decipher redox metabolism in tomato flower.

PRESENTING AUTHOR: ***Rémy Cordazzo, INRAE, BFP UMR 1332, France***

SUB THEME: ***Imaging MS***

Redox metabolites play a vital role in regulating signalling pathways essential for both developmental and environmental responses in plants. These responses encompass critical processes such as the development of reproductive organs, including pollen formation in flowers and early fruit growth. Preliminary transcriptomic and metabolomic findings suggest that a precise balance between reactive oxygen species and antioxidants is necessary for tomato flower fertility. For a better understanding of redox mechanisms, it is essential to map the spatial distribution of these compounds within the flower.

We developed mass spectrometry imaging (MSi) methods using MALDI and DESI techniques, which are typically suited for large molecules like proteins and lipids. However, analysing small polar compounds such as redox metabolites (oxidised and reduced ascorbic acid and glutathione, NAD(P)(H) and chlorogenic acid) presented challenges. We optimised the MS imaging workflow, from sample preparation to MS analyses. Key challenges included the ionisation and detection of small analytes and their diffusion due to polarity (logP between -0.4 and -7.1). We thus optimised MALDI source parameters, laser frequency, intensity and compared different matrices (9AA for negative mode, DHB and CHCA for positive mode) to address ionisation issues. Matrix crystallisation was enhanced by focusing on sprayer height, layer number and drying time, but also matrix concentration and solvent. To minimise metabolite diffusion, we tested various embedding media (CMC, gelatin), thaw-mounting, and double-sided tape for section adhesion onto an ITO-coated glass slide.

These methodological advancements enabled the differential spatial mapping of central and specialised antioxidants, such as ascorbic and chlorogenic acids, in heat-stressed tomato flowers. Future work will compare DESI with MALDI MS imaging. Overall, this method development will facilitate redox studies in plants, particularly under environmental stresses like temperature waves, which significantly impact fruit production, in tomato and fruit trees particularly.

2262 B — Evolutionary metabologenomics of Streptomyces specialized metabolism: a population-level look into chemical diversity

PRESENTING AUTHOR: ***G. Nayeb Ghanbar Hosseini, Institute of Biology Leiden - Instituut Biologie Leiden (IBL), Netherlands***

CO-AUTHORS: ***Jordi Weijers, Paco Barona Gomez***

SUB THEME: ***Imaging MS***

Streptomyces species exhibit extensive chemical diversity even within populations. These soil-dwelling bacteria display notable diversity, not only at the level of their specialized metabolomes but also in terms of phenotypic and morphotypic heterogeneity. Central to this heterogeneity is the ability of these microbes to evolve and adapt to ecological changes, including heterogeneity of the Streptomyces colony, particularly antibiotic biosynthesis, which is a subject of significant scientific interest. Here we hypothesize that this heterogeneity arises from microevolutionary pressures throughout microscale environmental gradients, driving the diversification of biosynthetic gene clusters (BGCs) and specialized metabolite production. As a result, we expect Streptomyces species to display strain-specific chemical diversity within a single population. To decipher this complexity we adopt a metabologenomic approach with taxonomic, temporal, and spatial resolution, that includes yearly soil population-level isolation, MALDI-TOF biotyping, genome mining, phylogenetics, LC-MS/MS and DESI-MS imagining. After three years, our approach has enabled a comprehensive assessment of the population dynamics of one new-to-science Streptomyces species. Characterization of members of this soil Streptomyces population, with common but also diverse phenotypes, morphotypes, and chemical diversity, highlights that heterogeneity arises from microevolutionary pressures. To characterize this chemical diversity, we employ Desorption Electrospray Ionization-Mass Spectrometry Imaging (DESI-MSI) to leverage with GNPS and genomic data. This approach enables the visualization and spatial distribution of metabolites within Streptomyces populations at the colony level. Localization of Clavulanic acid and Cephamycin, and their dynamics between them, in Streptomyces katsurahamanus highlights that DESI-MSI is a powerful approach to disentangling chemical diversity with spatial resolution. Overall, our work shows that integrating high spatial resolution imaging with mass spectral and genomic data provides novel insights into the heterogeneity and metabolic complexity of soil-derived Streptomyces populations.

2263 B — Comprehensive Metabolite Profiling in Single-Cell Systems via Dual-Modal MALDI-Mass Spectrometry Imaging

PRESENTING AUTHOR: ***Jie Yuan, Shanghai Institute of Materia Medica. Chinese Academy of Science, China***

CO-AUTHORS: ***Xiafei Li, Xinxin Shen, Pei Xiong, Nanlin Zhu, Yang Ye, Jia Liu***

SUB THEME: ***Imaging MS***

The development of spatial multi-omics technologies, particularly matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), has revolutionized our ability to map metabolic processes at single-cell resolution. However, current techniques face challenges in minimizing matrix interferences and achieving comprehensive metabolite detection across multiple ionization modes. In this study, we present a novel dual-modal MSI workflow that leverages the pairing of 1,5-diaminonaphthalene (DAN) and its hydrochloric salt (DAN-HCl) matrices for sequential detection in positive and negative ion modes, respectively. This approach significantly enhanced metabolite coverage, spanning both lipid-based and non-lipid small molecules, while eliminating the need for solvent cleaning steps. Applied to a co-culture of cholangiocarcinoma (CCLP1) and hepatic stellate (LX2) cells, the workflow revealed significant metabolic distinctions, including differential accumulation of glycerolipids and energy-related metabolites, highlighting the unique metabolic profiles of each cell type. Additionally, several unidentified metabolites were detected, indicating the potential to discover novel metabolic variations. These findings establish our method as a robust tool for single-cell spatial metabolomics, with broad applicability in studying complex cellular interactions and advancing both research and clinical applications.

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TECHNOLOGY ADVANCEMENTS

2264 B — Deep Metabolome Profiling Using mzmine and Self-Supervised Deep Learning Molecular Networking on a novel timsTOF instrument

PRESENTING AUTHOR: *Lucy Woods, mzio GmbH, Germany*

CO-AUTHORS: *Robin Schmid, Ansgar Korf, Steffen Heuckeroth, Roman Bushuiev, Tomas Pluskal, Nikolas Kessler, Aiko Barsch, Claudia Martelli, Sven Meyer, Heiko Neuweger, Matthew Lewis*

SUB THEME: *Ion mobility spectrometry*

Metabolomic experiments generate vast amounts of data, in which only a small proportion of compounds can be annotated. Trapped ion mobility spectrometry (TIMS) enhances peak capacity and enables mobility-resolved MS2 spectra collection via parallel accumulation-serial fragmentation (PASEF), leading to deeper insights per injection. Transforming these complex datasets into knowledge requires advanced data mining strategies, including molecular networking for exploring unknown chemical space.

We present a workflow for deep metabolome profiling using a novel timsTOF instrument, mzmine data processing, and interactive molecular networking. Two data-dependent acquisition (DDA) LC-MS2 methods were developed: one without TIMS (autoMSMS) and another with TIMS (PASEF). mzmine processing covered raw data import, spectral processing, 3D feature detection (LC-MS2), 4D feature detection (LC-TIMS-MS2), ion identity and feature-based molecular networking (FBMN), and spectral library matching using MSnLib and MassBank of North America (MoNA). For FBMN, we applied both modified cosine similarity and the recently introduced DreaMS (Deep Representations Empowering the Annotation of Mass Spectra) transformer-based neural network for spectral similarity.

mzmine enabled rapid comparison of both acquisition methods. The LC-TIMS-MS2 approach using PASEF outperformed conventional DDA LC-MS2 in total detected features, acquired MS2 spectra, and annotated compounds. Mobility-resolved MS2 acquisition via PASEF reduced chimeric spectra, while the LC-TIMS-MS2 method generated more connected molecular networks with fewer singleton nodes compared to the conventional LC-MS2 method. The DreaMS neural network further minimized singleton nodes compared to modified cosine similarity, which is tuned for single modifications. In contrast, DreaMS connects structural analogs with multiple or complex modifications, forming complementary networks.

The combination of a novel timsTOF instrument and mzmine enhances feature detection, spectral acquisition, and annotation. TIMS-PASEF reduces chimeric spectra, while DreaMS improves molecular networking by linking structural analogs, advancing metabolomics research.

2265 B — Dendritic polyesters for CCS calibration in positive and negative mode

PRESENTING AUTHOR: *Jens Sommertune, POLYMER FACTORY SWEDEN AB, Sweden*

CO-AUTHORS: *Cedric Thom, Oliver Schmitz*

SUB THEME: *Ion mobility spectrometry*

Structurally flawless, symmetrical and polar bis-MPA based dendrimers have recently been proven to be excellent candidates for ion mobility mass spectrometry (IM-MS) calibrations - for both collision cross section (CCS) and mass accuracy[1]. Presented work has been focusing on positive mode in ESI and MALDI sources. Here, we present results for studies performed in negative mode which is deemed important for the lipidomic and metabolomic field.

This poster presents the results of DT CCS value studies performed on an Agilent 1290 Infinity II Bio HPLC and discusses the potential benefit to the IM-MS society.

Ref:

[1]Utilization of bis-MPA Dendrimers for the Calibration of Ion Mobility Collision Cross Section Calculations

Emily R. Sekera, Árpád Somogyi, Zoltan Takáts, Florian Stappert, Cedric Thom, Oliver J. Schmitz, Claudia Moeckel, Béla Paizs, and Jens Sommertune

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TECHNOLOGY ADVANCEMENTS

2266 B — Novel LC-MS workflow for simultaneous targeted and untargeted metabolomics and lipidomics profiling of plasma and urine in large-scale studies

PRESENTING AUTHOR: *Christian Schultewolter, Bayer AG, Germany*

CO-AUTHORS: *Zhipeng Wang, Oleksandr Boychenko*

SUB THEME: *New developments in instrumentation*

Metabolomics plays a crucial role in supporting drug discovery, advancing biomarkers identification and understanding fundamental biological processes. The success of untargeted metabolomics studies relies on the unambiguous identification of metabolites, which is essential for gaining acceptance among interdisciplinary research teams. To address this challenge, we developed a novel LC-MS methodology that combines, in one sequence, a targeted selected ion monitoring (tSIM) approach capable of quantifying approximately 800 metabolites and lipids using an internal reference database, which includes MS2 data and retention times, merged with untargeted deep profiling through ddMS2 acquisition.

We combined micro-flow LC (50 µL/min, C18) methods for the separation of metabolites and lipids with Orbitrap MS1 and Astral MS2 untargeted data acquisition and tSIM. This increased the number of high-confidence metabolites by approximately two times, due to the increased sensitivity of tSIM quantification in comparison with the untargeted Orbitrap/Orbitrap ddMS2 or AcquireX workflow. The automated switch between water/acetonitrile and water/acetonitrile/isopropanol mobile phases enabled sequential profiling of metabolites and lipids on the same column without user interaction. The long-term robustness, retention time stability, and signal reproducibility of the novel LC-MS workflow were confirmed on plasma and urine samples. To enable the reproducibility of large cohort analyses, we used 24 spiked heavy-labeled compounds that encompass a complete retention elution range (including amino acids, carboxylic acids, dipeptides, steroids, vitamins, and lipids) to monitor system performance and QC samples to enable post-acquisition data correction for untargeted profiling. Overall, our metabolomics-lipidomics workflow showed robust performance for plasma and urine.

This metabolomics platform aids in understanding disease, discovering new biomarkers, and confirming targeted metabolic and lipidomic changes that are translated from tissues to biofluids. We are committed to further enhancing metabolome depth by expanding our library of reference compounds to comprehensively characterize the metabolome in biofluids and tissue.

2267 B — Accuracy in Global Profiling: The Role of Instrument Sensitivity

PRESENTING AUTHOR: *Aiko Barsch, Bruker Daltonics GmbH & Co KG, Germany*

CO-AUTHORS: *Sven W. Meyer, Claudia Martelli, Cristian De Gobba, Nikolas Kessler, Matthew R. Lewis*

SUB THEME: *New developments in instrumentation*

The quantitation of analytes introduced for mass spectrometry via electrospray ionization is often influenced by the presence of other analytes, a phenomenon known as the “matrix effect.” This effect can be measured and corrected in targeted analyses using isotopically labelled internal standards. However, applying these principles to global profiling studies is impractical due to the broad scope of analytes and lack of prior knowledge of their identities, compromising measurement accuracy. Emerging strategies involve large mixtures of internal standards and extrapolation from proxy standards. This study explores the role and value of instrument sensitivity in mass spectrometry.

A mixture of flavonoids and isotopically labelled internal standards was combined with NIST SRM human urine at various concentrations in a dilution series. The samples were analyzed by reversed-phase chromatography coupled to high sensitivity TIMS-MS. Analyte ionization suppression was measured across the retention time range and at each urine sample dilution. The urine metabolic profiles were evaluated for depth and data richness.

Inaccurate measurement of metabolic profiles can confound statistical analysis, obscuring or erroneously creating biomarker signatures. Conventional data quality assessment in global profiling studies focuses on the precision of replicated measurements in an unchanging matrix (typically a “pooled QC”), overlooking the matrix effect. Greater urine sample dilution was associated with reduced matrix effect, indicating that measurement accuracy is proportional to sample dilution. The extent of the effect depended on the local matrix environment, with the most heavily suppressed areas being early column breakthroughs. High sample dilution negatively affected profile depth and richness, which can be compensated by increasing instrument sensitivity. Our observations indicate a four-fold decrease in signal suppression with a commensurate ten-fold sample dilution enabled by high sensitivity mass spectrometry. Thus, mass spectrometry sensitivity directly enhances profile accuracy, improving global profiling studies and biomarker discovery.

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TECHNOLOGY ADVANCEMENTS

2268 B — In-depth structural annotation of acylcarnitines using a novel Q-ToF-MS and electron-induced dissociation

PRESENTING AUTHOR: *Valentina Ramundi, Helmholtz-Munich, Germany*
CO-AUTHORS: *Michael Witting*
SUB THEME: *New developments in instrumentation*

Acylcarnitines are crucial intermediates in mitochondrial energy metabolism, facilitating the transport of fatty acids into the mitochondrial matrix for β-oxidation and ATP production (Indiveri et al., 2011). Disruptions in acylcarnitine metabolism are strongly associated with metabolic, cardiovascular, and neurodegenerative diseases, making them valuable biomarkers (McCann et al., 2021) for early diagnosis and disease monitoring. In particular, abnormal acylcarnitine profiles are key indicators of mitochondrial fatty acid oxidation disorders, such as carnitine-acylcarnitine translocase (CACT) deficiency, which presents severe metabolic and cardiac complications in infancy. Despite their clinical relevance, the structural complexity of acylcarnitines has challenged conventional mass spectrometry techniques like collision-induced dissociation (CID), which often fall short of providing detailed structural information. To address this, electron-activated dissociation (EAD)-based MS/MS has emerged as a powerful alternative (Wang et al., n.d.), enabling high-resolution structural characterization of fatty acid chain lengths and double bond positions via sequential -CH_2 losses. In positive ionization mode, EAD allows complete structural elucidation, and two highly specific and reproducible fragment ions (m/z 144.1077 and 162.1132) offer promise as diagnostic markers for rapid screening (van der Hooft et al., 2015). We utilized a novel Q-ToF-MS system with enhanced sensitivity and EAD capabilities to perform detailed structural elucidation of acylcarnitines from NIST 1950 reference plasma. The results demonstrated a clear enhancement in sensitivity, signal intensity, and structural detail using the novel instrumentation with MS2 accumulation times compatible with UHPLC applications. Our analysis shows that with the setup used, we can locate positions of double bonds, hydroxy groups, and methyl branches.

2269 B — Resolving the polar metabolome using porous graphitic carbon stationary phase for reversed phase liquid chromatography.

PRESENTING AUTHOR: *Chris Pook, Liggins Institute, The University Of Auckland, New Zealand*
CO-AUTHORS: *Francesca Day, Farha Ramzan, Justin O'Sullivan*
SUB THEME: *New developments in instrumentation*

Despite advances in stationary phase and column design primary metabolites such as sugars, amino acids, phosphorylates and the carboxylic acid intermediates of the Krebs Cycle remain poorly resolved by reversed phase chromatography. While HILIC or gas chromatography can resolve polar metabolites both have established limitations in the simplicity of sample preparation and analytical complexity. We set out to develop and validate a powerful, fast and simple method using Porous Graphitic Carbon [PGC] reversed stationary phase combined with aqueous trichloroacetic acid deproteinisation and extraction and conventional high resolution mass spectrometric detection for untargeted polar metabolomics of human serum.

Our method resolves a significant fraction of the polar chemical space covering a logP range of −9 to 6. It resolves very small, polar metabolites such as ethanolamine and choline, to larger, polar metabolites such as glutathione conjugates, and mid-polarity metabolites such as nucleoside phosphates. The performance of the method was validated using commercially available isotopically labelled metabolite standard mixtures with Coefficient of Variation <0.16 for ten standards and recovery of 60-140% for eight. Analysis of extract dilution series showed good quantitative performance with 80% of the 274 features annotated by MS-DIAL software showing r2 values >0.8. The method is highly complimentary to our existing mid-polarity reversed phase metabolomics method with 41% of 637 annotated features unique to the PGC method reported here and only 13% common to both methods.

Our method shows excellent performance at capturing qualitative and quantitative information about an important and previously poorly accessible fraction of the metabolome that includes many other metabolites with demonstrated relevance to human health and disease. Examples identified include microbially-derived metabolites such as the obesogen delta-valerobetaine and the insulin sensitivity modulator indole propionate. The method is fast and simple and uses mobile phases compatible with other reversed phase analysis. This potentiates high-throughput, automated analysis.

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TECHNOLOGY ADVANCEMENTS

2270 B — Boosting the profiling of clinical metabolomics projects using a novel trapped ion mobility spectrometry TOF-MS

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CO-AUTHORS: *Denis A. Ispan, Boris Dymov, Patrick Boschmann, Stefanie Wernisch, Lucia Laugwitz, Chrtistoph Laske, Bernhard Krämer, Thomas Gasser, Sven W. Meyer, Matthew R. Lewis, Christoph Trautwein*

SUB THEME: *New developments in instrumentation*

Despite massive advancements in mass spectrometry (MS) in the past years, the detection of very low abundant metabolites in in LC-MS based untargeted metabolomics is challenging. Also, false positive annotations can be an issue, especially in large-scale profiling.

Here, we compare the profiling results from established timsTOF 4D metabolomics workflows with data generated with a novel instrument optimized for small molecules. It provides unprecedented depth of coverage of the overall metabolome running in mobility-enhanced 4D acquisition mode.

We performed optimized 4D metabolomics profilings for several biofluids from our daily (pre-)clinical research on a timsTOF Pro 2: A) cerebrospinal fluid (CSF) samples from patients suffering from Alzheimer's (AD) and Parkinson's disease (PD), B) human urine extracts from neuropediatric metachromatic leukodystrophy (MLD) patients and C) blood serum from female patients with various stages of endometriosis. All sample types were analyzed using dedicated 4D-LC-timsTOF-MS/MS workflows, via HILIC and RP chromatography in both polarities. Additionally, the samples were analyzed on a novel timsTOF high-sensitivity instrument for small molecule analysis in HILIC positive mode.

The incorporation of CCS values for metabolite detection resulted in improved data confidence of the resulting annotations. For example, using our established workflows (Bruker MetaboBASE® and NIST spectral libraries), we achieved 115 high-quality annotations in CSF from AD and PD patients. High-quality annotations are characterized by high matching scores across the 4 selectivity dimensions of accurate mass, MS/MS, retention time, and ion mobility. Improved small molecule coverage on the novel timsTOF instrument manifested as significant increase of signal intensities across analyzed samples, yielding >50% additional features and annotations that were previously of poor quality (low annotation score). This new development opens the door for analysis of very small sample amounts, but most importantly offers even deeper phenotyping possibilities in metabolomics and lipidomics research.

2271 B — Dual metabolomics-proteomics extraction of murine bone marrow-isolated cells

PRESENTING AUTHOR: *Julie Courraud, University of Athens, Greece*

CO-AUTHORS: *Ioanna Barla, Panagiota Efstathia Nikolaou, Maria Voumvouraki, Nikolaos Thomaidis*

SUB THEME: *New multi-omics workflows*

With the increasing accessibility of HRMS and computational tools for multi-omics integration, there is growing interest in combining metabolomics and proteomics. However, sample availability often limits such analyses, especially in preclinical models. We designed pilot experiments to evaluate simultaneous extraction protocols capable of yielding high-quality metabolites and proteins from a single biological sample. Bone marrow is highly relevant to cancer, immunology, and cardiology research. Bone marrow cells were isolated from four (N=4) C57BL/6 mice (femurs and tibias). The marrow was flushed with 2 mL PBS/2% FBS. Cells were centrifuged at 300 g for 6 min and washed with PBS after red blood cell lysis. Three extraction solvents were tested (6 replicates, 1×10^6 cells each): 80% methanol, 80% acetone, and 100% acetone. After a brief sonication, samples were left to settle for 1h at -20°C in the dark, then spun down for 10 min at 15,000 g. The metabolite-rich supernatant was dried under vacuum and stored at −80°C. The remaining protein pellet was redissolved in 50 µL trifluoroacetic acid, neutralized with 2 M Tris base, and quantified using the Lowry method. Two micrograms of proteins were reduced & alkylated (TCEP/CAA), and digested with trypsin overnight. Peptides were purified onto C18 stage tips before analysis. The metabolomics platform consisted of a Dionex UltiMate 3000 RSLC UHPLC system (ThermoFisher Scientific) coupled with a MaXis impact Q-TOF MS (Bruker). The proteomics platform consisted of a nanoElute 2 coupled with a timsTOF fleX MS (Bruker) operated in diaPASEF. Raw files were processed with TASQ (Bruker) and FragPipe v22.0, respectively. Technical repeatability, including features coefficient of variations across the six replicates, will be presented. Feature-Based Molecular Networking will allow the comparison of the metabolite coverage of the three different extraction solvents. Advantages and limitations of each solvent will be discussed.

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TECHNOLOGY ADVANCEMENTS

2272 B — 1H NMR Pure Shift Metabolomic Analysis of Black Tea

PRESENTING AUTHOR: *Han Wang, University Of Manchester, United Kingdom*
CO-AUTHORS: *Laura Castañar, Ralph Adams, Stephen Fowler, Gareth Morris, Mathias Nilsson*
SUB THEME: *NMR metabolomics*

Pure shift NMR provides a significant improvement in resolution, by suppressing homonuclear scalar couplings, but at the cost of sensitivity. This work compares the untargeted metabolomic analysis results obtained using different pure shift methods (PSYCHE, TSE-PSYCHE, and 2DJ-PSYCHE) with those from conventional 1D 1H and 2D J-resolved experiments. The comparison was made on a selected set of black teas from four regions and of two cultivars. The efficiency of these spectra for tea classification and metabolite identification was evaluated with chemometric methods including principal component analysis (PCA), fold change (FC) analysis, and t-test in order to find potential biomarkers for sample classification. PCA suggests that despite the lower sensitivity, the enhanced resolution of PSYCHE gave similar classification power of the different varieties of black tea to standard 1D 1H NMR. PSYCHE also makes relative quantification possible even in the most crowded region of the conventional 1H NMR spectrum. The feasibility of using 1D projections of 2DJ-PSYCHE spectra for an NMR metabolomics study was evaluated for the first time. A pure shift 1D 1H NMR spectrum and a phase-sensitive 2D J-spectrum can be obtained in a single experiment using the 2DJ PSYCHE method; this approach is particularly attractive for NMR metabolomic studies.

2273 B — DIA acquisition and data processing opportunities in untargeted metabolomics

PRESENTING AUTHOR: *Gordana Ivosev, SCIEX, Canada*
CO-AUTHORS: *Pradeep Narayanasamy, David Cox, Jinal Patel, Huini Du, Anjali Chelur, Cuong Le*
SUB THEME: *Non-targeted and semi-targeted methods*

Data-dependent acquisition (DDA) is widely used for LC-MS-based qualitative and quantitative analysis of small molecules but faces challenges like missing MS/MS spectra and inaccuracies in MS1 quantitation due to analyte co-elution. Standard Data-Independent Acquisition (DIA) methods can provide superior qualitative and quantitative results, as seen in the field of proteomics. However, the adoption for small molecule analysis is limited by fragments from co-isolated precursors that may or may not be chromatographically separated, incomplete libraries and false positives during data processing.

Here, we explore a novel DIA method, ZT Scan, and data processing approach to address the limitations of DDA and standard DIA methods for small molecule analysis.

Using metabolites extracted from NIST 1950 human plasma and spiked with pesticides and isotopically labeled metabolites, samples were prepared and analyzed using Agilent 1290 Infinity HPLC coupled with a SCIEX ZenoTOF system. A 12-minute reversed phase separation gradient and CID-based fragmentation were used. The acquisition methods included DDA, MRMHr, SWATH, and ZT Scan. Different settings were assessed for qualitative and quantitative performances and throughput.

ZT Scan involves sliding quadrupole isolation window across the precursor m/z range while continuously recording MS/MS events within TOF pulses. Fragment spectra are produced by summing TOF pulses that overlap with a quadrupole isolation window and then condense around a narrow precursor range. Fragment intensity profiles along the chromatographic and quadrupole dimensions are used to collate related fragments and associate them with an accurate precursor. The set of fragment ions, precursor mass and retention time enable identification by spectral matching against library containing metabolites and pesticides and quantitation of analytes using fragment ion signals.

Results revealed that ZT Scan, coupled with novel data processing, significantly improved the metabolite identification rate over DDA and SWATH while preserving MS2-based quantitation depth and accuracy. Thus highlighting an advantageous technique for untargeted analysis.

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TECHNOLOGY ADVANCEMENTS

2274 B — Moving Beyond ddMS2: Improving Annotation Confidence in Untargeted Metabolomics Using Higher Resolution MS and Parallel Ion Trap Experiments

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SUB THEME: *Non-targeted and semi-targeted methods*

In metabolomics, both known and unknown compounds need characterization, with mass spectrometry playing a crucial role. Mass spectrometry determines elemental formulas using accurate precursor m/z values along with resolved isotopic patterns while structural composition is investigated using fragmentation data. However, as m/z values increase, the number of potential formulas rises, complicating accurate determination. Isomers and isobars further challenge characterization. This study explores increasing Orbitrap resolution to enhance elemental formula annotation using isotopic fine patterns and improved annotation confidence with additional MSn or breakdown curve data via parallel ion trap scans.

Chromatography involved methanol and water with 0.1% formic acid on a C18 Thermo Scientific™ Hypersil GOLD™ column using a Thermo Scientific™ Vanquish Horizon™ HPLC. An extract of NIST1950 plasma was analyzed on a Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer at resolutions from 60,000 to 1,000,000. AcquireX™ sequences included five injections with ddMS2 and either ddMS3 or a five-point breakdown curves in the ion trap. Data were processed using Thermo Scientific™ Compound Discoverer™ and Freestyle™.

Preliminary data was evaluated for elemental composition predictions at different resolutions with a focus on complex isotopic fine patterns. For instance, a compound with m/z 496.3396 had three possible formulas within 0.35 ppm mass accuracy. At 60K resolution, the A2 pattern was unresolved, but at 1M resolution, a 15N +13C peak and 18O peak ruled out two of the potential formulas.

Annotation confidence was further evaluated with ddMS3 and breakdown curve data using Compound Discoverer. Isomers with similar MS2 spectra, like paracetamol and 2-acetamidophenol, were distinguishable at the MS3 level. Breakdown curve data showing ion intensities at different energy levels also helped differentiate isomers such as theophylline and paraxanthine.

Combining high-resolution MS with diverse fragmentation strategies enhances annotation confidence. Future work will explore the impacts of higher resolution and additional fragmentation on experimental throughput.

2275 B — Untargeted profiling of the urine metabolome using ion-exchange chromatography-mass spectrometry (IC-MS)

PRESENTING AUTHOR: *Isabelle Legge, University Of Oxford, United Kingdom*

CO-AUTHORS: *Mia Hippisley, Tereza Kacerova, Rachel Williams, John Sidda, John Walsby-Tickle, James McCullagh*

SUB THEME: *Non-targeted and semi-targeted methods*

Urine metabolomics offers a window into system-wide changes in metabolism that occur in diseases such as diabetes, cancer and inborn errors in metabolism. In addition, urine biomarkers can be useful for early detection and diagnosis, monitoring drug treatments and responses, side effects, diet and nutritional studies, xenobiotic metabolism, and a person's exposures. However, urine contains highly polar and ionic metabolites, with many conjugated to enhance their polarity for excretion. This makes selecting an appropriate analytical platform for comprehensive metabolite characterisation an important consideration. Whilst reversed-phase, ion-pairing, ion-exchange, mixed-mode, gas-phase (with derivatisation), super-critical and HILIC chromatography have all been used in urine metabolomics, HILIC-MS/MS is probably the most common. This method is not without limitations, however, including poor reproducibility, peak shapes and low coverage of some classes of ionic metabolites, including organic acids and phosphorylated metabolites. Recently ion-exchange chromatography-mass spectrometry (IC-MS) has provided an alternative approach.

In this study we developed and validated a urine metabolomics protocol using IC-MS. We used a Dionex ICS-5000+ Ion-chromatography system coupled to a Thermo Q Exactive hybrid quadrupole-Orbitrap mass spectrometer and evaluated a range of solvent extraction and urine normalisation approaches. We analysed batches of up to 120 urine samples comparing a range of disease cohorts alongside standards, blanks and quality control samples as well as authentic standards. We performed targeted and untargeted method validation, including Rt stability, linearity, LLODs, %CVs and matrix effects for selected metabolites. We illustrate the results comparing urine from a range of cancer and non-cancer patients.

In this study we have established and validated an ion-exchange chromatography-mass spectrometry protocol for urine metabolomics which complements existing methods and provides a number of benefits. These include highly polar and ionic metabolite coverage, retention time stability, sensitivity and selectivity, flexible sample preparation (solvent choice), simplified matrices and robustness.

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TECHNOLOGY ADVANCEMENTS

2276 B — Plasma Benchmark: a multi-platform LC-MS profiling of the human fasting plasma metabolome

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SUB THEME: *Non-targeted and semi-targeted methods*

The human plasma metabolome has been studied in detail. While thousands of characterized plasma metabolites are theoretically within the reach of LC-MS analytical platforms, the number of well-known and repeatedly detected metabolites is much smaller and poorly standardized. We aimed to compile a database of the 'usual suspects of the plasma metabolome' by analyzing reference plasma in three independent laboratories utilizing similar untargeted LC-MS methodology and determining the known and unidentified metabolites that fulfilled our requirements of robust detection. We characterized 288 such metabolites, of which 236 were putatively identified and 52 were unknown. The combination of four analytical modes was essential for the analytical overage. There were differences between chemical classes in fulfilling the robustness requirements. We developed an online tool (https://plasmabenchmark.utu.fi/) to visualize the benchmark metabolome and the key characteristics of the metabolites. This study sets a framework for a more reproducible detection and reporting of key human plasma metabolites in typical LC-MS metabolomics assays.

2277 B — Untargeted information-dependent acquisition analysis of microbially-conjugated bile acids and N-acyl amides

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CO-AUTHORS: *Matilda Kråkström, Santosh Lamichhane, Pieter C. Dorrestein, Alex Dickens, Matej Orešič*

SUB THEME: *Non-targeted and semi-targeted methods*

Emerging studies suggest that gut microbe-derived lipids play a crucial role in the regulation of host metabolism. Gut microbes can conjugate bile acids (BAs) in the intestine, and increasing evidence suggests these microbiome-conjugated BAs influence host health, including the immune system development in early life. N-acyl amides (NAAs) are a large and diverse group of lipids and some are already known to be important signaling molecules, such as endocannabinoids. Recent evidence suggests that more compounds belonging to the NAA family are present in biological systems than previously thought. These studies have motivated the development of a semi-quantitative liquid chromatography-mass spectrometry (LC-MS) method for comprehensive profiling of the microbially-conjugated BAs and NAAs. We have exploited standard mixtures produced by combinatorial chemistry and reference spectral libraries in the GNPS database. We have analyzed conjugated BAs in human fecal samples from infants (n=300) on the Waters Premier liquid chromatography system equipped with two parallel analytical columns, connected to a high-resolution mass spectrometer (Sciex ZenoTOF). In total, 89 of the analytes were detected in at least part of the samples, and fragmentation spectra confirmed 57 % of the identifications in samples. For lysine and ornithine conjugates, two corresponding chromatographic peaks suggested the presence of isomers in both standards and samples. In the case of NAAs (n=1,426), several chromatographic gradient adjustments and acquisition method optimizations, including collision energy (CE), have been performed to achieve the desired fragmentation. A CE of 25 was optimal for most of the conjugates, however, some of these conjugates also required lower or higher energies. NAAs are low in abundance in biological samples, thus the analytical method optimization is necessary to enrich the analytes. This study highlights the method development strategy for reliable analyses of recently discovered analytes within biological samples.

2278 B — Thin Layer Chromatography / Mass Spectrometry - a Simple Alternative for Phospholipid Profiling

PRESENTING AUTHOR: *Hannah Koller, Leipzig University, Germany*

CO-AUTHORS: *Johanna Schubarth, Jürgen Schiller, Claudia Wiesner*

SUB THEME: *Novel approaches in lipidomics*

Phospholipids are important biomolecules involved in membrane formation, signaling, trafficking, and metabolism, which makes them promising biomarkers for various diseases. However, the large number of different molecular species and the complex integration into biological matrices make their analysis challenging. In addition, problems such as carry-over, increased noise due to non-specific extraction and column ageing due to interactions of the surfactant-like species with the stationary phase affect the performance of conventional liquid chromatography with tandem mass spectrometry (LC-MS/MS) as a reference method.

As an alternative, our study consolidates Thin-Layer-Chromatography (TLC) as a valuable tool for phospholipid analysis, particularly in combination with automated Matrix-Assisted-Laser-Desorption (MALDI) and Desorption-Electrospray-Ionization (DESI) directly from the TLC surface. We explored the potential of TLC-MS as a memory-effect free method for the relative quantification of phospholipid profiles compared to that from a conventional LC-MS/MS workflow. A semi-quantitative approach was used to understand the differences obtained by the two surface ionization techniques in an experiment on phospholipid profiles across different extraction protocols.

Our results confirmed previous findings: liquid-liquid extraction based on the combination of chloroform and methanol shows advantages over other common extraction protocols in terms of the number of detected phospholipids and method reproducibility. While LC-MS/MS provides the broadest coverage and highest sensitivity, TLC-MS methods still reliably detect major phospholipid species, supporting their use for an initial screening. In an extension of previous studies, however, we compared the lipid profiles obtained by MALDI and DESI ionization and discuss the differences with respect to the corresponding ionization mechanisms. This work demonstrates the utility of TLC-MS as a viable and informative tool in lipidomics and explores the integration of surface ionization techniques with TLC for fast and efficient phospholipid analysis.

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TECHNOLOGY ADVANCEMENTS

2279 B — Characterisation of triacylglycerols in milk at the double bond positional level using oxygen attachment dissociation (OAD).

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CO-AUTHORS: *Emily G. Armitage, Alan Barnes, Georgios Theodoridis, Neil J Loftus*
SUB THEME: *Novel approaches in lipidomics*

Introduction and Aim

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

Methods

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

Results

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

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

Conclusion

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

2280 B — Exploring dermal interstitial fluid as an alternative specimen for forensic drug testing: Focus on methamphetamine and ketamine

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SUB THEME: *Novel samples/matrices*

Dermal interstitial fluid (d-ISF) has emerged as a promising bioanalytical specimen due to its strong correlation with blood in reflecting endogenous compound levels. However, its potential application in forensic drug testing remains unexplored. This study evaluates d-ISF as an alternative specimen for confirming methamphetamine (MA) and ketamine (KE) ingestion. To assess its suitability, we conducted a pharmacokinetic comparison between d-ISF and plasma following intravenous administration of 1 or 5 mg/kg MA or KE in rats. The concentrations of MA and its primary metabolite amphetamine (AP), as well as KE and its metabolites, norketamine (NKE) and hydroxynorketamine (HNKE), in d-ISF were highly concordant with those in plasma. Key pharmacokinetic parameters, including half-life, area under the concentration-time curve, volume of distribution, and terminal elimination rate constant, were comparable between d-ISF and plasma at both doses. These findings highlight d-ISF as a viable alternative matrix for forensic drug testing, offering a minimally invasive yet reliable approach for drug monitoring and bioanalysis.

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TECHNOLOGY ADVANCEMENTS

2281 B — Exploring the salivary metabolome: key drivers and insights from 1,436 European Children

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CO-AUTHORS: ***Emile Callemeyn, Kimberly de Windt, Vera Plekhova, Kathleen Wijnant, Pablo Vangeenderhuysen, Beata Pomian, Stefaan De Henauw, Nathalie Michels, Heli Viljakainen, Marja Leppänen, Timo A. Lakka, Karolien Van De Maele, Nele Baeck, Ruth De Bruyne, Sander Lefere, Anja Geerts, Matthijs Vynck, Lynn Vanhaecke***

SUB THEME: ***Novel samples/matrices***

Alarming statistics reveal that one in three school-aged European children is overweight or obese, a trend accompanied by a declining mental well-being, both impacting their quality of life. Therefore, this study investigated saliva as a non-invasive biofluid to assess how its metabolome reflects age, weight (BMIz, waist-to-height and body fat percentage), psychology (depressive feelings, self-esteem, perceived stress, eating behavior and body image) and lifestyle (sleep, physical activity and screen time), while also considering associations to dietary intake and its microbiome.

Saliva samples from 1,436 European participants (7-18 years) across two discovery (FAME; n=539 and Fin-HIT; n=475) and two validation cohorts (PANIC; n=339 and OPERA; n=112) were analyzed using our in-house developed and validated validated salivary ultra-high-performance liquid chromatography high-resolution mass spectrometry method (Wijnant et al. 2020, AC). Associations were assessed using Spearman's correlation, univariate statistics, and Mummichog analysis.

The discovery cohorts revealed >3,500 untargeted and 188 consistently detected targeted metabolites (Tier 1 identified). Weight status and age showed the strongest correlations with the salivary metabolome, followed by psychological parameters and lifestyle. Multiple metabolites, particularly amino acids and their derivates, were positively linked to weight-related and psychological parameters across cohorts, confirming studies in other biofluids. Several of these weight-associated metabolites also correlated with bacterial phyla reported as perturbed in overweight, including Bacteroidetes and Firmicutes. Interestingly, the artificial sweetener acesulfame K, was detected in saliva in all cohorts and correlated positively with artificially sweetened food intake and weight-related and psychological parameters. Notably, children with overweight and/or high stress reported higher consumption of these foods.

These findings highlight saliva as a promising biofluid for non-invasive pediatric health screening, reflecting metabolic changes and dietary habits linked to weight status, mental wellbeing and the microbiome. The consistent association of acesulfame K with weight and psychological stress points towards an association between these, warranting further investigation.

2282 B — A workflow for simultaneous quantitation and discovery analysis using Revident LC-QTOF to follow CRISPRi knockdowns in E. coli metabolism

PRESENTING AUTHOR: ***Anna Walke, Agilent Technologies, Germany***

CO-AUTHORS: ***Stefan Schaebler, Karen E. Yannell, Daniel Cuthbertson***

SUB THEME: ***Other***

With large cohort studies in metabolomics, researchers need to maximize the amount of information from metabolomics samples to reduce the need for reinjection. Often, the scope of metabolomics experiments is quantification or untargeted discovery separately. Unfortunately, the strategies for data collection and evaluation for these experiments are highly different. Herein, a workflow including data acquisition and chemometrics analysis is established that combines targeted and untargeted experiments in a single injection to increase efficiency and information returns per each sample.

Amino acid and TCA cycle organic acid standards were used to establish a matrix calibration in untreated E. coli controls. For knockdown experiments aroC CRISPRi strains were isolated[1]. Using data-independent acquisition (DIA) in addition to traditional data dependent (DDA) and targeted approaches for MS/MS acquisition, amino and organic acids were quantified in control and knockdown E. coli. Additionally, metabolites differentiating the groups were evaluated with multivariate statistical tools with a combination of 3rd party as well as vendor software.

DIA data were evaluated by targeted data mining using a HILIC database containing retention times and spectra for 500+ metabolites[2], minimizing false positive identifications. For most analytes, the linear working range was low nM to high µM concentrations showing sufficient dynamic range for real sample measurements. Reproducibility of both the DIA and DDA measurements was good with low coefficient of variation between replicate injections (<12%). At fixed scan rates DIA resulted in enhanced data points per LC peak, improving feature finding and quantitation.

This work showcases the use of DIA QTOF data for the two most common experiments in metabolomics: Quantitation and untargeted discovery. The approach enables a shared data acquisition strategy and effectively limits the number of injections per sample compared to traditional approaches.

1: Donati, S. et al. https://doi.org/10.1016/j.cels.2020.10.011.

2: Yannell, K et al. ASMS poster ThP-085. 2024.

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TECHNOLOGY ADVANCEMENTS

2283 B — Perspicacité-AI: An AI-Pipeline for Accelerating Scientific Education and Research

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SUB THEME: *Other*

Recently, emerging tools such as LLM have gained attention in scientific research and education. However, their limitations — notably outdated knowledge and untrackable provenance — hinder their ability to keep pace with rapid advancements in scientific fields, like metabolomics. Some of these challenges can be addressed by coupling LLM with Retrieval-Augmented Generation (RAG) techniques, which combine relevant data retrieval with generative AI. Here, we developed Perspicacité-AI, a free and open source agentic literature assistant with advanced RAG capabilities designed for scientific research and education.

Perspicacité-AI required the development of the Bibt2KB python package, which seamlessly transforms open-access bibliographic references into a structured and searchable Facebook AI Similarity Search (FAISS) knowledge base (KB). The generated KB is used by the pipeline's framework to deploy an AI-assistant enhanced by RAG, ensuring that each question asked is grounded in relevant literature and documents. The framework features different advanced modes of document search and question-answering, including Perspicacité-Profound, an iterative, structured reasoning workflow suitable for answering complex scientific questions with the trackable facts and sources. Furthermore, the pipeline supports major open source and proprietary LLM providers, broadening the access for different users.

Perspicacité-AI also incorporates a novel reranking function for document selection, Sigmoid Weighted RRF (SW-RRF). The benchmarking of SW-RRF showed up to 3.5% improvement over the traditional Reciprocal Reranking Function (RRF) for all tested metrics: Recall, Precision, F1, Mean Reciprocal Rank (MRR), Average Precision (AP) and Normalized Discounted Cumulative Gain (NDCG) at 1, 3, 5 and 10 documents retrieved ($p < 0.05$) at no computational expenses.

Initial applications of Perspicacité-AI in computational metabolomics [metaboguide.holobiomicslab.eu] demonstrated effective chatbot responses with accurate citations, and while still ongoing, initial evaluations of the pipeline show promising results, not only mitigating some of the limitations of standalone LLMs but empowering scientific literature with generative AI.

2284 B — Chiral LC-MS/MS approach for separation and quantification of 2-hydroxyglutaric acid and Lactate as potential biomarkers

PRESENTING AUTHOR: *Kefan Cheng, University of Tuebingen, Germany*
CO-AUTHORS: *Adrian Sievers-Engler, Siavash Mansouri, Peter Freisinger, Saskia Biskup, Rajkumar Savai, Yi Zhong, Franziska Poeske, Katarina Pejic, Patrick Inci, Sophie Schnepf, Christoph Trautwein, Michael Lämmerhofer*
SUB THEME: *Other*

Chiral analysis of 2-hydroxycarboxylic acids (2-HCAs) is essential due to their enantiomer-specific biological roles, particularly for 2-hydroxyglutaric acid (2-HGA), where (D)-2-HGA is recognized as an oncometabolite associated with IDH-mutant tumors and epigenetic dysregulation[1]. Building on the work by Calderon et al. [2], who established a chiral separation method for 2-HCAs using Cinchonan carbamate-based weak anion exchangers, this study aimed to further develop and optimize a chiral LC-MS/MS method, with a focus on clinical applicability. CHIRALPAK® QN-AX columns (150 × 2.1 mm and 150 × 4.6 mm) were evaluated using an Agilent 1290 Infinity UHPLC system coupled to an API 4000 LC-MS/MS (Sciex) and an Agilent 1290 Infinity II UHPLC coupled to a QTrap 4500 LC-MS/MS (Sciex), respectively. Systematic evaluation of elution modes, mobile phase compositions, and other column parameters were performed. As a result, baseline enantioseparation was achieved for 2-HGA and lactate. Finally, the developed method was validated with human serum samples from glioma patients with IDH mutations, confirming its applicability to complex biological matrices.

[1]P. Wang, J. Wu, S. Ma, L. Zhang, J. Yao, K.A. Hoadley, M.D. Wilkerson, C.M. Perou, K.L. Guan, D. Ye, Y. Xiong, Oncometabolite D-2-Hydroxyglutarate Inhibits ALKBH DNA Repair Enzymes and Sensitizes IDH Mutant Cells to Alkylating Agents, Cell Rep 13 (2015) 2353–2361.

[2]C. Calderón, M. Lämmerhofer, Chiral separation of short chain aliphatic hydroxycarboxylic acids on cinchonan carbamate-based weak chiral anion exchangers and zwitterionic chiral ion exchangers, J Chromatogr A 1487 (2017) 194–200.

2285 B — Multi-targeted analysis of steroids by liquid chromatography-tandem mass spectrometry

PRESENTING AUTHOR: *Min Su, University of Tübingen, Germany*
CO-AUTHORS: *Bernhard Drotleff, Zoè Bürger, Birgit Derntl, Michael Lämmerhofer*
SUB THEME: *Quantitation in metabolomics*

In the field of steroid analysis within diverse biological matrices (plasma, saliva), the pursuit of precise, accurate and reliable quantification of endogenous and exogenous steroids at low concentrations remains a persistent challenge. In this study, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed to address this challenge. Employing 1,2-dimethylimidazole-5-sulfonyl chloride for estrogen derivatization, we aimed to enhance sensitivity of estrogens quantification in human plasma. This derivatization reagent provides higher intensity compared to the commonly used dansyl chloride, along with analyte-specific fragmentation, thereby enhancing confidence in method selectivity [1].

By simultaneous detecting both derivatized estrogens and non-derivatized other steroids, the method enables low-level quantification. Stable isotope-labeled standards were utilized as surrogate calibrants along with complementary stable isotope-labelled internal standards to ensure matrix-matched calibration of steroids in the actual biological matrix. Within the surrogate calibration strategy, we evaluated parallelism between the response of analytes and corresponding surrogate calibrants in the matrix of interest. Coupled with a solid-phase-extraction sample preparation approach, our method achieves picogram-level quantification of steroids in human plasma.

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TECHNOLOGY ADVANCEMENTS

2286 B — Comparative Evaluation of µLC-MS/MS and Derivatization Approaches for Oxylipin

PRESENTING AUTHOR: *Zijing Xu, University Of Tuebingen, Germany*
CO-AUTHORS: *Michael Laemmerhofer*
SUB THEME: *Quantitation in metabolomics*

Oxylipins, bioactive lipid mediators derived from the oxidation of polyunsaturated fatty acids, play pivotal roles in various physiological processes including inflammation, immunity, and lipid metabolism. However, due to the low concentration in biological samples, complex matrix effect and multitudinous structural isomers, it is hard to detect and quantify the oxylipins accurately and precisely.

In this study, we systematically compared three analytical strategies based on liquid chromatography–mass spectrometry (LC-MS) for oxylipin detection and quantification. The first method is microLC-MS/MS for direct analysis without derivatization. It offers high sensitivity and environmental sustainability, requiring only one-tenth of the solvent volume compared to conventional LC. The second approach employs N, N-dimethylethylenediamine (DEPA) as a charge-reversal derivatization reagent, enhancing electrospray ionization (ESI) efficiency and signal intensity by using UPLC-MS/MS systems. The third strategy is 4-(aminomethyl)phenylpyridinium iodide (AMPP) derivatization. The positively charged AMPP tag enhances ionization efficiency and generates structure-specific ion pairs upon fragmentation, improving isomer discrimination.

Our results highlight the strengths and limitations of each method, providing guidance for selecting appropriate analytical strategies for oxylipin quantification in complex biological matrices.

2287 B — Cellular Heterogeneity Analysis: An Innovative Approach to Single-Cell Metabolomics

PRESENTING AUTHOR: *Maud Heuillet, Metatoul_MTH, France*
CO-AUTHORS: *Claire-Line Marais, Mickael Maucourt, Nicolas Richard, Mickael Castelain, Floriant Bellvert*
SUB THEME: *Single-cell metabolomics and lipidomics*

Cellular heterogeneity, arising from variations in gene expression, physiological environment, and cell age, significantly influences the phenotype of cultured cells. A better understanding of this heterogeneity is essential in all fields, from biotechnology to healthcare. Single-cell analysis provides a wealth of information for studying cellular physiology and pathology.

Among single-cell “omics” approaches, metabolomics stands out by offering rapid and dynamic insights into cellular functions due to the metabolome’s immediate response to environmental changes (seconds or less).

However, single-cell metabolomics faces substantial challenges, including low metabolite levels, their endogenous and exogenous presence, and the inability to amplify metabolites.

Recent advancements in mass spectrometry approaches now enable single cell analysis mainly by miniaturizing cell handling, incubation, and enhancing chip-coupling concepts for analyte ionization through the interfacing of microfluidic chips and mass spectrometers.

Here we propose an innovative miniaturized system that combines microfluidics with nanoESI-MS for high-throughput on-line detection of cellular metabolites. On a specific homemade chip, Suspended cells are isolated from a fermentor by trapping, rinsed on-line to remove exogenous metabolites, lysed during gas-assisted electrospray, and monitored in real-time via MS analysis.

This system is currently being optimized and validated using synthetic cells with a specific lipid composition of PC, PG, and PE. Once validated, it will be applied to more complex cell cultures to study cellular heterogeneity.

We anticipate that this technology will serve as a foundation for more sophisticated designs, with the medium-term goal of coupling metabolomic and genomic analyses of single cells to gain a comprehensive understanding of cellular heterogeneity.

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TECHNOLOGY ADVANCEMENTS

2288 B — Decoding Tumoral Heterogeneity in Glioblastoma: Integrating Targeted and Untargeted Metabolomics for Brain Tumor Analysis

PRESENTING AUTHOR: *Christina Brenner, University Of Vienna, Austria*
CO-AUTHORS: *Lisa Panzenböck, Sagar Acharya, Harald Schöny, Gilbert Hangel, Gunda Köllensperger*
SUB THEME: *Small volume metabolomics technologies*

Glioblastoma, an aggressive brain tumor, ranges amongst the most malignant cancers and is characterized by its low survival rates.[1] A big challenge in diagnosis and treatment is the high tumoral heterogeneity associated with this disease, which has been confirmed by imaging techniques. However, this aspect has often remained elusive in molecular analysis techniques, such as mass spectrometry-based metabolomics. Clinical metabolomics is routinely performed on representative tissue samples, masking spatially localized differences.[2] This study aims to push the boundaries of UHPLC-MS/MS analysis to provide a comprehensive view of the heterogeneity in glioma metabolism and use untargeted analysis to elucidate underlying pathological mechanisms.

Prior to surgery, patients underwent magnetic resonance spectroscopy imaging. Metabolically active hotspots were sampled during tumor resection, immediately flash-frozen and later homogenized. Metabolites and lipids were extracted via a two-phase protocol with 13C-labelled biomass for internal normalization.[3] Extracts were separated on a dual HPLC system (RP, HILIC), and analyzed on a high-resolution orbitrap instrument in both polarities via data-dependent acquisition.[4] For data evaluation, Skyline and MZmine were used for quantitative and untargeted analysis, respectively.[5,6]

Preliminary experiments confirmed the possibility for detection and quantification of key oncometabolites down to sub-mg amounts of tissue. The number of identifications showed no information loss during untargeted analysis. This highlights the possibility for miniaturized sampling methods, enabling higher spatial resolution and less invasive sampling avenues.

Samples from multiple patients were analyzed and show substantial heterogeneity in both metabolomics and lipidomics data, both on concentration levels of single metabolites and molecular fingerprints over different brain loci. By unifying accurate quantification with untargeted analysis in the context of glioblastoma, this study provides a unique platform to explore novel avenues for more precise diagnostic strategies or therapeutic developments.

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[2]Want,Nat.Prot.,2013,doi:10.1038/nprot.2012.135
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[4]Schwaiger,Analyst,2019,doi:10.1039/c8an01219a
[5]Schmid,Heuckeroth,Korf,Nat.Biotech.,2023,doi:10.1038/s41587-023-01690-2
[6]Adams,J.Prot.Res.,2020,doi:10.1021/acs.jproteome.9b00640

2289 B — Standardized quantitative solution targeting over 1,200 metabolites in human plasma, urine and feces using optimized calibration standards, quality controls, and acquisition methods

PRESENTING AUTHOR: *Stefan Ledinger, biocrates life sciences ag, Austria*
CO-AUTHORS: *Gordian Adam, Hai Pham Tuan, Cornelia Röhring, Ulf Sommer, Stefan Ledinger, Guido Dallmann, Alice Limonciel*
SUB THEME: *Standardization, Reproducibility, QA/QC and ring trials*

Robust and reproducible results are the cornerstone of any scientific finding and require standardized and quantitative methods. To answer this need, we developed a metabolomics kit with unprecedented coverage, targeting 1,233 metabolites across 49 biochemical classes of small molecules and lipids. The kit includes optimized calibration standards, quality controls, and acquisition methods to enable robust absolute quantification and generation of reproducible results across mass spectrometry platforms. The kit was validated for use with human plasma and urine samples and tested with human feces samples. Here, we investigated the detectability of the targeted metabolites in human plasma, urine, and feces samples.

Plasma (n=11), urine (n=10) and feces (n=10) samples from healthy human subjects (absence of medical diagnosis) were analyzed. A total of 40 µL of plasma, urine or feces sample extracts were pipetted onto patented filter plates, followed by derivatization, extraction, and dilution. Measurements were performed on a SCIEX 5500+ triple quadrupole mass spectrometer using matrix-optimized acquisition methods. Raw data processing, quantification and statistical analysis was performed in the kit's workflow manager.

In total, the number of metabolites detected in at least half of the human plasma, feces and urine samples was 1,011, 910, and 501, respectively. As expected for this matrix, the metabolites detected in urine were primarily small molecules rather than lipids. The median CV of metabolites detected in plasma, urine and feces was 10%, 6% and 9%, respectively. In urine samples, coverage of different organic acid classes was particularly broad - except for fatty acids.

We designed and applied a standardized, quality-controlled kit that provides quantitative metabolic profiling relevant to multiple biological matrices to study the impact of the microbiome, metabolic health, and disease metabolism.

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TECHNOLOGY ADVANCEMENTS

2290 B — Discovery of Robust Objective Biomarkers for Predicting the Preanalytical Quality of Human Plasma and Serum for Metabolomics

PRESENTING AUTHOR: *G.A. Nagana Gowda, Univesity Of Washington, United States*
CO-AUTHORS: *Vadim Pascua, Lucas Hill, Danijel Djukovic, Daniel Raftery*
SUB THEME: *Standardization, Reproducibility, QA/QC and ring trials*

Recent efforts in our laboratory have focused on the measurement of unstable/labile metabolites, including the major coenzymes and antioxidants, which has led to the discovery of two promising biomarkers of blood sample quality. The coenzymes and antioxidants are notoriously unstable and are extremely sensitive to specimen harvesting, extraction and measurement conditions. Investigation of whole blood stored on ice showed that these metabolites displayed unusual dynamics, and showed that the dynamics also alters the levels of plasma and serum metabolite levels. In particular, in cold blood, the anomalous dynamics of adenosine triphosphate (ATP) result in the progressive accumulation of adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine and hypoxanthine. While the negatively charged phosphorus metabolites, ATP, ADP, AMP, and IMP, are confined to red blood cells (RBCs), the non-phosphorus metabolites, inosine and hypoxanthine, are excreted into plasma/serum. The plasma/serum levels of inosine and hypoxanthine depend on the temperature of blood and the plasma/serum contact time with the RBCs, and hence they represent robust biomarkers for evaluating the preanalytical quality of plasma/serum. These two biomarkers are highly specific since they are generally absent in fresh plasma/serum and are highly sensitive since they are derived from ATP, one of the most abundant metabolites in blood (>1mM). Further, whether blood was kept at room temperature or on ice could be predicted based on inosine levels. An analysis of >2000 plasma/serum samples processed for metabolomics-centric analyses showed alarmingly high levels of inosine and hypoxanthine. The results highlight the gravity of sample quality challenges with high risk of grossly inaccurate measurements and incorrect study outcomes. The discovery of these robust biomarkers, which can be easily measured in protein precipitated blood by both NMR and mass spectrometry, provides new ways to address the longstanding and underappreciated preanalytical sample quality challenges in the blood metabolomics field.

2291 B — Dual-phase LC: serial column isocratic separation of isobaric hexosyl-ceramides

PRESENTING AUTHOR: *Marius-Ionuț Iurașcu, Centogene Gmbh, Germany*
CO-AUTHORS: *Steffen Fischer, Peter Bauer*
SUB THEME: *Targeted methods*

LC/MRM-MS analysis is a powerful method to detect and quantify biomolecules based on three distinct properties: retention time (RT), precursor m/z and fragment m/z. However, there are mixtures that are difficult to breakdown since they comprise molecules that only differ either by the presence of a double bond and/or that are isobaric diastereomers. While aliphatic molecules are usually separated at the LC level using reverse phase (RP) columns, diastereomers like sugars are separated using hydrophilic interaction (HILIC) columns.

We are reporting here the separation of such a mixture of compounds where the naturally occurring ^{13}C isomers give rise to a situation where four isobaric compounds share one MRM channel (812.7 > 264.3): GlcCer(d18:1/24:0), GalCer(d18:1/24:0), $^{13}C_2$-GlcCer(d18:1/24:1), and $^{13}C_2$-GalCer(d18:1/24:1).

The analytes have been measured as pure standards and as a mixture using three MRM LC-MS methods using a RP C18 column, a HILIC column, and both columns coupled in series. We have been able to separate the single and double bond isomers using an RP column, while using a HILIC column we were able to separate between the Glucosyl and Galactosyl isomers. The separation of this complex mixture of isobaric isomers of hexosyl-ceramides has proven impossible on a single LC column. The two LC methods have an opposite gradient profile, the RP columns elutes under an increasing organic phase gradient while the HILIC column elutes under a decreasing organic phase gradient.

After optimizing the LC gradient, we have found a common isocratic gradient that can be used with both columns to separate each pair of isomers. Furthermore, by coupling the two LC columns in series inside one LC system, we were able to separate all four isobaric compounds with the use of one single LC pump system.

2292 B — Simultaneous determination of multiple neurochemicals in mouse brain tissue by liquid chromatography-tandem mass spectrometry

PRESENTING AUTHOR: *Sung-hee Cho, Korea Research Institute of Chemical Technology, South Korea*
CO-AUTHORS: *Jangjae Lee, Yunji Kim*
SUB THEME: *Targeted methods*

Neurochemicals play critical roles in modulating neuronal communication and brain functions, with their dysregulation resulting in neuropsychiatric disorders. The accurate quantification of these signaling molecules is essential for understanding disease mechanisms and developing therapeutics. To address the numerous drawbacks of current neurochemical analysis techniques, we herein developed and validated a liquid chromatography-tandem mass spectrometry method for the simultaneous quantification of 55 neurochemicals in biological samples. This method employs protein precipitation and a fluorophenyl column for chromatographic separation and does not require derivatization. The mobile phase, organic solvent, and column oven temperature were optimized to enhance sensitivity and selectivity. The validated method exhibited an excellent linearity (r2 > 0.990), limits of detection ranging from 0.15 to 75 ng/mL, limits of quantitation ranging from 0.5 to 250 ng/mL, and AOAC guideline–compliant intra- and interday precisions and accuracies. Using this method, we achieved separation within 13 min and quantified 45 neurochemicals in different mouse brain regions, revealing region-specific distributions. The developed approach holds promise for neuroscience research, clinical diagnostics, and the development of drug for neuropsychiatric disorder treatment.

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TECHNOLOGY ADVANCEMENTS

2293 B — Improving Selectivity and Sensitivity of Lipid Mediator Analyses by Coupling Nano-flow Chromatography with the Stellar Mass Spectrometer

PRESENTING AUTHOR: *Ciara Myer, Thermo Fisher Scientific, United States*

CO-AUTHORS: *Thiago Mattos, Rahul Deshpande, Susan Bird*

SUB THEME: *Targeted methods*

Introduction

Signaling lipids play a central role in biology, mediating a wide range of cellular processes. Over the last few decades, the role of lipids as mediators of inflammation has become increasingly recognized. This process involves biochemical pathways that help inflamed tissues return to homeostasis. Detecting and quantifying these lipids in biological matrices is challenging due to their chemical instability, structural similarity, and low concentrations (typically from ppt to ppb).

Methods

Lipid mediator analysis typically employs LC-MS/MS with SRM/MRM on triple quadrupole mass spectrometers and high-flow UHPLC systems. We introduced a targeted approach using a nano-flow UHPLC system (500 nL/min) coupled with a mass spectrometer featuring a dual pressure linear ion trap detector.

Preliminary Data

The mass spectrometer's PRM capability allows monitoring of multiple transitions with both HCD and CID fragmentation at speeds up to 140 Hz. This method enabled reliable detection and quantification of dozens of lipid species in under 30 minutes, using less than 20 µL of solvent per sample. Comparative analysis with high-flow chromatography (0.3 mL/min) using two different mass spectrometers, including the conventional triple quadrupole mass spectrometer, showed higher sensitivity with the nano-flow method. The nano-flow MS method exhibited remarkable selectivity and sensitivity, with LOQs reaching low ppt levels.

2294 B — High-Throughput and Highly Selective Quantitative Lipidomics Approach with the Stellar Mass Spectrometer

PRESENTING AUTHOR: *Joao Mokochinski, Thermo Fisher Scientific, United Kingdom*

CO-AUTHORS: *Brittany Lee, Joao Mokochinski, Cristina Jacob, Rahul Deshpande, Susan Bird, Magdalena Vagnerova, Hector Gallart-Ayala, Julijana Ivanisevic*

SUB THEME: *Targeted methods*

The rising incidence of metabolic disorders necessitates advanced lipidomics methods for high-throughput lipid panel analysis. Precise quantification of lipid species, especially those with varying fatty acid compositions, is critical due to their distinct metabolic roles and biological functions, influencing clinical outcomes. Accurate measurement of these species is essential for better risk prediction and diagnostics. Technological advancements in mass spectrometry have improved the specificity and selectivity of lipid analysis, facilitating translational research, biomarker discovery, and clinical applications. These developments are vital for understanding the complex roles of lipids in health and disease.

We employed a mass spectrometer integrating a quadrupole and dual-cell linear ion trap to assess lipid panels. Using a HILIC-MS/MS platform, we initially measured over 1,000 lipids in the 1950 NIST plasma reference material within 12 minutes. This mass spectrometer employs multiple activation types, including beam-type and resonance collision-induced dissociations, MS3 capabilities, and parallel reaction monitoring (PRM), enhancing specificity and sensitivity for isomeric and structurally similar lipids. Data processed with Skyline enabled the development of a targeted lipidomics panel.

The mass spectrometer significantly improved the specificity and sensitivity of lipidomics workflows, allowing rapid acquisition of full MS2 spectra and comprehensive lipid annotation. Utilizing LipidCreator software, we developed a robust targeted lipidomics panel, achieving an average of 3 transitions per analyte compared to a single transition previously. The mass spectrometer increased lipid species diversity by 50% and enhanced points across a peak by 20%, demonstrating superior performance. Extending this methodology to breast milk samples, we quantified a wide range of lipid species with high sensitivity and specificity, providing insights into the nutritional and biological composition of breast milk. The mass spectrometer's capabilities highlight its transformative impact on lipid analysis in complex samples, supporting neonatal health research and clinical lipidomics.

The mass spectrometer delivers unprecedented specificity, sensitivity, and throughput.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3000 C — Next level of reproducible, comparable and integrable Metabolomics: An ELIXIR Implementation Study

PRESENTING AUTHOR: ***Thomas Payne, EMBL-EBI, United Kingdom***

CO-AUTHORS: ***Franck Giacomoni, Magalie Weber, Olivier Filangi, Egon Willighagen, Denise Slenter, Monica Chagoyen, Elliott J. Price, Helge Hecht, Nils Hoffmann, Noemi Tejera, Peter Horvatovich, Helena Schnitzer, Clara Musicco, Fabrizio Mastrorocco, Luca De Martino, Kim Kultima, Steffen Neumann***

SUB THEME: ***Collaborative and open data science***

The ELIXIR Metabolomics Community relies on the development, adoption and continuous use of standards, formats and data processing and analysis solutions. Challenges persist in ensuring high-quality reported metadata, sufficiently contextualised results and interoperable and reusable datasets that hinder integrating metabolomics data with other omics or other studies.

We introduce the ELIXIR Implementation Study to progress towards the Next level of Reproducible, Comparable and Integrable Metabolomics (RCI-M). RCI-M has been designed to address these issues by connecting key international standards with ELIXIR resources, and creating associated community guidelines and training materials.

Based on the ELIXIR FAIRification framework, the RCI-M project will:

1) increase interoperability and re-use of public metabolomics datasets and workflows through enhanced and extended open data standards, resources and new semantic annotations; 2) define, ensure and establish quality control for study baselines in Metabolomics and Exposomics; and 3) facilitate metabolomic data interpretation and meta-analysis integration with multi-omics and systems biology studies.

As a first necessary step, RCI-M will create a Semantic Metabolomics Data Model to standardise metadata, ensuring unambiguous re-use of metabolomics studies and results. The model will focus on integrating key ontologies, providing open training initiatives, and enhancing the interoperability and reproducibility of processing and analysis of metabolomics data through the production of open guidelines for annotation steps. By linking with ELIXIR's Deposition Databases, the ISA Framework, and other services, RCI-M seeks to boost interconnection with ELIXIR Platforms, other ELIXIR Communities (e.g., System Biology, Food & Nutrition, Galaxy, Proteomics, Toxicology, RDA and Single-Cell Omics), the FAIR Cookbook and Bioschemas.org communities.

We thus facilitate new ambitious and innovative solutions using semantically enriched data for inter-comparison of studies in the healthcare, life science, clinical and plant domains, and lay the foundation for AI-enabled metabolomics.

3001 C — Making metabolomics data FAIR and AI ready to accelerate research and enable personalized medicine

PRESENTING AUTHOR: ***Thomas Hankemeier, Leiden University/Metabolomics & Analytics Centre, Netherlands***

SUB THEME: ***Collaborative and open data science***

To effectively apply AI in metabolomics and big data research, data from multiple sources must be integrated. These sources include clinical studies, cohorts, and diverse data types, allowing insights from individual variations. However, enforcing standards for integration has largely failed, as data owners make independent choices, leading to poor discoverability, limited accessibility, and weak interoperability.

A more effective approach is making data machine-actionable through FAIR principles: Findable, Accessible, Interoperable, and Reusable. This method enhances AI compatibility without requiring changes to existing data models, giving data owners full control over their FAIR implementation. Actually, AI itself can assist in FAIRifying metabolomics data.

In this presentation, we share our experience in making metabolomics data FAIR. We co-founded the Leiden Institute for FAIR and Equitable Sciences to collaborate with various groups and companies on developing FAIR data solutions. Our vision is a decentralized FAIR metadata network, based on minimal open standards, ensuring machine-actionability and cross-domain data reuse while accommodating domain-specific needs. This infrastructure allows data providers to set equitable access conditions, ensuring responsible reuse of both open and privacy-sensitive data.

We will discuss challenges in FAIRifying metabolomics data and our collaborations in the Netherlands and beyond. Once data is FAIR, it can be exchanged, combined, and analyzed more effectively. Additionally, FAIR Digital Twins allow visiting algorithms to query data without direct access, enhancing security and usability.

Finally, we will outline how FAIR data supports metabolomics research and personalized medicine, enabling better data integration and analysis for scientific and clinical advancements.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3002 C — MetaboCrates: An open-source pipeline for quality-aware analysis of targeted metabolomics data

PRESENTING AUTHOR: *Michał Burdukiewicz, Medical University Of Bialystok, Poland*
CO-AUTHORS: *Krystyna Grzesiak, Joanna Pokora, Jarosław Chilimoniuk, Adrian Godlewski, Adam Krętowski, Rafał Kolenda, Michał Ciborowski*
SUB THEME: *Data processing & software tools*

MetaboCrates is an open computational framework for preprocessing and exploratory analysis of quantitative metabolomics data acquired using targeted mass spectrometry kits, including those developed by Biocrates. While vendor software typically supports initial acquisition and normalization, reproducible downstream processing remains manual mainly and fragmented. MetaboCrates addresses this gap by providing a standardized environment for post-acquisition data curation and diagnostic evaluation, catering to the specific needs of targeted metabolomics.

The platform implements rule-based imputation strategies, including limit-of-quantification (LOQ) filtering, to reduce noise and bias in low-abundance features. It includes statistical diagnostics such as PCA for identifying batch effects, technical variation, and outliers. Each step is accompanied by interpretable visual outputs, supporting transparency and reproducibility across workflows.

MetaboCrates is released as an R package with an optional web interface for interactive use. It can be deployed in secure local environments or remote servers, allowing reproducible processing of sensitive data. The code and documentation are available at https://biogenies.info/metabocrates, with a demo instance at https://biogenies.info/metabocrates-ws.

3003 C — PySPRESSO: Simple Python Module for Multi-Batch LCMS Data processing

PRESENTING AUTHOR: *Jan Macháň, Palacky University Olomouc, Czech Republic*
CO-AUTHORS: *Richard Masař, Sára Filipová, Lukáš Najdekr*
SUB THEME: *Data processing & software tools*

In this work, we present an open-source off-line Python module that is designed to simplify the processing of large “multi-batch” LCMS data. This tool includes various filters, correction methods, and statistical tools that allow users to build pipelines and customize their settings to the specific needs of their data. We conceived the module as an all-in-one solution for data processing following the use of “peak-picking” software such as mzmine (mzio) or Compound Discoverer (Thermo Fisher Scientific). PySPRESSO effectively addresses the problems of batch effect and other analytical errors. In addition to data processing, the main advantages are the ability to generate images suitable for presentations or publications and the subsequent creation of a reporting PDF file that allows users to follow each process applied onto the data step by step. The ability to work off-line with “multi-batch” LCMS data, including those with many missing values, is particularly useful for cases of confidential or excessively large files that are not suitable for uploading to popular online tools. The user can run the workflow locally and interpret the data very quickly by themselves. The code is written entirely in Python, ensuring accessibility and easy implementation of new filters and data transformations. With Python as the main machine learning language, it is also ready to integrate various AI models for feature prediction, data classification, or for the addition of other correction methods such as SERRF, for example. This module can contribute not only to accelerating data analysis and evaluation, but also to improve reproducibility and unify approaches across scientific teams thanks to its fully accessible code. Additionally, we are currently working on a UI to simplify the use of PySPRESSO for all non-programmers. We welcome anyone willing to participate in development or willing to help us test PySPRESSO.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3004 C — Missing data imputation in metabolomics data using truncated Gaussian infinite factor analysis

PRESENTING AUTHOR: *Kate Finucane, University College Dublin, Ireland*
CO-AUTHORS: *Lorraine Brennan, Isobel Claire Gormley*
SUB THEME: *Data processing & software tools*

Metabolomics data acquired using mass spectrometry contain highly correlated variables, typically with many more variables than observations. Missing data are prevalent: missing at random (MAR) missingness occurs due to acquisition or processing error, while missing not at random (MNAR) missingness occurs when true values lie below a threshold for detection. This work imputes missing values in metabolomics data, accounting for their physical constraints and varying missingness types, thereby providing complete datasets, with uncertainty quantification, for further analysis.

To facilitate imputation in metabolomics data in a statistically and physically principled manner, a truncated Gaussian infinite factor analysis (tGIFA) model is proposed. The truncated Gaussian assumption ensures cognisance of the data's physical constraints when imputing, and the assumption of an infinite number of latent factors obviates the need to select the optimal number. The tGIFA approach infers the missingness mechanism of each missing value and imputes entries either within the range of the observed data (MAR) or between zero and the detection limit (MNAR). A Bayesian inferential approach provides uncertainty quantification for missingness types and imputed values.

A simulation study is performed to assess tGIFA's performance. Simulated datasets are constructed so the prevalence and type of missingness mimic real metabolomics data, with 2-3% of entries missing by both MAR and MNAR mechanisms. Imputation results are compared to other state-of-the-art imputation methods and assessed against the known true simulated values. Results from tGIFA handle MAR and MNAR identification and imputation more accurately than random forest and singular value decomposition imputation methods, with more physically meaningful imputed values than non-truncated GIFA. Application to urinary liquid chromatography mass spectrometry data with 2.69% missingness results in complete data with MAR and MNAR entries identified and appropriately imputed, with associated uncertainty, prepared for subsequent metabolomics analyses.

Open-source R code accompanies tGIFA, facilitating its widespread use.

3005 C — Databases are a speedy, small, and simple solution for mass-spectrometry data storage and access

PRESENTING AUTHOR: *William Kumler, University Of Washington, United States*
CO-AUTHORS: *Anitra Ingalls*
SUB THEME: *Data processing & software tools*

Mass spectrometry still lacks a high-performance data storage solution, despite the introduction of a dozen or more formats over the past decade. This issue has become increasingly problematic with the increase in file sizes due to higher resolution and faster scanning instruments. Here, we propose a simple database schema for storing m/z and intensity array information and compare it to existing alternatives in terms of disk size, search and retrieval speed, and ease of use. We also evaluate multiple database types, from the widely used SQLite to the more modern Apache Parquet and DuckDB formats.

Our findings show that ion chromatogram extraction, retention time range queries, and MS/MS search speeds can be improved by approximately 100x for a single file while simultaneously requiring very little memory and reducing disk usage with DuckDB. Moreover, this format preserves an intuitive, language-agnostic access model via SQL, which is gaining popularity among mass spectrometrists.

We argue that scan-based, single-file architectures are likely to be outpaced in the near future by software designs that seamlessly scale to the cloud, integrate across entire datasets, and execute exploratory queries in fractions of a second rather than minutes or hours.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3006 C — MetabolomicsHub: International Data Exchange and Data Representation Standards for Metabolomics

PRESENTING AUTHOR: *Ozgur Yurekten, EMBL-EBI, United Kingdom*

CO-AUTHORS: *Thomas Payne, Eoin Fahy, Mano Maurya, Srinivasan Ramachandran, Brian C. DeFelice, Nils Hoffmann, Yasin El Abiead, Pieter Dorrestein, Shankar Subramaniam, Juan Antonio Vizcaino*

SUB THEME: *Data repositories*

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

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

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

3007 C — MetaboLights - Open Access Metabolomics Data Repository

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CO-AUTHORS: *Ozgur Yurekten, Felix Xavier Amaladoss, Callum Martin, Mark Williams, Thomas Payne, Juan Antonio Vizcaíno*

SUB THEME: *Data repositories*

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

MetaboLights is a global cross-species and cross-technique database for metabolomics studies, including raw experimental data and the associated metadata, covering also metabolite structures and their reference spectra. The adoption of standards (data/metadata) and the usage of ontologies with expert curation adds value and enables the reanalysis of studies by the community, as well as compliance to FAIR (findability, accessibility, interoperability and reusability) data principles.

The repository accepts individual and programmatic submissions, accommodating contributions from companies, Phenome Centres, and other large-scale laboratories. The team collaborates with international repository partners and contributes to the development of tools that facilitate the discovery and integration of metabolomics and multi-omics research, as well as standardisation efforts (mQACC, OORF and HUPO-PSI).

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

New to 2025, full MetaboLights accession numbers are assigned only after the data submission is finalised, ensuring the completeness and compliance of the submitted datasets, standardising further all new available datasets . The team has developed the new study Validation Framework v2 and new minimum submission requirements, to scale the submission process, improving reporting and speeding up data release. MetaboLights manual curation is now retained only in certain cases, reducing the required time for studies to be publicly available.

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

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3008 C MetExploreViz, online visualisation tool for metabolic networks

PRESENTING AUTHOR: *Jean-clément Gallardo, INRAE, France*
CO-AUTHORS: *Marion Liotier, Florence Vinson, Fabien Jourdan, Ludovic Cottret*
SUB THEME: *Data Visualization*

The study of metabolism is now an integral part of research projects aimed at understanding organism responses to biotic and abiotic stress. In this context, Genome-Scale Metabolic Networks (GSMNs) offer a framework for describing the metabolic functioning of living organisms. They represent all biochemical reactions occurring within a cell or tissue, providing a structure for interpreting metabolomic, transcriptomic, and fluxomic data. In particular, GSMNs encode the interplay between metabolites (substrates and products of reactions) which can be measured using metabolomics.

The key challenge is to extract knowledge from the thousands of connected elements in GSMNs. To help address these challenges, the bioinformatics community have developed network visualisation tools to aid in data interpretation. The goal of this discipline is to capture the essence of complex datasets and propose the most effective ways to represent them. Beyond GSMNs, representing biological data as networks is a particularly effective approach for visualizing diverse data types in the context of systems biology.

Our tool, MetExploreViz (https://forgemia.inra.fr/metexplore/metexploreviz-ui), has been developed since 2018 to facilitate the understanding and exploration of metabolic knowledge and related omics data. MetExploreViz is an online tool dedicated to the visualisation of metabolic networks. The most recent release offers technical enhancements and a standalone website. It offers a range of features such as visualisation of metabolic networks as interactive graphs, mapping of omics data directly onto the network, visualisation of flux distributions and application of a hierarchical layout to improve readability and interpretation.

These advancements reinforce MetExploreViz as a versatile and accessible solution for researchers seeking to extract meaningful insights from complex metabolic datasets. MetExploreViz is also part of the MetExplore ecosystem, a database dedicated to metabolic networks that provides users with a variety of analysis and interpretation tools, making it a comprehensive platform for metabolomics research.

3009 C Biostatistical Monitoring of Secondary Effects on Metabolic and Endocannabinoid Changes in Biological Studies

PRESENTING AUTHOR: *Eva Cifkova, University of Hradec Kralove, Czech Republic*
CO-AUTHORS: *Miroslav Lisa, Frantisek Staud*
SUB THEME: *Data Visualization*

In biological research, observed outcomes are often determined by the interplay of multiple factors. Although biological studies typically focus on the primary effects of key variables, a deep understanding necessitates the investigation of secondary effects. To achieve a comprehensive interpretation of our animal model, we employed a reversed-phase liquid chromatography with mass spectrometry (RP-LC/MS) platform (1) for the detailed analysis of polar metabolites and endocannabinoids in a well-characterized cohort of rat placenta and brain samples. This cohort was designed to include both treated and control groups and also took into account biological variations that represent secondary influences, such as gender and litter size. Multivariate statistical methods were applied to determine key metabolic alterations that distinguish our experimental groups. Subsequently, we focused on identifying and quantifying secondary effects and their influence on the relationships observed between our primary variables. This approach allowed us to move beyond a simplified perception of direct effects and fully appreciate the complex, multi-faceted nature of the biological responses observed in our rat placenta and brain cohort.

This study was supported by the Czech Health Research Council (NW24-07-00129), and Czech Science Foundation (22-13967S).

(1) Cifkova E, Neuerova Z and Lísa M. Reversed-phase liquid chromatography/mass spectrometry approach for (un)targeted analysis of polar to mid-polar metabolites. Talanta 2025; 291:127853.

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3010 C — Enhanced Identity Spectrum Search with AI/ML Confidence Scoring for HRAM Data

PRESENTING AUTHOR: *Gábor Zsemlye, HighChem sro., Slovakia*
CO-AUTHORS: *Marynka Ulaszewska, Gábor Zsemlye, Juraj Lutišan, Maria Falaq, Rajesh Jha, Samuel Benkovič, Tim Stratton, Michal Raab*
SUB THEME: *Machine Learning and AI*

Spectra annotation is a significant challenge in untargeted small molecules analysis. Comparing unknown spectra against spectral libraries is the initial step in annotation workflows. Ideally, the identification algorithm provides clear high-ranking scores, indicating the correct library hit. However,ambiguous results often occur due to isomeric and isobaric species producing similar or poorly specific spectra, leading to multiple high-scored hits. We propose new AI/ML confidence scoring system for spectra identification of Orbitrap data against the mzCloud spectral library.

We used a histogram gradient boosting model to replicate a scientist's behavior in compound identification through library searches, simulating multiple searches and learning from true and false hits. Training and validation data were sourced from mzCloud's curated spectral library. To ensure variability, we selected 1600 compounds from Autoprocessed and Reference libraries, with an additional 1600 compounds for false hits. We created 170 model input features reflecting fragmentation spectra complexity, metadata, and matching pairs query-hit, etc.

New AI/ML model was validated against analytical standards from mzCloud, while validation with real samples still must occur. We compared performance of new model against four scoring algorithms: NIST, Cosine, HighChem-HighRes and older confidence score from 2016. Our method achieved satisfactory results outperforming other scoring systems. Specifically, cosine score correctly ranked only 82.5% of compounds, old confidence 91.3%, while new AI/ML confidence achieved 93.6% correct rankings. The ROC AUC at compound level for true hits, reached 0.90, while cosine and old confidence 0.77 and 0.70, respectively. The ROC AUC for new AI/ML confidence at spectrum pair level when including true positives and false negatives reached 0.95, while cosine and old confidence 0.53 and 0.54, respectively. Further validation will focus on model performance for isobaric and isomeric compound species, as well as deeper analysis of different collision energy regions for spectra and compounds where AI/ML model performed with lower accuracy.

3011 C — Predicting molecular structures from multi-stage MSn fragmentation trees using graph neural networks and DreaMS foundation model

PRESENTING AUTHOR: *Filip Jozefov, IOCB Prague, Czechia*
CO-AUTHORS: *Roman Bushuiev, Josef Sivic, Tomáš Pluskal*
SUB THEME: *Machine Learning and AI*

Molecular identification from mass spectrometry data is a longstanding challenge in metabolomics due to complex fragmentation patterns and inherent spectral ambiguity. To tackle these issues, we establish neural network baselines that leverage multi-stage MSn spectra. Our study focuses on two key tasks: (i) Retrieval, which ranks candidate molecular graphs from chemical databases based on predicted molecular representations, and (ii) De Novo generation, which reconstructs molecular graphs from spectral data. Our experiments compare two distinct spectral representations, raw spectra and DreaMS (1) embeddings. Besides that, we assess the additional benefit of incorporating known molecular formulas as a bonus task.

To facilitate these tasks, we extend the MassSpecGym (2) benchmark by introducing MassSpecGymMSn, a novel dataset that captures the hierarchical fragmentation of molecules through multi-stage MSn trees. By automating the preprocessing of our MSn dataset, our framework efficiently organizes 16,476 fragmentation trees. We process the data hierarchically to support molecular retrieval and de novo generation tasks. Notably, each fragmentation tree captures a distinct stage of molecular fragmentation, with 5,292 trees reaching the maximum depth of MS5.

Results demonstrate that integrating deeper fragmentation levels significantly improves predictive performance. Notably, adopting DreaMS (1) embeddings leads to higher accuracy, underlining the crucial role of advanced foundation models in mass spectrometry. We exploit internal representation analyses employing centered kernel alignment and statistical tests to understand further what is changing in the model with a deeper fragmentation stage or different spectra representation. We validate these improvements and probe the underlying mechanisms; we show that deeper trees yield richer representation. Moreover, our investigation of models with the bonus task reveals a significant shift in internal representations for the same predictive task.

1.Bushuiev R, et al. DreaMS. Nat. Biotechnology. 2025. doi:10.26434/chemrxiv-2023-kss3r-v2

2.Bushuiev R, et al. Mass Spec Gym. NeurIPS 2024. doi:10.48550/arXiv.2410.23326

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3012 C — Unlocking latent phenotypic predictive power in unstructured molecular data via self-supervised machine intelligence

PRESENTING AUTHOR: ***Baljit Ubhi, Matterworks, United States***
CO-AUTHORS: ***Gabriel Asher, Lea Amar, Baljit Ubhi, Jennifer Campbell, Niall O'Connor, Jack Geremia***
SUB THEME: ***Machine Learning and AI***

Traditional mass spectrometry (MS)-based omics workflows rely on human-curated features such as peaks and MS/MS identifications. While effective, these approaches force structure onto inherently unstructured data, discarding large volumes of raw signals that may contain valuable predictive information. To overcome these limitations, we developed Large Spectral Models (LSMs)—self-supervised foundation models trained on diverse, unlabeled MS spectra. LSMs learn a semantic representation of raw mass spectral data, enabling phenotype prediction directly from instrument output without prior peak picking or molecular annotation.

In this study, we fine-tuned LSMs on small, labeled phenotype datasets to demonstrate predictive capabilities in real-world biological contexts. Using public LC-MS data from Sah et al. (2024) on ovarian and cervical cancer patients, we trained a predictive model using raw MS spectra alone. The LSM-based approach significantly outperformed a conventional supervised method based on manually selected lipid biomarkers. Our model achieved mean accuracy of 0.96, sensitivity of 0.98, and specificity of 0.95, with a tenfold reduction in false negative error rates.

Importantly, LSM embeddings captured latent biochemical signals not detected through traditional workflows. Visualization of the reduced LSM representations revealed clear phenotypic clustering, and model benchmarking across 1,000 test/train splits confirmed reproducibility and robustness. By removing the need for feature engineering or annotation, LSMs reduce time-to-insight and expand access to predictive biology for users with limited data science expertise.

This work illustrates a scalable, application-agnostic path for phenotype prediction from raw MS data. As a first-of-its-kind foundation model in mass spectrometry, the LSM approach unlocks unstructured molecular information previously discarded, redefining how spectral data can drive diagnostics, translational research, and precision medicine.

3013 C — Computational Strategies for Metabolomics Data Analysis

PRESENTING AUTHOR: ***Elena Dracheva, Umeå University, Sweden***
CO-AUTHORS: ***Katie Bennett, Johan Trygg***
SUB THEME: ***Metabolic modeling***

The increasing complexity and volume of metabolomics data have intensified the demand for robust, transparent, and reproducible computational approaches. As metabolomics becomes more integral to systems biology and translational research, advanced methods for data processing, statistical analysis, and biological interpretation are essential for extracting meaningful insights from high-dimensional datasets.

This work highlights key computational strategies commonly used in metabolomics research, with an emphasis on multivariate modelling, statistical validation, and biological interpretation. A major focus is placed on selecting appropriate statistical and machine learning models that address the high dimensionality and collinearity characteristic of metabolomics data.

We examine challenges such as model overfitting, false discovery rates, and biological variability, and present strategies to enhance the robustness and interpretability of results. The importance of reproducibility and the adoption of standardized workflows is emphasized, as these are critical for ensuring consistency and comparability across different experimental contexts.

Through case studies from plant biology and human health, we demonstrate how computational approaches can uncover meaningful metabolic patterns. These examples illustrate the versatility and cross-disciplinary relevance of computational metabolomics in life science research.

By evaluating current analytical practices and identifying areas for methodological refinement, this work supports the continued development of computational metabolomics as a vital component of systems biology. Emphasis is placed on informed model selection and biologically grounded data interpretation. Addressing common sources of variability and promoting standardized analytical workflows are highlighted as priorities for improving the reliability and biological validity of metabolomics studies across diverse research domains.

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★ AWARD WINNERS

COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3014 C — MSLipidMapper: a multiomics data mining environment using untargeted lipidomics data.

PRESENTING AUTHOR: ★ *Takaki Oka, Tokyo University of Agriculture and Technology, Japan*
CO-AUTHORS: *Kozo Nishida, Hiroshi Tsugawa*
SUB THEME: *Metabolic networks*

Lipids play essential roles as membranes, energy storage, and signaling molecules, with functions driven by the structural diversity of lipidome. While untargeted lipidomics can characterize over 1,000 lipid molecules per sample, current lipidomics software lacks the capability to visualize lipid diversity created through the complex interplay of de novo and remodeling pathways. Furthermore, there is no bioinformatics environment that can integrate data from other omics layers and visualize the lipid metabolism, making it difficult to derive biological interpretations.

In this study, we developed MSLipidMapper, a pathway visualization tool to automate lipid molecular mapping onto metabolic pathways by using untargeted lipidomics data. MSLipidMapper features user-friendly operability and high extensibility as software by modularizing each component. This tool accepts quantitated lipidome data. When using MS-DIAL output specifically, MSLipidMapper users can obtain omics-projected pathway diagrams without any modifications to the output tables by using the Shiny application. Within MSLipidMapper, lipidome data is converted to the generic omics data container SummarizedExperiment, from which plots (box plots, dot plots, etc.) are generated for each lipid for projection. Cytoscape is used to project omics data onto pathways. The plots are then made available to Cytoscape as a REST service using the plumber API generator. This platform provides pathway maps that include 179 lipid subclasses supported by MS-DIAL as nodes with knowledge-based enzymatic reactions as edges, enabling hierarchical visualization of lipid expression at both subclass and molecular species levels. Using this platform, we conducted a re-analysis of the kidney lipidome data from an aging mice study. The results revealed that galactosylceramide shows a male-specific accumulation, while ceramide molecules with C20 fatty acyl chains show female-specific increases correlating with CerS4 (Ceramide Synthase 4). This study demonstrates that bioinformatics technology enhances the reuse of lipidomics data by enabling a data-driven metabolic research platform that can provide new biological insights.

3015 C — Automatic Metadata Generation via Network Analysis And Large Language Models

PRESENTING AUTHOR: *Prajit Rajkumar, University Of California, San Diego, United States*
CO-AUTHORS: *Haoqi Nina Zhao, Pieter C. Dorrestein*
SUB THEME: *Metabolite identification/annotation*

Proper classification of chemicals with relevant metadata is necessary for facilitating scientific discoveries and forming associations between metabolites and exposure sources, pathologies, or other descriptors. However, annotating chemicals with metadata is a labor intensive task that grows larger as more data is deposited into the public domain. Our [prior work](https://pypi.org/project/chemsource/) has shown that large language models can be used to successfully automate the annotation of chemicals with metadata with high accuracy. However, the coverage of this method, which relies on using descriptions of chemicals found in prior literature, still needs improvement, as many chemicals do not have usable descriptors that can be retrieved from prior literature. Here, we extend chemical classification via large language models to have greater coverage via molecular networking and pathway analysis. We focus on the task of classifying chemicals by exposure source, and we hypothesize that molecules that differ by one node within a molecular network are likely to have similar exposure sources. Preliminary results show that analysis of exposure sources via a molecular network over previously classified chemicals can extend coverage by an additional 5 to 15% of previous classifications. More comprehensive analysis via classification of molecules outside the initial molecular network and an analysis of the progression of molecules through known pathways is likely to further extend the coverage of chemicals we achieve through this method.

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3016 C — MS2LDA 2.0: Boosting natural product discovery by substructure identification in metabolomics data

PRESENTING AUTHOR: *Laura Rosina Torres Ortega, Wageningen University, Netherlands*

CO-AUTHORS: *J. J.Justin van der Hooft, Jonas Dietrich*

SUB THEME: *Metabolite identification/annotation*

Natural products are bioactive compounds analyzed using mass spectrometry (MS), with computational tools aiming their identification through spectral library matching, networking analyses, or machine learning. While full structure annotation is common, substructure-based approaches provide critical insights into biosynthetic relationships and compound classification. For over a decade, MS2LDA has enabled substructure discovery by identifying recurring spectral patterns (Mass2Motifs) in MS/MS data. However, its scalability, reliance on expert interpretation, and lack of integration with modern MS frameworks remain limitations.

Here, we introduce MS2LDA 2.0, which improves computational efficiency, achieving a from 3x up to 10x speedup in runtime. We integrated Spec2Vec for automated Mass2Motif annotation guidance, reducing manual curation efforts. Benchmarking against MotifDB's largest MotifSet (66 Mass2Motifs), the automated annotation guidance showed a 97% median annotation accuracy when compared to the manual annotations.

To validate MS2LDA 2.0 for natural product discovery, we applied it to a previously studied mushroom dataset where GNPS and SIRIUS failed to identify three bioactive compound families. MS2LDA 2.0 successfully generated Mass2Motifs linked to these families. For the erinacerin family, six Mass2Motifs were identified, with automated substructure guidance accurately predicting substructures for erinacerins N and F, covering 24 of 25 molecular family nodes. Additionally, MS2LDA 2.0 uncovered structural relationships overlooked by GNPS molecular networking, offering new hypotheses on unlinked nodes sharing Mass2Motifs.

In conclusion, MS2LDA 2.0 significantly enhances speed, usability, and integration with tools like matchms and molecular networking. By enabling more efficient and automated substructure annotation, it uncovers molecular relationships that conventional tools fail to detect. This advancement boosts natural product research, opening new avenues for bioactive compound discovery and structural elucidation.

3017 C — Integrating Metabolomic and Metabarcoding Data from Fecal Samples of Nellore Steers to Explore Host-Microbiome Interactions

PRESENTING AUTHOR: *Aline Morais, USP, Brazil*

CO-AUTHORS: *Banny Silva Barbosa Correia, Liz Jianinne Fuentes Rojas, Jennifer Jessica Bruscadin, Hanne Christine Bertram, Daniel Rodrigues Cardoso, Alexandre Berndt, Luciana Correia de Almeida Regitano*

SUB THEME: *Multi-omics/Integrative omics*

The hologenome concept clarifies host-microbiome symbiosis, where, for instance, metabolic end-products support animal growth, development, and system modulation. This study investigated whether the biological datasets ASV (Amplicon Sequence Variant) abundance and metabolites concentration were drivers of phenotypic traits, including residual feed intake (RFI), rib eye area (REA), subcutaneous fat thickness (SFT) and reactivity test (REATEST). Data were collected from 100 Bos indicus fecal samples of bulls aged between 18 and 24 months, belonging to the 2020 edition of the genetic improvement program (PNAT). The exploratory analyses were divided into four associations: I- Metabolites and ASVs; II- Phenotype scores from performance test and ASVs; III - Metabolites and phenotype scores from performance test; and IV - ASVs, metabolites and phenotypes. The final biological network revealed the following main correlations. [Eubacterium]_coprostanoligenes_group, a cholesterol-reducing bacteria, showed positive and negative correlations with SFT and REATEST, respectively, with the latest being negatively associated with anxiety disorder. Some strong negative correlations were found between Eubacterium_coprostanoligenes_group, Oscillospiraceae, lactate, succinate, formate, and SFT. The metabolites lactate, succinate, and formate are the main energy sources for microbial short-chain fatty acids (SCFA) producers. In addition, the negative correlation with excreted precursors of SCFA may indicate lower SCFA production by the microbiota. Negative correlations between excreted aspartate, glycine, butyrate, propionate, acetate, N-acetyl glycine and SFT, as well as between RFI and acetate, may also indicate the further metabolization and absorption of these metabolites as relevant for the phenotypes . Also, findings of positive correlations between SCFA-producing bacteria Oscillospiraceae, Lachnospiraceae, and fecal SCFA were reported. The pairwise findings demonstrated the potential of biological datasets to discover biomarkers in order to optimize the costs in animal production.

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3018 C — Establishing a bioinformatics pipeline for the analysis of biotransformation experiments

PRESENTING AUTHOR: *David Rüscher, Leibniz-institute For Plant Biochemistry, Germany*
CO-AUTHORS: *Arianne Schnabel, Steffen Neumann*
SUB THEME: *Other*

Carnosic acid and its derivative carnosol are major ingredients of rosemary extracts. Due to their antioxidant properties, they are commonly used as food preservatives and have a plethora of current and potential use cases in medicine and pharmacology. The aim of our project is to produce novel compounds derived from carnosic acid or carnosol through biotransformation. Therefore, yeast cells were transformed with different combinations of enzymes and exposed to carnosic acid. Subsequently, extracts of these cells were subjected to untargeted UPLC-IMS-QTOF-MS/MS measurements to identify potentially novel compounds. The analysis of such data, however, is challenging due to the high number of combinatorial events, enzyme promiscuity and function. Therefore, we established a robust bioinformatics pipeline to mine for and characterize potential novel compounds and minimize the number of false positives by incorporating both statistical methods and biochemical knowledge.

The raw LC-MS/MS data was processed using mzMine and the resulting feature tables analyzed in R. For the streamlined incorporation of both environments, we created the R package mzReactionMineR that allows for the generation of R specific SummarizedExperiment data classes and simplifies integration with other packages like the RforMassSpectrometry universe. The statistical analysis was performed using the limma package due to its fast implementation of linear model fits allowing for proper definition of the blocking design of the experiment without sacrificing throughput. To avoid false positives, the resulting list of potential candidates was filtered for complete absence in any control samples across blocks.

Lastly, we generated mass difference networks of precursor ions and MS/MS feature networks to find likely substrate product pairs. This allows for the data-driven annotation of the chemical structure and the most likely modification of the newly formed substrates, which can later be validated using NMR measurements.

3019 C — Chemical Fingerprinting of Trees Across Romania: Integrating Manual Curation with Machine Learning-Based Classification

PRESENTING AUTHOR: *Georgia Angelidou, Max Planck Institute for Terrestrial Microbiology, Germany*
CO-AUTHORS: *Alexandru Nicolescu, Francois-Xavier Lehr, Andrei Mocan, Nicole Paczia*
SUB THEME: *Other*

Understanding the chemical diversity of plant metabolites across different environments provides valuable insights into ecological adaptation. In this study, we investigated the metabolic profiles of Artemisia alba Turra samples collected from six distinct locations in Romania using LC-MS/MS-based untargeted metabolomics. Our goal was to explore location-specific chemical variations and evaluate data analysis workflows for metabolite annotation and classification. Two complementary approaches were used to process and interpret the MS/MS datasets. The first was a bottom-up strategy using Compound Discoverer, where we developed an in-house manually curated library to identify metabolites based on accurate mass, retention time, and fragmentation patterns. This approach allowed for targeted compound identification and facilitated a high-confidence annotation of known metabolites. The second approach leveraged SIRIUS and CANOPUS, advanced computational tools for de novo molecular formula prediction, and chemical class assignment. SIRIUS enabled the structural elucidation of unknown compounds by reconstructing fragmentation trees, while CANOPUS predicted chemical classes using machine learning and the ClassyFire ontology. This top-down strategy allowed for broader annotation coverage, including compounds absent from databases, and enabled us to assess chemical diversity at the class level across locations. Comparative analysis revealed both overlapping and complementary findings between the two workflows. While the in-house library approach offered high confidence for known compounds, the SIRIUS/CANOPUS pipeline expanded our understanding of chemical class-level variation and uncovered putative novel metabolites. We present a benchmark comparison highlighting the strengths and limitations of each method in terms of coverage, confidence, and biological insight. By combining targeted and untargeted strategies, this study showcases how tools like SIRIUS and CANOPUS deepen our understanding of plant metabolite diversity in response to ecological variation

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COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3020 C **TICQ of Approval: An LC-MS-Based Quality Assessment of Sample and Instrument Performance using Total Ion Current**

PRESENTING AUTHOR: ***Vimalnath Nambiar, Murdoch University, Australia***

CO-AUTHORS: ***Shabarinath Nambiar, Theresa Schiemer, Monique J Ryan, Jayden Lee Roberts, Luke Whiley, Kok Wai Wong, Guanjin Wang, Elaine Holmes, Julien Wist***

SUB THEME: ***Other***

The integration of liquid chromatography (LC) with mass spectrometry (MS) has revolutionised the field of metabolic phenotyping studies, yet data acquired from these instruments pose significant challenges due to their susceptibility to sample and instrument variations. Consequently, ensuring data quality and reliability has become a time-consuming bottleneck in high-throughput LC-MS data processing pipelines, requiring manual in-person assessment of each acquired sample. To address this, we present an analytical methodology employing total ion current (TIC) for rapid sample quality and instrument performance evaluation.

Our study utilised 3415 control samples obtained using LC-quadrupole time-of-flight-MS instruments. These samples include matrix-free analytical quality control and calibration samples of different concentration levels along with long-term biological references of different matrices. Using an in-house data preparation pipeline, the mass-to-charge values stored within the data were filtered against 73 known compounds of interest encompassing mass calibration references, internal standards, and endogenous analytes.

Our analytical workflow involved a preliminary check on the total number of spectrum data of each sample followed by univariate and multivariate statistical analyses, including principal component, time series, and data distribution analyses. These models assessed the TIC across chromatographic regions, revealing influences of sample quality and instrument variability through deviation-based thresholds. Further examination of the TIC of extracted ions also enabled the monitoring of compound stability over time.

By combining the use of TIC with statistical modelling approaches, our study offers a swift systematic approach to evaluate sample quality and instrument performance without the need for manual assessment. Through automation and reporting features, the proposed methodology significantly lowers the time and resources required while enabling researchers to consistently ensure the quality and reliability of their data. By mitigating the impact of sample and instrument variances, we can facilitate more accurate and reproducible metabolic phenotyping studies, paving the way for deeper insights into biological systems.



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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3050 C — A streamlined LC-HRMS approach for Lipidomic and Metabolomic profiling applied to age-related macular degeneration (AMD) patients

PRESENTING AUTHOR: *Marcos Bouza, IPLA-CSIC, Spain*

CO-AUTHORS: *Clara de Lorenzo González, Oumeyma Sabry El Makkas, Pablo Alvarez, Beatriz Fernández, Rosario Pereiro, Héctor González-Iglesias*

SUB THEME: *Ageing research*

The growing importance of lipidomics and metabolomics in clinical research requires analytical platforms that are not only sensitive and wide-ranging, but also fast, reproducible, and easy to implement. This study introduces a comprehensive LC-HRMS-based workflow designed for untargeted analysis of lipids and metabolites from small volumes of serum, optimized for large-scale clinical applications. The method employs a unified extraction using methanol:MTBE (1:1, v/v), allowing simultaneous isolation of lipidomic and metabolomic components. It incorporates normalization with isotopically labeled internal standards and leverages a semi-automated Python script for streamlined data processing.

The entire protocol, from sample preparation to data analysis, was completed within 24 hours for 40 serum samples, underscoring its suitability for high-throughput studies. Using dual-polarity iterative MS/MS, the approach enabled the detection of more than 500 distinct lipids across 27 classes and over 500 metabolite features, supporting integrated lipid-metabolite profiling. The use of internal standards significantly enhanced reproducibility and precision in the case of lipidomics, achieving relative standard deviations of 6% and 7% in positive and negative ionization modes, respectively.

When applied to serum samples from individuals with age-related macular degeneration (AMD), the workflow identified notable alterations in docosahexaenoic acid-enriched triglycerides, sphingomyelins, and oxidized fatty acids.

3051 C — Metabolomic analysis of in Pre-Elderly Adults: A Multicenter Prospective Cohort Study

PRESENTING AUTHOR: *Sarah Shin, Korea Institute of Oriental Medicine, South Korea*

CO-AUTHORS: *You Mee Ahn, Jeeyoun Jung*

SUB THEME: *Ageing research*

Declines in physical and cognitive functions due to aging may occur concurrently or independently. However, the corresponding metabolic alterations remain insufficiently understood. This study aimed to investigate group-specific metabolomic profiles and related pathways based on combined physical and cognitive function, in order to identify potential biomarkers of health status.

Data were collected from 1,000 pre-elderly adults aged 50 and older who were enrolled in the Korean Medicine for Aging Cohort (KoMAC), launched in August 2023 as part of a multicenter prospective cohort study.

Cognitive function was assessed using the Montreal Cognitive Assessment (MoCA) and categorized into cognitively normal and mild cognitive impairment (MCI) groups. Physical function was measured using a core endurance test, and participants were stratified into three subgroups as follows: Group 1: Normal cognitive and physical functions; Group 2: Impaired cognitive and physical functions; Group 3: Normal cognitive function with impaired physical function.

Untargeted metabolomic profiling was conducted using LC-QTOF-MS, and batch effects were normalized via the systematic error removal using random forest (SERRF) algorithm. Significant metabolites were selected based on variable importance in projection (VIP) score derived from partial least squares discriminant analysis, with a threshold of VIP > 1.0, followed by pathway enrichment analysis to interpret biological relevance.

Distinct metabolomic profiles were observed among the groups, with significant changes particularly noted in glycine and serine metabolic pathways associated with functional status.

These findings support the relevance of metabolomic profiling in detecting early physiological changes associated with age-related physical and cognitive decline. Furthermore, the identification of function-specific metabolic biomarkers provides a foundation for early risk assessment and the development of personalized intervention strategies to promote healthy aging.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3052 C — Non-Targeted Lipidomics Reveals Age-Related Changes in Surfactant Lipid Profiles of Mouse Lungs

PRESENTING AUTHOR: *Miina Ozeki, Nissui Corporation, Japan*
CO-AUTHORS: *Taro Sakamoto, Seizo Sato*
SUB THEME: *Ageing research*

Pulmonary surfactants, primarily composed of phospholipids, play a crucial role in maintaining alveolar stability and modulating innate immunity. Among these lipids, phosphatidylcholine (PC) and phosphatidylglycerol (PG) are key constituents. However, how their molecular profiles change with aging remains poorly understood.

In this study, we conducted a non-targeted lipidomic analysis to investigate age-associated alterations in surfactant lipid composition in mouse lungs. Lung tissues were collected from young and aged mice, and lipids were extracted using the Folch method with internal standards. LC-MS/MS was performed in both positive and negative ionization modes using a data-dependent acquisition (DDA) strategy. Raw data were processed using MS-DIAL, and statistical analysis was conducted to identify significant differences between the age groups.

The results revealed a significant increase in polyunsaturated fatty acid (PUFA)-containing PC and PG species, particularly those incorporating arachidonic acid (C20:4) and docosahexaenoic acid (C22:6), in aged lungs. Phosphatidylcholine and phosphatidylglycerol, the major components of pulmonary surfactants, are generally known to contain saturated and monounsaturated fatty acids such as palmitic acid (C16:0) and oleic acid (C18:1). Our findings suggest that aging is associated with an increase in PUFA-containing molecular species within these classes.

Our findings highlight the dynamic nature of surfactant lipid profiles during aging and their potential implications for respiratory function and immune regulation. The PUFA-rich lipid species identified in this study may serve as candidate biomarkers for lung aging. Future research integrating structural lipidomics with functional assessments will help elucidate the biological significance of these compositional changes.

3053 C — Contrasting Associations of Plasma and Urine Tryptophan-Kynurenine Pathway Metabolites with Diabetic Kidney Disease Progression: A Dual-Matrix Metabolomic Analysis

PRESENTING AUTHOR: *Kee Voon Chua, Duke-NUS, Malaysia*
CO-AUTHORS: *Jian-Jun Liu, Keven Ang, Su Chi Lim, Jianhong Ching*
SUB THEME: *Biomarker validation and clinical translation*

Introduction and Aim: The tryptophan-kynurenine pathway has emerged as a potential mediator in diabetic kidney disease (DKD) pathophysiology. However, the relative utility of plasma versus urine metabolites as biomarkers of disease progression remains unclear. We investigated whether metabolites from this pathway in different biological matrices provide complementary or redundant information regarding risk of end-stage kidney disease (ESKD) in patients with type 2 diabetes.

Methods: We measured tryptophan, kynurenine, 3-hydroxykynurenine, kynurenic acid, and xanthurenic acid concentrations in both plasma (n=1,915) and urine (n=2,057) from patients with type 2 diabetes. The primary outcome was a composite of incident ESKD (eGFR <15 mL/min/1.73m^2, sustained dialysis, or renal death). Secondary outcomes included annual eGFR decline and all-cause mortality. Associations were assessed using Cox proportional hazards models adjusted for clinical risk factors including baseline eGFR and albuminuria.

Results: We observed striking matrix-specific associations with ESKD risk. In plasma, tryptophan (HR 0.62 [95% CI 0.51, 0.75]), kynurenic acid (HR 0.74 [0.60, 0.91]), and xanthurenic acid (HR 0.74 [0.60, 0.91]) were inversely associated with ESKD risk. Conversely, in urine, tryptophan showed a borderline positive association (HR 1.18 [1.00-1.39]), while kynurenine (HR 1.60 [1.16-2.22]), 3-hydroxykynurenine (HR 1.64 [1.17-2.31]), and kynurenic acid (HR 1.47 [1.10-1.97]) were positively associated with renal outcomes. The kynurenine-to-tryptophan ratio was consistently associated with increased risk in both matrices. Urine metabolites correlated with tubular injury markers (dkk-3, MCP-1, KIM-1), and their associations with outcomes were stronger in patients with established CKD.

Conclusion: Plasma and urine tryptophan-kynurenine pathway metabolites demonstrate opposing associations with DKD progression, suggesting they reflect different pathophysiological processes. While plasma metabolites may indicate systemic metabolic disturbances, urine metabolites appear to reflect local tubular dysfunction. This dual-matrix approach provides complementary information for risk stratification in diabetic kidney disease and identifies potential therapeutic targets for intervention.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3054 C — Using Metabolomics as a Diagnostic Tool for Early Detection of Fracture-Related Infections: Insights from a Pilot Study

PRESENTING AUTHOR: *Theresa Schiemer, Riga Technical University, Latvia*
CO-AUTHORS: *Claudia Siverino, Fintan Tomas Moriarty, Kristaps Klavins*
SUB THEME: *Biomarker validation and clinical translation*

Fracture-related infection (FRI) is a major complication in orthopedic trauma surgery. Diagnosing FRI can be challenging due to its varied clinical presentations, and the limitations of current diagnostic tools. Metabolomics has emerged as a valuable resource for exploring host-pathogen interactions at the level of small molecules. Understanding the role of host metabolism in FRI is essential for identifying specific metabolic pathways involved during infections and for developing diagnostic biomarkers. We used LC-MS-based targeted metabolomics profiling on serum samples from three cohorts: healthy individuals (n=9), fracture patients without infection (n=9), and FRI patients (n=21) with confirmed Staphylococcus aureus (n=6) or S. epidermidis (n=15) infection, to compare global metabolic changes.

After median normalization due to different total metabolite concentrations, we found significant differences across all three groups – mainly in amino acids and their derivatives. An increase of tryptophan (FDR 0.001) and a decrease in threonine (FDR 0.007) and arginine (FDR 0.04) were specific for FRI patients compared to both healthy and non-infected fracture patients, highlighting specific metabolic alterations associated with infection rather than fracture. Additionally, a predictive model based on the ratios of alanine to glutamic acid and arginine to tryptophan successfully differentiated between healthy individuals and FRI patients, while also classifying fracture patients as non-infected.

This study represents the first metabolic profiling of serum from patients with FRI. Although the research is limited by the small group size and the need for data normalization to overcome cohort differences, the findings show potential. Serum metabolites could serve as effective biomarkers for FRI in a clinical setting as a simple predictive model was able to distinguish FRI patients from both healthy controls and patients with fracture.

3055 C — High-Throughput UPLC-MS/MS Quantification of Lyso-Gb3 and Its Analogues from Dried Blood Spots for Clinical Application in Fabry Disease

PRESENTING AUTHOR: *Rosario Stimoli, University of Naples Federico II, Italy*
CO-AUTHORS: *Fabrizio Merciai, Eduardo Sommella, Pietro Campiglia*
SUB THEME: *Biomarker validation and clinical translation*

Rapid and accurate quantification of lyso-globotriaosylsphingosine (lyso-Gb3) and its structural analogues is critically needed in the clinical management of Fabry disease, where α-galactosidase A deficiency leads to biomarker accumulation. In this contribution We developed a high-throughput UPLC-MS/MS method for simultaneous determination of lyso-Gb3 and its analogues in dried blood spots (DBS), optimized for speed, robustness, and clinical applicability. The method uses a short, narrow-bore CSH column (50 × 2.1 mm, 1.7 µm) under finely tuned flow rate, temperature, and gradient conditions. Detection was performed in multiple reaction monitoring (MRM) on a triple quadrupole MS analyzer, using electrospray ionization in positive mode. The assay exhibited excellent linearity (R^2 = 0.999) and sensitivity, with a limit of detection (LOD) of 0.0050 nM and a limit of quantification (LOQ) of 0.015 nM. Using isotopically labeled internal standard (lyso-Gb3-d_7) evaluate extraction efficiency and matrix effects (10.7%). were evaluated, the extraction protocol yielded excellent recovery (97.2%) and was consistent across clinically relevant concentrations. Method precision was confirmed by intraday CVs of 0.43% (retention time) and 4.85% (peak area), and interday CVs below 0.3% (RT) and 3.3% (AUC). Total runtime, including sample injection and column reconditioning, was limited to 4.5 minutes, comprising column re-equilibration. This rapid and reliable assay is suitable for routine clinical implementation and pharmacological monitoring in Fabry disease. As proof of concept, samples of patients treated with enzyme replacement therapy (ERT) or migalastat were analyzed.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3056 C — An investigation of host and microbial metabolite profiles in non-small cell lung cancer patients with varying responses to immunotherapy

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SUB THEME: *Cancer*

Over the past few years, immune checkpoint inhibitors (ICIs) have emerged as the treatment of choice for advanced non-small cell lung cancer (NSCLC). Though, only a limited number of patients has demonstrated a successful response to ICI therapy. A number of studies have reported metabolic dysregulation and microbiome dysbiosis as being involved in cancer development and treatment responses. Consequently, various metabolite markers, such as amino acids, lipids, and fecal metabolites, have been proposed for predicting ICI treatment outcomes. Even so, there is still a lack of detailed analysis of circulating metabolites among treatment response groups.

In this study, blood samples were collected from 64 NSCLC patients who received ICI therapy. Samples were collected before and after treatment. Among these, there were 43 matched cases from the hyperprogressive disease (HPD; n=12) and non-hyperprogressive disease (Non-HPD; n=31) groups. The abundances of host and microbial metabolites, including amino acids, fatty acids, organic acids, sugars, sugar alcohols, phospholipids, uremic toxins and bile acids in plasma were determined.

We observed differences in both metabolite levels and correlation patterns between the HPD and Non-HPD groups. More differential host metabolites were found after ICI treatment compared to before therapy. In addition, phosphatidylcholine (PC) 16:0/18:0, 4-guanidinobutyric acid, and N-phenylacetylglycine could potentially represent the differences between the two response groups.

Our findings suggest differences in patients' metabolic status in response to the treatment. Variations in membrane lipids, such as PC 16:0/18:0, might reflect the impact of membrane composition on the immune system. Furthermore, the presence of microbial metabolites previously reported to be involved in inflammation and host immune system may contribute to the differences in treatment responses observed among patients.

3058 C — Metabolic Analysis of Cisplatin Resistance in Head and Neck Squamous Cell Carcinoma

PRESENTING AUTHOR: *Nicole Edel, Max-Delbrück Center, BIMSB, Germany*

CO-AUTHORS: *Sabrina Geisberger, Stefan Kempa*

SUB THEME: *Cancer*

Head and neck squamous cell carcinoma (HNSCC) is among the most prevalent cancer types globally. Cisplatin (CDDP) remains a cornerstone in the chemotherapy of various solid tumors. However, treatment failure often occurs, particularly after relapse, due to both inter- and intratumoral heterogeneity that contributes to resistance.

To explore the metabolic basis of CDDP resistance, we utilized CDDP-sensitive (Sen) and CDDP-resistant (Res) subclones of the FaDu HNSCC cell line. Integrative metabolomic and proteomic analysis revealed that Res cells exhibit elevated expression of proteins involved in nucleotide synthesis compared to Sen cells. This was further supported by nucleotide profiling, which showed a reduction in nucleotide pools in Sen cells following CDDP treatment Strikingly, upon treatment the Res display nucleotide levels reflective of their resistance profile.

Additionally, we assessed extracellular nucleoside levels as a potential surrogate for intracellular nucleotide status. We found that a positive treatment response was associated with increased levels of unmodified nucleosides and decreased levels of methylated nucleosides. Given previous findings of elevated modified nucleosides in the urine of cancer patients, our data suggest that extracellular nucleoside profiles could serve as non-invasive biomarkers for monitoring treatment response. Therefore, we are currently investigating nucleoside levels in a PDX mouse model under CDDP therapy.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3059 C — Discovery of Microbial Metabolite Biomarkers for Early Prediction of Immunotherapy Response in Non-Small Cell Lung Cancer

PRESENTING AUTHOR: ***Pingping Zhu, Radboud University, Netherlands***
CO-AUTHORS: ***Robert S Jansen***
SUB THEME: ***Cancer***

Lung cancer is the leading cause of cancer incidence and mortality worldwide, with non-small cell lung cancer (NSCLC) comprising approximately 85% of cases. Immunotherapy is considered a breakthrough for NSCLC treatment. However, not all patients respond properly to it, and the underlying mechanism remains unclear. Gut microbial metabolites, particularly short-chain fatty acids (SCFAs), are believed to play a key role in immune regulation and may enhance the immunotherapy response in NSCLC. Our study aims to identify microbial metabolite biomarkers for early immunotherapy response prediction by exploring the fecal metabolome of 200 NSCLC patients from the DEDICATION-1 study (NCT04909684).

A targeted RPLC-MS metabolomics method was developed for absolute quantification of key gut microbial metabolites, including SCFAs, bile acids, and indole derivatives. To ensure reliable analysis of SCFAs in the RP column, a pre-analytical derivatization with 3-nitrophenylhydrazine was employed to convert the targets to their 3-nitrophenylhydrazones. Derivatization conditions, such as reaction time and reagent concentration, were optimized for method reliability. For fecal sample preparation, freeze-drying is commonly recommended before extraction to control water content variation among fecal samples. However, being volatile organic acids, SCFAs are likely to evaporate during the freeze-drying process. Therefore, we investigated the impact of freeze-drying on the targets by comparing their signals in 150 mg wet samples and those freeze-dried for 24h, 48h, and 72h. Samples were extracted with 50% acetonitrile in water (mg:µL, 1:10) before LC-MS analysis.

Our results show that with 3-nitrophenylhydrazine derivatization, the developed RPLC-MS method achieved reliable and sufficient separation of the targeted metabolites. SCFAs show no significant change after freeze-drying for 24 ,48, and 72 hours compared to wet samples. This optimized method will be fully validated and applied to final fecal sample analysis. Additionally, we will develop an untargeted method to explore unknown microbial metabolites in the future.

3060 C — Toward SN- and double bond position resolved untargeted lipidomics using OAD/CID-DIA with ammonium bicarbonate solvent condition

PRESENTING AUTHOR: ***Bujinlkham Buyantogtokh, Tokyo University Of Technology And Agriculture, Japan***
SUB THEME: ***Cancer***

Elucidating lipid structures is crucial to understand biological functions and metabolic pathways. Recently, structural lipidomics techniques untangling multimodal tandem mass spectral information have been developed to characterize lipid class, chain composition, double bond (C=C) location, and SN positional isomers. Nevertheless, compared to the widely used collision-induced dissociation (CID), these techniques often suffer from lower sensitivity and more complex spectral patterns, posing challenges in annotation accuracy and the evaluation of structural isomers ratio. We present a novel hybrid method, called OAD/CID MS/MS, which integrates the mass fragmentations of oxygen attachment dissociation (OAD) and CID, where two independent spectra are integrated and acquired as a single spectrum. The OAD technique selectively cleaves double-bond sites, while CID produces lipid class and acyl-chain-specific fragments. We also evaluated the mass fragmentation patterns of phosphatidylcholine (PC) with a liquid chromatography condition using ammonium bicarbonate, providing diagnostic product ions to resolve SN positional isomers in negative ion mode. Finally, we employed a data-independent acquisition (DIA) technique to quantify the C=C and SN positional isomers by using the MS2 chromatograms, thereby eliminating the need for complete chromatographic separation.

We validated the method using lipid isomer standards, PC 16:0/18:1(9), PC 18:1(9)/16:0, and PC 16:0/18:1(11) where the fragmentation efficiencies for each C=C position and SN positional isomer were within the range of 100 nM to 1 mM ($Y=0.99X+2.5$; $R^2=0.99$ and $Y=1.16X–6.3$; $R^2=0.97$, respectively). We also found that the limit of detections (LODs) for the diagnostic ions used to determine the C=C position and SN position was 10 nM and 25 nM, respectively. Further validations with NIST SRM 1950 human plasma and mouse brain lipid extracts indicated that our approaches can provide the confidence ratios of C=C and SN positional isomers when compared to previously reported results. Finally, our OAD/CID-DIA technique was applied to elucidate cancer cell line-specific lipidomes.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3061 C — Investigating the role for elevated 2-hydroxyglutarate (2-HG) in IDH1 mutant glioblastoma cells using $^{13}C_5$ 2-HG as a metabolic tracer

PRESENTING AUTHOR: *Yiquan Chen, University Of Oxford, United Kingdom*

CO-AUTHORS: *James McCullagh*

SUB THEME: *Cancer*

Mutations in the metabolic genes IDH1 and IDH2 are found in over 70% of grade II and III gliomas and lead to a change in enzyme function which produces 2-hydroxyglutrate (2-HG) at high µmolar levels in mutant cancer cells. The presence of IDH mutations is associated with oncogenesis, methylation of the genome and inhibition of 2-oxoglutarate (2-OG) dependent oxygenase enzymes but whether 2-HG directly interconverts with other metabolites is less well understood. The aim of this study was to investigate this question using a ^{13}C tracer approach in mutant and wild-type LN18 glioblastoma cells.

Mutant IDH (mutIDH) and wild type (wtIDH) glioblastoma cells (LN18 cell lines) were cultured using media supplemented with $U^{13}C_5$ 2-HG and cell samples were analysed using untargeted anion-exchange chromatography-mass spectrometry (IC-MS) and reversed-phase liquid chromatography-mass spectrometry (RPLC-MS).

A database of 458 metabolite standards and an untargeted metabolomic analysis platform, X^{13}CMS were used to search for isotope abundance patterns indicative of ^{13}C enrichment beyond natural abundance levels. Results showed no evidence for the conversion of 2-HG back to 2-OG by comparing 2-OG and 2-HG isotopomer distributions and evidence of 2-HG-derived carbon being present in other common metabolites, suggesting no interconversion. However, one 2-HG-derived product was detected intracellularly. This was shown to contain a phosphate group and its identity was commensurate with a phosphorylated form of 2-HG, present in the IDH1 mutant cell line. Further validation experiments are being pursued.

In summary, our ^{13}C labelled 2-HG tracer experiments in glioblastoma cells, provided evidence that endogenous 2-HG was not extensively interconverted into other metabolites, including its precursor 2-OG, but there was a evidence of a novel 2-HG-derived product which we provide evidence for here for the first time.

3062 C — Dysregulated Branched Chain Amino Acids Pathway as a Metabolic Signature of Lung Cancer Cells

PRESENTING AUTHOR: *Sara Abdulkader, Weill Cornell Medicine Qatar, Qatar*

SUB THEME: *Cancer*

Introduction: Lung cancer remains the leading cause of cancer-related mortality worldwide. Given that cancer is recognized as disease of dysregulated metabolism, further understanding of its underlying metabolic alterations is critical for identifying novel therapeutic targets.

Untargeted metabolic profiling provides an overview on metabolic composition and can therefore be useful in identifying cancer-associated metabolic alterations. Nevertheless, studies on lung cancer cell metabolism remain limited. This study aims to characterize metabolic dysregulation in lung cancer.

Methods: Untargeted metabolomic profiling was performed on 21 lung cell lines, including 16 lung cancer cell lines and 5 normal lung fibroblast cell lines. Quantitative polymerase chain reaction (qPCR) and Western blot analysis were performed to assess the expression of genes implicated in the metabolic dysregulations identified through metabolomic profiling.

Results:

A total of 367 metabolites were identified, across various metabolic pathways, including peptides, amino acids, carbohydrates, energy metabolism, cofactors and vitamins, lipids, nucleotides, and xenobiotics. Principal component analysis (PCA) revealed clear metabolic distinctions, with lipid, amino acid, and carbohydrate metabolism contributing most to the separation between lung cancer cell lines and normal lung fibroblast cell lines. The branched chain amino acid (BCAA) pathway was significantly altered across lung cancer cell lines. BCAAs (leucine, valine and isoleucine) were downregulated, while acylcarnitines (isovalerylcarnitine (C5), butyrylcarnitine (C4), and 2-methylbutyrylcarnitine (C5)) were upregulated in lung cancer cells. Genes involved in the catabolism of these metabolites showed significant dysregulation at both mRNA and protein levels, further confirming alterations in this pathway.

Conclusion: This study highlights BCAA metabolism as dysregulated in lung cancer, suggesting its role in cancer progression, and underscoring its potential as a therapeutic target.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3063 C — Plasma metabolomics reveals N-arachidonylglycine as a potential biomarker in acute coronary syndrome

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SUB THEME: *Cardiovascular diseases*

Background: Acute coronary syndrome (ACS) refers to a range of conditions relating to an abrupt reduction in blood flow to the heart and is a leading cause of cardiovascular morbidity and mortality. Traditional biomarkers of this condition, such as cardiac troponin, have limitations because their changes are not exclusively found in ACS, highlighting the need for new biomarkers that enhance diagnostic accuracy and guide therapeutic interventions. The exploration of alternative diagnostic tools is essential. This study aimed to identify unique metabolomic profiles, novel alternative potential biomarkers, and their associated pathways in ACS patients using metabolomic techniques.

Methods: We conducted an untargeted metabolomic analysis using liquid chromatography-tandem mass spectrometry on plasma samples from 25 ACS patients and 36 healthy controls in Thailand. Partial least-squares discriminant analysis (PLS-DA) and differential metabolite abundance were employed to distinguish the metabolomic profiles of ACS patients from those of healthy controls, identifying key metabolites that differentiate these groups. Pathway enrichment analysis using a KEGG-based database was also conducted to investigate ACS-related changes in biochemical pathways.

Results: Principal component analysis and PLS-DA demonstrated clear separation between ACS patients and healthy controls based on metabolomic profiles. Five metabolites—namely, NP-006862, 2-monolinolenin, fatty acid (18:0) (11R,12S-methylene-octadecanoic acid), N-arachidonylglycine, and (1E)-1-(allyldisulfanyl)-3-(methylsulfinyl)-1-propene—were significantly more abundant in ACS patients. The ACS group also exhibited significant alterations in 64 biochemical pathways compared with the control group. Arachidonic acid metabolism emerged as the most profoundly dysregulated pathway ($p < 7 \times 10\text{-}9$), strongly suggesting its involvement in the pathogenesis of ACS.

Conclusions: N-arachidonylglycine was identified as a novel potential biomarker of ACS based on its association with the disease in PLS-DA and pathway analysis. This study highlights the importance of combining advanced metabolomic techniques with multivariate statistical methods to identify novel biomarkers for ACS, thereby enhancing early diagnosis and facilitating more targeted therapeutic interventions.

3064 C — Untargeted lipidomic profiling of plasma from patients with ST-elevation myocardial infarction

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CO-AUTHORS: *Štěpán Hudec, Martin Rada, Veronika Kučerová, Lukáš Najdekr*
SUB THEME: *Cardiovascular diseases*

Cardiovascular diseases (CVDs) are the leading cause of death in developed and developing countries, taking approximately 20.5 million lives each year. However, according to a report by the World Heart Federation, up to 80 % of premature heart attacks and strokes could be prevented by the correct use of more specific diagnostic tools. An untargeted lipidomic approach seems to be a suitable tool for the search for potential new biomarkers. Here in our work, we present data from analyses of lipid profiles of patients after ST-elevation myocardial infarction (STEMI) compared to the control group.

We have collected nine patient plasma samples from the Department of Cardiology and nine control plasma samples from the Department of Clinical Biochemistry. The human plasma samples were analysed using reversed-phase liquid chromatography coupled with a mass spectrometer (RP-HPLC/MS). The data matrix was processed using Compound Discoverer 3.3 SP3 (Thermo Fisher Scientific, USA).

The untargeted lipidomics with “peak rating” filtering in Compound Discoverer 3.3 SP3 yielded 875 putatively annotated compounds in positive and 631 in negative ionisation mode. Fragmentation spectra acquisition and molecular networking enabled structural grouping and identification of potential biomarker candidates. Lipid compounds were successfully annotated using machine learning algorithms (SIRIUS, University of Jena). Using S-plots and volcano plots, ceramides (namely, Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:1), and Cer(d18:2/24:1)) were evaluated as the most significant compounds in patient samples. In the Mayo Clinic hospital system, Cer(d18:1/16:0), Cer(d18:1/18:0), and Cer(d18:1/24:1) levels, along with their ratios to Cer(d18:1/24:0), are used to assess the risk of adverse severe CVD events over one to five years.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3065 C — 13C-labeled scyllo-inositol to investigate its metabolism in mammalian cells

PRESENTING AUTHOR: ***Meike Marie Amma, LeibnForschungsverbund Berlin e. V. (FMP), Germany***

SUB THEME: ***Cellular metabolism***

Myo-inositol is the most abundant inositol isomer in humans and can be metabolized into different soluble inositol phosphates and various phosphatidylinositides. Dysregulation of myo-inositol metabolism is associated with several diseases, including cancer and diabetes. Scyllo-inositol is the second most abundant inositol isomer in the human brain and is associated with several neurological disorders. However, very little is known about its exact physiological role and its metabolic fate.

To address such questions, we developed a concise synthesis of [13C6]scyllo-inositol by combining the oxidative potential of Gluconobactor oxydans with chemical reduction.

First, uptake and potential metabolites of [13C6]scyllo-inositol under [12C6]myo-inositol starvation were explored by NMR. After incubation for eight days, uptake of [13C6]scyllo-inositol was observed in HCT116, HEK293T and HepG2 cells.

We also investigated A172 cells, a glioblastoma derived cell line with reported high uptake and metabolism of myo-inositol. We demonstrated the uptake of [13C6]scyllo-inositol in A172 cells by NMR and HILIC-MS/MS analysis for 15 days. Additionally, the formation of [13C6]myo-inositol was only detected after acidic, but not organic extraction. We currently investigate our hypothesis that [13C6]myo-inositol metabolized from [13C6]scyllo-inositol is extracted from cell membrane bound phosphatidylinositides.

3066 C — HepG2 cells may not be reliable allies for the study of hepatic gluconeogenesis

PRESENTING AUTHOR: ***Charlotte Lemoine, Umons, Belgium***

CO-AUTHORS: ***Jean-Marie Colet, Anne-Emilie Declèves, Vanessa Tagliatti, Karima Dalil***

SUB THEME: ***Cellular metabolism***

Every cell in the human body needs energy to sustain its metabolic functions. To this respect, glucose regulation is essential, especially the gluconeogenesis pathway that converts non-hexose precursors, such as glycerol, lactate, pyruvate and glucogenic amino acids into glucose moieties. It is known that gluconeogenesis mainly occurs in the liver and, to a lesser extent, in kidneys. During fasting and under stress conditions, renal gluconeogenesis may account for 40% of systemic gluconeogenesis. To this respect, our lab is interested in liver-to-kidney crosstalk. To do so, we intend to build an in vitro model allowing the communication between hepatocytes and renal cells. Initially, we decided to use the HepG2 hepatocyte cell line mainly for its ease of implementation as well as for the numerous publications demonstrating their usefulness in various applications.

We started with and the assessment of their metabolic skills by using a 1H-NMR -based metabolomic approach. The gluconeogenesis pathway was stimulated by adding a cocktail of known precursors (lactate, pyruvate, cAMP and dexamethasone). However, the metabolomic findings revealed that HepG2 cells essentially displayed a Warburg-like profile, as expected for cancer cells, but with very limited or no gluconeogenesis activity even under stimulation.

In conclusions, the HepG2 cell line does not seem like a reliable ally to study gluconeogenesis and another model is needed. Primary hepatocytes could represent a valuable alternative. However, their use should be coupled with MS-based metabolomic instead of NMR due to a limitation in cell number.

3067 C — Imaging of metabolites in cancer cells: Integration of MALDI-MSI and microscopy for spatial single cell metabolomics

PRESENTING AUTHOR: ***Hanna Bednarz, Universität Bielefeld, Germany***

SUB THEME: ***Cellular metabolism***

The increase in spatial resolution and sensitivity in MALDI-MS imaging increasingly enables the analysis of single cells. The individuality of single cells is the basic element in analysis by fluorescence microscopy, high-content screening and flow cytometry. In order to characterize metabolic individuality, approaches to the analyses of cells as single, individual objects are required in a similar way for MALDI MSI. We have used different software-approaches in order to analyze results of MALDI-MSI of single cells of melanoma and lung adenocarcinoma cell lines. A striking metabolic heterogeneity can be observed even between cells within one single cultivation. Different cell lines, even those originating from metastases of the same individual can be distinguished by their different metabolic profiles. Ion maps can be segmented to obtain metabolic signatures of individual cells. MALDI-MSI results can be combined with microscopical imaging, and cell morphology can be correlated with metabolic features.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3068 C — Metabolomic analysis of gastrocnemius and soleus muscles in mice treated with SGLT-2 inhibitors under normal and high-fat diets

PRESENTING AUTHOR: *Yejin Kim, Incheon National University, South Korea*
CO-AUTHORS: *Jae Geun Kim, Jae Kwang Kim*
SUB THEME: *Endocrine and metabolic diseases*

Sodium-glucose cotransporter 2 (SGLT-2) inhibitors are effective in managing type 2 diabetes by lowering blood glucose levels independently of insulin, thereby reducing the risk of hypoglycemia and promoting weight loss. However, their potential effects on skeletal muscle mass and strength warrant further investigation, as the underlying mechanisms within muscle tissue remain largely uncharacterized. This study aimed to evaluate the potential impact of SGLT-2 inhibitors on the skeletal muscles (gastrocnemius and soleus) of mice fed either a normal or high-fat diet, using a metabolomics-based approach.

Eight-week-old mice were randomly assigned to four groups (n = 6 per group): normal diet (V), normal diet with SGLT-2 inhibitor (S), high-fat diet (HV), and high-fat diet with SGLT-2 inhibitor (HS), and were treated for six weeks.

Metabolic profiling of skeletal muscle tissues was performed using gas chromatography–time-of-flight mass spectrometry (GC–TOF/MS). A total of 42 hydrophilic and lipophilic metabolites—including intermediates of the TCA cycle, amino acids, organic acids, sugars, fatty acids, and sterols—were identified. Multivariate statistical analysis of gastrocnemius muscle metabolites revealed significant differences among the four groups, driven by both diet and SGLT-2 inhibitor treatment. Notably, alterations were observed in pyruvate metabolism, the glucose–alanine cycle, inositol metabolism, and beta-alanine metabolism in the gastrocnemius. In contrast, multivariate analysis of soleus muscle metabolites showed that dietary differences had a stronger influence than SGLT-2 inhibitor treatment, particularly affecting fatty acid metabolism. These findings suggest that SGLT-2 inhibitors may influence fatty acid and ketone body utilization, as well as amino acid and glucose metabolism, specifically in the gastrocnemius muscle. Moreover, the study highlights differential metabolic responses to SGLT-2 inhibition between the gastrocnemius and soleus muscles.

3069 C — Evaluation of the Diagnostic Performance of Metabolite Risk Scores for Type 2 Diabetes

PRESENTING AUTHOR: *Hye Jin Yoo, Inha University, South Korea*
SUB THEME: *Endocrine and metabolic diseases*

Background/purpose: Type 2 diabetes (T2D) has well-established biomarkers for diagnosis, including blood glucose and HbA1c. However, glucose levels are influenced by various factors and exhibit limited reproducibility. While HbA1c is a more stable indicator, it is useful for assessing long-term glycemic control rather than the current condition. Therefore, this study aimed to evaluate the diagnostic performance of the metabolite risk score (MRS) established using newly identified biomarkers through metabolomics.

Methods: Each of discovery and replication cohorts comprised 100 study subjects, with 50 healthy individuals and 50 patients with T2D in each cohort. Plasma samples from the subjects were obtained through the Korea Biobank Network (IRB of Inha University: approval No. 221004-1AR4). Global metabolomics was conducted (UHPLC-MS/MS), and an MRS was computed using a weighted approach, represented by the following equation: $\sum \beta i Mi$, where 'β' indicates standardized β coefficients (weights), 'M' indicates z-score, and 'i' refers to each key biomarker.

Results: A total of 58 metabolites were identified after excluding unknown and drug-related metabolites and were further screened based on two criteria: 1) a VIP score ≥1.5, and 2) an adjusted p-value <0.05. Subsequently, linear regression analysis was conducted to validate the key metabolites. As a result, five metabolites—3-(6,7-dimethoxy-1,3-benzodioxol-5-yl)-2-propen-1-ol, L-pyroglutamic acid, N-(3-acetamidopropyl)pyrrolidin-2-one, 1,7-dimethyluric acid, and 4-vinylphenol sulfate—emerged as being associated with T2D. The MRS was derived using the five key metabolites in the discovery cohort, and a cut-off value for the MRS was obtained. Based on this cut-off, the replication cohort was re-grouped. The re-grouping achieved an accuracy of 98%, and the MRS demonstrated enhanced diagnostic abilities (99.7%) compared to glucose levels (95.1%) and HbA1c (98.0%).

Discussion: The present study proposes a novel diagnostic system using the MRS, which incorporates several significant biomarkers with assigned weights. This approach offers a new perspective on utilizing biomarkers and is expected to enhance diagnostic accuracy.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3070 C — Untargeted urine metabolomics identifies metabolic signatures of diabetes progression in high sugar consumers

PRESENTING AUTHOR: *Miso Nam, Korea Food Research Institute, South Korea*
CO-AUTHORS: *Min-Sun Kim*
SUB THEME: *Endocrine and metabolic diseases*

High dietary sugar intake is a known risk factor for type 2 diabetes mellitus (T2DM), but not all individuals with high sugar consumption develop the disease. This indicates the need for biomarkers that can identify individuals at higher metabolic risk, particularly among high sugar consumers. In this study, we conducted untargeted urine metabolomics to identify metabolites associated with dietary sugar intake and to detect metabolic differences across glycemic status groups.

Urine samples were collected from a cohort of adults categorized into normoglycemic, prediabetic, and diabetic groups based on clinical criteria. Dietary intake was assessed using a validated food frequency questionnaire (FFQ), and sugar intake was normalized to total caloric intake. Untargeted metabolite profiling was conducted using an 800 MHz nuclear magnetic resonance (NMR) spectrometer. Multivariate analysis using partial least squares discriminant analysis (PLS-DA) revealed a distinct separation of the diabetic group from the normoglycemic and prediabetic groups. Among the metabolites contributing to the separation observed in the PLS-DA score plot, fumarate and citrate—both intermediates of the tricarboxylic acid (TCA) cycle—were identified as discriminative metabolites and were subsequently examined for their associations with dietary sugar intake and glycemic status. Sugar intake was significantly higher in the prediabetic and diabetic groups compared to the normoglycemic group, but there was no difference between prediabetes and diabetes. In contrast, urinary fumarate and citrate levels were significantly elevated in the diabetic group, while remaining comparable between the normoglycemic and prediabetic groups. Among individuals with high sugar intake, elevated levels of these metabolites were significantly associated with diabetes status.

These results suggest that urinary TCA cycle intermediates may be useful metabolic markers for detecting diabetes progression in high-risk individuals with elevated sugar consumption.

3071 C — Sex-Specific Endocrine and Reproductive Disruption in Zebrafish (Danio rerio) Induced by Environmental Contaminants: TDCPP, Sodium Perchlorate, and 4-tert-Octylphenol

PRESENTING AUTHOR: *Jangjae Lee, Korea Research Institute Of Chemical Technology, South Korea*
CO-AUTHORS: *Sung-Hee Cho*
SUB THEME: *Endocrine and metabolic diseases*

This study investigates the endocrine-disrupting and reproductive effects of three widely detected environmental chemicals—tris(1,3-dichloro-2-propyl) phosphate (TDCPP), sodium perchlorate (SP), and 4-tert-octylphenol (OP)—in zebrafish (Danio rerio). Over a 21-day exposure period, various biomarkers were measured, including thyroid hormones (THs), steroid hormones, vitellogenin (VTG) mRNA, aromatase activity, and oxidative stress markers.

TDCPP, an organophosphate flame retardant, showed significant sex-specific toxicokinetics, with females exhibiting higher accumulation and slower clearance compared to males. This disparity correlated with oxidative stress and disrupted hormone synthesis, particularly affecting steroid and thyroid pathways, leading to reproductive impairment.

SP exposure resulted in reduced TH levels, which directly impacted steroid hormone synthesis and reproductive outcomes. Correlation analysis revealed a strong interaction between thyroid and gonadal hormone axes, highlighting their interdependence under chemical stress.

OP, a degradation product of alkylphenol ethoxylates, induced estrogenic effects in females and both estrogenic and anti-androgenic effects in males. Females displayed increased hepatic VTG mRNA and estradiol levels, while males experienced reduced androgen receptor expression and androgen levels. These disruptions emphasized the dual-mode endocrine interference of OP.

Overall, this study underscores the importance of assessing sex-specific responses in ecotoxicology and reveals critical insights into the hormonal interplay disrupted by environmental contaminants. These findings contribute to a broader understanding of chemical-induced endocrine disruption, informing environmental risk assessment and regulatory policies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3072 C — Association between bisphenol A, S and F exposure and thyroid function in adults

PRESENTING AUTHOR: ***Po-Chin Huang, National Health Research Institutes, Taiwan***
CO-AUTHORS: ***Han-Bin Huang***
SUB THEME: ***Epidemiology***

While most studies have focused on the health risks of bisphenol A (BPA), little is known about the adverse effects of its alternatives—bisphenol S (BPS) and bisphenol F (BPF)—especially in adults and concerning thyroid function. This study aimed to assess exposure levels and daily intake (DI) of BPA, BPS, and BPF in Taiwanese adults and to evaluate the distribution of hazard indices and associations between individual and combined bisphenol exposures and thyroid function indicators.

This cross-sectional study used data from the Taiwan Environmental Survey for Toxicants. Blood and urine samples were analyzed to measure thyroid function indicators—thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), and free thyroxine (FT4)—as well as urinary concentrations and DI of BPA, BPS, and BPF. A total of 262 adults were included. Generalized linear models (GLMs) were applied to assess associations between bisphenol exposure and thyroid function. Additionally, quantile g-computation (q-g-comp) models and Bayesian kernel machine regression (BKMR) were used to evaluate the overall effects of bisphenol mixtures.

Our findings indicate that BPA exposure was significantly positively correlated with FT4 ($\beta = 0.027$) and SPINA-GT ($\beta = 0.079$). Daily intake of BPA ($\beta = 0.054$) and BPS ($\beta = 0.039$) was positively correlated with T4, but significantly negatively correlated with the T3/T4 ratio ($\beta = -0.047$ for BPA; $\beta = -0.052$ for BPS). Additionally, BPA DI was negatively correlated with the TSH/T4 ratio ($\beta = -0.144$) and positively correlated with SPINA-GT ($\beta = 0.115$). The q-g-comp model for mixed exposure showed a positive correlation with T4 ($\psi = 0.025$). Furthermore, combined DI of BPA, BPS, and BPF was positively correlated with T4 ($\beta = 0.040$) and negatively with the T3/T4 ratio ($\beta = -0.041$, $p<0.05$).

These findings suggest that exposure to BPA, BPS, and BPF may influence thyroid hormone balance in adults, warranting further longitudinal studies.

3073 C — Exposure to Environmental Agents and Health Risks Among Vector Control Workers in Taiwan

PRESENTING AUTHOR: ***Mei-Lien Chen, National Yang Ming Chiao Tung University, Taiwan***
CO-AUTHORS: ***Zheng Xiong Wang, Yu-Fang Huang***
SUB THEME: ***Epidemiology***

Vector control is essential for public health and maintaining environmental sanitation. Workers could be exposed to environmental agents (EA) through inhalation and dermal contact during operation. This study investigates the work patterns, EA use, external and internal exposure level, and their correlation with urinary EA metabolites and oxidative stress among the vector control workers in Taiwan.

Personal air samples (n=20) were collected from three vector control companies in August 2021 along with workers' pre-and post-shift urine samples (n=40) and completed questionnaires. Eight EA including chlorpyrifos, fenitrothion, cypermethrin, permethrin, alpha-cypermethrin, deltamethrin, imidacloprid, and fipronil were analyzed. Urinary metabolites included TCPy, DCCA and 3-PBA, IMI, and FP/FP-SFO and oxidative stress biomarkers (8-OHdG, 8-NO2Gua, HNE-MA, 8-iso-PGF2α) were also assessed.

One-hundred workers completed the questionnaire survey. It found that the workers were mostly young males. Only about 60% of workers had a license or training for the job. Indoor vector control (72%) focused on cockroaches, mosquitoes, and flies' control, with pyrethroids being the most commonly used pesticides, followed by imidacloprid. Workers reported that standing for long hours, repetitive hand movements, carrying heavy objects, and unnatural postures were common during work. The use of safety equipment was minimal, while 80% of them underwent annual health checkups and 8% had abnormal blood acetylcholinesterase activity.

Field sampling revealed that all air samples were detected EAs except fenitrothion. Urinary metabolites were detected in over 95% of samples for TCPy, 3-PBA and IMI, followed by FP in 85%, DCCA in 35-45%, and FP-SFO in less than 10%. We found significant correlations among urinary metabolites of IMI, FP and oxidative stress biomarkers. Use of personal protective equipment did not affect IMI or oxidative stress levels. This study highlights the occupational risks in vector control industry and underscores the need for improved safety control measures to protect health of the workers.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3074 C — Robustness Assessment of an LC-HRMS Platform for Integrated High-Throughput Exposomics and Metabolomics

PRESENTING AUTHOR: *Julia Füreder, University Of Vienna, Austria*
CO-AUTHORS: *Delia Castilla Fernández, Yunyun Gu, Kolawole Ayeni, Giulia Guerra, Benedikt Warth*
SUB THEME: *Exposome research*

The exposome is defined as an integrated compilation of physical, chemical, biological, and psychosocial influences that impact biology and has a profound impact on health. Exposome-wide association studies (ExWAS) attempt to systematically discover relationships between disease phenotypes and environmental exposures and warrant large-scale measurements of human samples. High-resolution mass spectrometry (HRMS) is the key technology proposed for future ExWAS for enhancing discovery-driven epidemiology. Reliable data quality throughout the analytical run is of great importance for high-throughput measurements. Here, we propose a workflow for integrated exposomics and metabolomics on an LC-HRMS platform providing adequate data quality without compromising time- and cost-efficiency. Commercially available human plasma samples (n = 156) were prepared using a protein precipitation protocol and fortified with 13 labeled internal standards. Extracts were pooled and re-aliquoted to ensure comparability. Furthermore, the sequence design included multiple quality control samples, such as solvent blanks, process blanks, >300 reference standards in neat solvent and matrix, the standard reference material (SRM) 1950, and a pooled extract of all samples. Samples were measured using the Vanquish Horizon, an ultra-high-performance liquid chromatography (UHPLC) system simultaneously employing a reversed phase (RP) and a hydrophilic interaction liquid chromatography (HILIC) column, coupled to an Orbitrap Exploris 480 equipped with an electrospray ionization source. Data was acquired in full scan mode with fast polarity switching and a resolution of 60.000. Peak picking was done using Skyline. Over 75% of internal standards showed a relative standard deviation (RSD) of less than 15% throughout a total of >280 injections per column. In addition, relevant endogenous and exogenous compounds were identified in the plasma samples with comparable performance throughout the sequence (RSD <15%). This workflow is intended to be applied to approximately 1000 human plasma and urine samples each from a breast cancer nested case-control study (ORDET, Italy).

3075 C — Expanding Chemical Space for Exposomics/Metabolomics in Dried Blood Spots

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SUB THEME: *Exposome research*

Dried blood spots (DBS) are a well-stablished matrix used in newborn screening programs around the world for the screening of metabolic diseases. As such, it holds great potential as a simple, non-invasive and alternative sample for the assessment of chemical exposure alongside with additional metabolic information via non-targeted analysis. In this work we present the results for the extraction optimization of 223 highly diverse exposure chemicals in dried blood spots, including bisphenols, phthalates, parabens, perfluoroalkyl substances (PFAS), polyphenols, flame retardants and more. Four different solvent composition were compared making using of quantitative DBS spiked before and after extraction, in order to asses compound recovery, matrix effects and additional analytical performance indicators for two concentration levels, using LC-HRMS (ZenoTOF 7600, SCIEX). The optimized method was applied to the pilot analysis of DBS collected in-house showcasing the capability to report real-life exposures in a quantitative manner, with 16 positive detections, including bisphenol A, PFOS, PFOA, cotinine, methyl/propylparaben, 2-naphtol. Untargeted data analysis was performed subsequently to obtain additional information on endogenous metabolites including but not limited to amino acids, acylcarnitines, hormones, lipids, biogenic amines and bile acids, at identification levels 1 or 2. Complementary, the sample set was used to further demonstrate the capabilities of a previously developed targeted/untargeted methodology for exposomics/metabolomics based on the combination of MRM and SWATH experiments in a single run. Finally, a similar evaluation was performed for dried milk spots using a high-sensitive low-resolution LC-MS system (QTRAP 7500, SCIEX) to investigate the potential of this additional matrix for exposomics as dried matrix spot. In the future, the method will be applied to the large-scale screening of DBS samples from newborn screening programmes to showcase the potential of such methodology to be implemented in the exposome-scale biomonitoring of newborns, as a vulnerable population to toxic effects.

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3076 C — Breast cancer exposomics using a robotic liquid-liquid-extraction-GC-HRMS workflow

PRESENTING AUTHOR: ***Delia Castilla-Fernández, University of Vienna, Austria***
CO-AUTHORS: ***Benedikt Warth***
SUB THEME: ***Exposome research***

Exposome research monitors relevant exogenous chemicals that might be associated with adverse health effects. However, the influence of the exposome in breast cancer, the most diagnosed cancer among women, has not been well defined yet. Therefore, exposome-wide association studies (ExWAS) are crucial for identifying correlations between chemical exposures and health outcomes in cancer research. ExWAS rely on large-scale sample cohorts and cutting-edge analytical technologies. The current work presents the design of the first ExWAS on breast cancer, enrolling >1000 participants from the ORDET cohort based on a nested case-control study design. Gas chromatography using electron impact ionization coupled to high-resolution mass spectrometry (GC-EI-HRMS) was optimized to identify and/or quantify relevant xenobiotics, including persistent organic pollutants (POPs), in biological samples. A targeted data acquisition strategy including >100 POPs was successfully combined with non-targeted analysis. For sample preparation, an online liquid-liquid extraction (LLE) of 100 µL plasma was established by a robotic autosampler. The established online LLE-GC-EI-HRMS workflow was conceived under a rigorous quality assurance and quality control (QA/QC) strategy and proposed an analytical batch design for analyzing samples from large cohort studies. The entire protocol, which lasted 24 min per sample, including robotic extraction and analysis, was fully automated and demonstrated to be robust over three days. Finally, the described robotic LLE-GC-EI-HRMS workflow was applied to measure the randomized plasma samples from the nested case-control study from the ORDET cohort. To conclude, this work presents a robust and high throughput automated LLE-GC-EI-HRMS workflow and preliminary results on plasma samples from the ORDET cohort in the framework of breast cancer ExWAS.

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3077 C — Metabolomic profiling of mammalian macrophages reveals butyrate's modulatory role in LPS-induced inflammation

PRESENTING AUTHOR: ***Bastian Bennühr, Technical University Braunschweig, Germany***
CO-AUTHORS: ***Emily Komfort, Karsten Hiller***
SUB THEME: ***Immunometabolism***

Butyrate is a short-chain fatty acid (SCFA) produced by the gut microbiota through the fermentation of dietary fibers. It has drawn attention for its anti-inflammatory effects, particularly its ability to suppress the pro-inflammatory activation of macrophages via inhibition of histone deacetylase 3 (HDAC3). Although most studies to date have focused on butyrate-mediated transcriptional regulation, the associated metabolic rewiring remains poorly understood. Given the tight link between cellular metabolism and immune cell function, we aimed to characterize butyrate-induced metabolic changes in both activated and resting macrophages, which might help uncover the molecular mechanisms underlying its anti-inflammatory effects.

Using three macrophage models, murine RAW 264.7 cells, primary murine bone marrow-derived macrophages (BMDMs), and human THP-1-derived macrophages, we treated the cells with varying concentrations of butyrate under both basal and LPS-stimulated conditions. Metabolites were extracted and measured by gas chromatography–mass spectrometry (GC-MS). The resulting data were processed and subjected to multivariate statistical analysis, including principal component analysis (PCA) and two-way analysis of variance (ANOVA), to identify significant metabolic alterations. We observed that in resting macrophages, butyrate induced distinct, dose-dependent metabolic changes across all macrophage models, including alterations in tricarboxylic acid (TCA) cycle intermediates and amino acid levels. Furthermore, butyrate partially reversed some LPS-induced metabolic alterations, suggesting a broader modulatory effect on inflammation-associated metabolic phenotypes.

These findings demonstrate that butyrate consistently reshapes macrophage metabolism in both resting and activated states, reinforcing its role as a metabolic regulator of immune function. This study contributes to our understanding of how microbial metabolites influence host immune responses and highlights the potential of SCFA-driven immunometabolic reprogramming in controlling inflammation.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3078 C — Metabolomics and lipidomics reveals mitochondrial dysfunction and altered energy metabolism in VLCAD deficient fibroblasts

PRESENTING AUTHOR: *Yana Sandlers, Cleveland State University, United States*
CO-AUTHORS: *Igor Radzikh, Olha Tsikhun*
SUB THEME: *Inborn errors of metabolism and neonatal screening*

Background: Very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) is an inherited autosomal recessive disorder of mitochondrial β-oxidation, a critical pathway for the catabolism of long-chain fatty acids. In individuals with VLCADD, impaired long-chain fatty acid utilization results in a spectrum of clinical manifestations. These include hypoketotic hypoglycemia, cardiomyopathy, rhabdomyolysis, and potentially life-threatening metabolic crises. Given the very limited treatment options currently available, a comprehensive understanding of the diverse metabolic abnormalities associated with VLCADD is essential for effective disease management.

Methods: Our study employed untargeted metabolomics and lipidomics (LC-QTOF) to study metabolic aberrations in fibroblasts derived from subjects with three different VLCAD mutations. Chromatographic separation was achieved with Atlantis Premier HILIC-Z (2.1mm x 150 mm, 1.7 µm) and T3 HSS (2.1mm x 100 mm, 2.1 µm) columns for polar metabolites and lipids respectively. Metabolomics and lipidomics data analysis was carried out with MassProfiler Professional (Agilent), Metaboanalyst 6.0 and Lipid Annotator platforms.

Results: Detailed and conservative analysis of cellular metabolome reveals fourteen significantly increased (FC≥2, $p<0.05$) and eighty-six significantly decreased metabolites (FC≤2, $p<0.05$) in VLCADD cells, emphasizing extensive metabolic disruptions. Orthogonal Partial Least Squares—Discriminant Analysis shows clear clustering of VLCADD vs control groups. Pathway and enrichment analyses demonstrate that most of the altered metabolites are associated with energy production, Krebs cycle, and mitochondrial function. VLCADD cells also demonstrate altered lipidome with significant increases in fatty acid oxidation intermediates, ceramides, lysophosphatidylcholine (LPC), triglycerides, and diglycerides, accompanied by a decrease in sphingomyelins.

Conclusions/Novel aspect: We identified secondary biochemical mechanisms contributing to VLCAD pathogenesis. Cells harboring VLCAD mutation exhibited alteration in complex lipids, mitochondrial dysfunction and deficient energy metabolism. Therefore, modulating mitochondrial energy metabolism warrants exploration as a complementary therapeutic strategy with the potential to mitigate the development of the clinical phenotype in VLCAD disease.

3079 C — Enhancing the Precision Diagnosis of Isovaleric Acidemia by Using Untargeted Metabolomics Profiling by Identifying Novel Biomarkers

PRESENTING AUTHOR: *Reem Almalki, KFSHRC, Saudi Arabia*
CO-AUTHORS: *Maha Al Mogren, Ahmad Alfares, Anas Abdel Rahman*
SUB THEME: *Inborn errors of metabolism and neonatal screening*

Background: Isovaleric Acidemia (IVA) is a rare inherited metabolic disorder caused by a deficiency in isovaleryl-CoA dehydrogenase, leading to the accumulation of toxic metabolites such as isovaleric acid. This accumulation results in neurological, metabolic, and systemic complications. Early diagnosis and intervention through newborn screening (NBS) are critical; however, traditional NBS methods face limitations, including focusing on a small number of targeted metabolites, difficulties detecting novel or unexpected biomarkers, and issues with false positive or incomplete detection. Method: To address these challenges, this study employed an untargeted metabolomic approach using high-performance liquid chromatography mass spectrometry (HPLC-MS) to profile metabolic alterations in IVA comprehensively. 32 dried blood spot (DBS) samples were analyzed, 16 from IVA patients and 16 age- and gender-matched healthy controls. Results: Our analysis revealed 1,314 significantly dysregulated metabolites (false discovery rate $p<0.05$, fold change >2), with 466 upregulated and 848 downregulated in IVA patients compared to controls. Among these, 121 were successfully identified as human endogenous metabolites. Pathway analysis indicated significant disruptions in phenylalanine, tyrosine, and tryptophan biosynthesis, alanine, aspartate, and glutamate metabolism, the citrate (TCA) cycle, and arginine biosynthesis. Isovaleryl carnitine, a key biomarker routinely used in NBS programs, was confirmed as elevated in IVA patients. Other metabolites such as methylmalonate and orotidylic acid were also elevated and may serve as complementary biomarkers. Conclusion: Our findings demonstrate that untargeted metabolomics enables detecting a broader range of metabolites, improving the sensitivity and specificity of IVA and other inborn errors of metabolism (IMDs) detection. This approach facilitates earlier diagnosis, uncovers novel biomarkers, and provides deeper insight into the complex metabolic alterations underlying these disorders.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3080 C — Unraveling metabolic signatures in SARS-CoV-2 variant infections using multiomics analysis

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CO-AUTHORS: *Jueun Lee, Geum-Sook Hwang*
SUB THEME: *Infectious diseases*

The rapid emergence of SARS-CoV-2 variants, especially delta and omicron, has significantly impacted the course of the COVID-19 pandemic. While these variants exhibit distinct clinical characteristics, the underlying immunometabolic mechanisms driving host responses remain incompletely understood.

To explore these mechanisms, we employed a hamster model infected via intranasal administration of either the delta or omicron variant. Lung tissues and serum samples were collected for untargeted metabolomic and transcriptomic analyses.

Histopathological examination confirmed acute inflammation and tissue damage in the lungs of infected animals. Integrated analysis of metabolites and gene expression revealed substantial alterations in key metabolic pathways, including arginine biosynthesis, glutathione metabolism, and tryptophan metabolism. These changes were consistently observed in both metabolomic and transcriptomic datasets. Notably, similar metabolic disruptions were identified in serum, indicating systemic metabolic reprogramming that mirrors changes in lung tissue. Furthermore, the altered pathways were closely associated with elevated levels of inflammatory cytokines, suggesting a strong link between immune activation and metabolic remodeling.

Our study highlights variant-specific immunometabolic alterations induced by SARS-CoV-2 and demonstrates the power of integrative multi-omics approaches to uncover host-pathogen interactions and inform potential therapeutic strategies.

3081 C — Host metabolic consequences of intracellular infection by members of the ESKAPE pathogens

PRESENTING AUTHOR: *Volker Behrends, University of West London, United Kingdom*
SUB THEME: *Infectious diseases*

Background – The members of ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species) are leading causes of hospital-acquired infections. Many are facultative intracellular pathogens and are thus able to invade both phagocytic and non-phagocytic cells. The emergence of multidrug-resistant strains, e.g. methicillin-resistant S. aureus (MRSA) has made treating infections by ESKAPE significantly more complicated. Therefore, there is an urgent need to find novel therapies.

Objectives - Our study aimed to identify and target host molecular pathways exploited by MRSA, Acinetobacter baumannii and Klebsiella pneumoniae during intracellular infection with a view of identifying possible host-directed therapies.

Methods - We used stable-isotope (carbon-13) labelling coupled to mass spectrometry and nuclear magnetic resonance techniques to investigate the impact of bacterial infection on host carbon metabolism. Identified pathways were targeted by pre-approved inhibitors.

Results - We found extensive metabolic re-routing and detected several distinct metabolic changes in infected cells. Our data suggested that both S. aureus as well as A. baumannii induced starvation-based autophagy. In agreement, drug-induced inhibition of AMPK, the host kinase triggering autophagic flux, hampered bacterial intracellular proliferation. In contrast, invasion by K. pneumoniae only led to modest changes in host metabolism.

Conclusion - Our findings show that targeting host pathways essential for bacterial survival is a viable therapeutic strategy against ESKAPE pathogen.

3082 C — A Systematic Review and Experimental Comparison of Blood Collection Tubes for Metabolomics Studies

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CO-AUTHORS: *Daylia Thet, Nureesun Mahamud, Eakachai Prompetchara, Tippawan Siritientong, Rossarin Tansawat*
SUB THEME: *Laboratory Medicine*

Metabolomics is the study of the complete set of small-molecule metabolites in a biological sample. In clinical trials, blood samples, both serum and plasma, are often used to explore related biological processes, offering insights into health, disease, and treatment responses. Blood can be collected using various tubes: red-cap for serum, and green-cap (heparinized) or lavender-cap (EDTA) for plasma. However, previous reports have shown that the type of collection tube can influence the number of metabolites detected, which may lead to incomplete interpretation of results. This study aimed to systematically review the number of metabolites detected using different blood collection tubes and to validate the findings with a pilot experiment. A systematic review was conducted using the Cochrane Library, ScienceDirect, PubMed, and Scopus. The study protocol was registered in PROSPERO (CRD42024595098). Eligible human studies using untargeted metabolomics with serum or plasma from different tube types were included. Data were categorized by analytical platform (NMR or MS), and study quality was assessed using the QUIPS tool. In total, twenty studies were included in the review. Three studies using NMR and seventeen studies using MS found that serum tubes yielded more metabolites. However, comparisons between heparin and EDTA tubes showed mixed results. Next, a wet lab experiment was conducted using blood from a healthy fasting volunteer, collected in heparin and EDTA tubes. Plasma samples were analyzed in triplicate using LC-qTOF. The results showed that about 90.58% of identified metabolites (567 out of 626) did not show significantly difference between the two groups. However, 7% (44 metabolites) were higher, and 2.4% (15 metabolites) were lower in the EDTA tube. Volcano plots indicated greater metabolite diversity in EDTA plasma. In conclusion, EDTA and heparin tubes can be used interchangeably for plasma metabolomics.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3083 C — Assessment of nicotinic acid and nicotinamide for microbial sterility of immune cell therapy products

PRESENTING AUTHOR: *Thy Truong, La Trobe University, Australia*

CO-AUTHORS: *Afsar Ahmed*

SUB THEME: *Laboratory Medicine*

Microbial sterility is an indispensable requirement for the therapeutic application of cell therapy products. while culture-based methods remain the industry standard, an array of rapid microbial testing methods (RMTMs) have potential implications for microbial sterility testings. This study utilises an optimised and validated ultra-performance liquid chromatography (UPLC) with electrospray ionisation and high-resolution accurate mass (HRAM) tandem mass spectrometry (LC-ESI-MS/MS) methodology for the targeted metabolic analysis of nicotinic acid (NA) and nicotinamide (NAM) as determinants for microbial contamination in cell cultures designed for therapeutic purposes. Using a validated sample extraction protocol to extract both NA and NAM as determinants for microbial contamination in our immune cell therapy products, we evaluated six reference microorganisms including bacteria, yeast, and fungi. Our study has demonstrated that the measurement of NAM alone rather than its comparative ratio with NA is a more consistent marker for microbial contamination under both aerobic and anaerobic-like conditions.

3084 C — Investigating the metabolic fate and changes in the endogenous metabolite profile of rat faecal samples following administration of the FFA1/GPR40 agonist Fasiglifam

PRESENTING AUTHOR: *Adam King, Waters Corporation, United Kingdom*

CO-AUTHORS: *Lee Gethings, Robert Plumb, Ian Wilson*

SUB THEME: *Microbiome research*

Intro: The diverse bacterial flora in complex life forms play a crucial role in normal metabolic processes by altering molecules from the host, diet, and xenobiotics. Understanding bacterial metabolism of xenobiotics is vital in drug development, as it can affect drug efficacy and generate harmful metabolites not produced by the host. Ultimately, this can help in the design of new medications, reducing risk and improving their effectiveness.

Aim: To investigate novel metabolites of the hepatotoxic drug Fasiglifam in rat faeces and highlight changes in endogenous metabolites related to dose response.

Methods: Fasiglifam and a drug-free vehicle were administered to 24 Sprague Dawley rats (15 male, 9 female) at different doses and routes (IV 5mg/kg; PO 10 & 50 mg/kg). Faecal samples were collected at pre-dose, 0-24, 24-48, and 48-96 hours. Samples were homogenized, and 50 mg of homogenate were extracted using MTBE:MeOH:H2O. The upper organic phase was used for untargeted lipidomic analysis, and both phases were combined for untargeted metabolomic analysis. Data were acquired on a cyclic ion mobility-enabled Q-Tof mass spectrometer by reversed-phase LC.

Results: MassMetaSite software was used to identify the drug metabolism products corresponding to those previously seen in plasma samples. Principle component analysis of the detected features showed good clustering of QCs and triplicate sample injections, with clear separation between drug and vehicle dosed groups and time points. Several metabolites linked to diurnal effects were noted alongside metabolites associated with stress highlighted in dosed rats. Notable differences in abundance of certain drug metabolites were seen between male and female rats.

Conclusions: The results highlighted the importance of considering bacterial metabolism in drug development to improve efficacy and reduce risks. Differences in metabolite abundance between male and female rats were also noted, emphasizing the need for gender-specific analysis in drug discovery.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3085 C — Matrix-specific differences in targeted metabolomics of a mixed host-microbiome metabolite panel to support microbiome research in plasma, urine, and feces

PRESENTING AUTHOR: *M. Gordian Adam, biocrates life sciences ag, Austria*
CO-AUTHORS: *Esther Wortmann, Tuan Hai Pham, Cornelia Röhring, Alice Limonciel*
SUB THEME: *Microbiome research*

Metabolomics is becoming a sought-after solution in microbiome research to explain microbiome-host interactions. Metabolomics studies come in two primary forms: (i) a small subset of well-characterized target metabolites, or (ii) a broader set of metabolites annotated from untargeted profiling. In this study, we applied a standardized and quantitative solution combining the high quality of targeted metabolomics and the high coverage of untargeted metabolomics. This kit solution enables the study of nearly 140 microbiome-related metabolites, combined with a measure of over 1,000 host metabolites related to human health.

Plasma, urine, and feces samples were obtained from five female and five male healthy individuals and measured with mass spectrometry. The kit's workflow manager was used for data evaluation and validation, including AI-driven peak picking, quantification of metabolite concentrations, and calculation of microbiome-associated metabolite sums and ratios.

Microbiome metabolites known to associate with chronic disease, immune response, inflammation, and neurological disease were detected in plasma, urine, and feces. Urine and plasma displayed comparable coverage of microbiome-related metabolites with 114 and 113 microbiome-related metabolites detected in at least half of the samples, respectively, while 88 were detected in feces. The detected microbiome metabolites have known implications for several disease-associated phenotypes, not restricted to the intestine, but also affecting brain function or the efficacy of chemotherapy.

Despite feces being most closely related to the microbiome, microbiome-derived metabolites are absorbed by the host and metabolized further, resulting in plasma and urine displaying superior coverage of microbiome-related metabolites compared to feces. The combination of plasma, feces, and urine allowed following metabolite absorption and metabolic pathways across matrices, providing a more holistic view than either matrix alone. This study demonstrates that a broad targeted metabolomics screening is a valuable option for microbiome research that facilitates obtaining a full picture of microbiome-host interaction metabolism, especially when measuring diverse matrices.

3086 C — Lipidomic characterization of anti-inflammatory gut microbiota-derived exosome

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CO-AUTHORS: *Juyoun Lee, Dong-Kyu Lee*
SUB THEME: *Microbiome research*

A substantial body of research has demonstrated a robust correlation between gut microbiota composition and various intestinal disorders, including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), highlighting the need for comprehensive analysis of extracellular vesicles (EVs) due to their critical roles in immune modulation, metabolic regulation, and intestinal barrier maintenance. In this study, an LC-HRMS-based analytical approach was developed for comprehensive lipidomic profiling of EVs and microbiomes. To enhance minor lipid species identification, a lipid ladder was constructed based on retention time patterns linked to fatty acyl chain length and unsaturation, providing a robust framework for precise lipid identification. A total of 126 lipid species were identified, including 25 phosphatidylcholines (PCs), 13 phosphatidylethanolamines (PEs), 13 diacylglycerols (DGs), 70 triglycerides (TGs), 2 ceramides (Cers), 1 dihydroceramide (DHCer), and 2 sphingomyelins (SMs). Additionally, 34 EV-specific lipids were identified, comprising 13 PCs, 7 PEs, 1 SM, 11 TGs, and 2 DGs. Partial Least Squares Discriminant Analysis (PLS-DA) clearly separated Group A strains from controls, with a significant upregulation of phospholipids, further validated by volcano plot analysis. Notably, EVs from Group A microbes exhibited elevated levels of PE, particularly odd-chain and unsaturated species. This study establishes a methodological framework for the lipidomic analysis of EVs derived from specific microbial strains, offering insights relevant to gut microbiota-related disease research. This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : RS-2023-00265981).

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3087 C — Impact of SARS-CoV-2 mRNA Vaccination on Gut Microbiome in a Mouse Model of Type 1 Diabetes

PRESENTING AUTHOR: ***Sang-Myeong Lee, College of Veterinary,Chungbuk National University, South Korea***
CO-AUTHORS: ***Jien Chae, So-Hyun Park***
SUB THEME: ***Microbiome research***

The impact of mRNA vaccines on autoimmune conditions in relation to gut microbiome alterations remains poorly understood. The present study evaluated changes in gut microbiome composition in Non-obese diabetic (NOD) mice, a model for spontaneous type 1 diabetes, following repeated intramuscular administration of SARS-CoV-2 spike mRNA encapsulated with lipid nanoparticles (LNP). NOD mice were vaccinated three times, and fecal samples were analyzed using next-generation sequencing (NGS). Analysis revealed significant changes in microbiome composition due to mRNA vaccination. Unique amplicon sequence variants (ASVs) were markedly fewer in vaccinated mice (356 ASVs) compared to unvaccinated controls (545 ASVs), with 353 ASVs shared by both groups. Alpha diversity analysis using Observed and Chao1 indices indicated that vaccinated mice had significantly lower microbial species richness and abundance than controls, reflecting a vaccine-associated reduction in gut microbial diversity. Shannon and Simpson indices further confirmed a decrease in microbial diversity and evenness in the vaccinated group. At the phylum level, vaccinated mice exhibited a notable increase in Proteobacteria, a phylum often associated with pathogenic bacteria and linked to intestinal inflammation and compromised barrier integrity. Additionally, vaccination induced specific functional shifts in the microbiome, characterized by increased populations of acetate and certain propionate-producing bacteria, while concurrently decreasing butyrate-producing Lachnospiraceae family members. These shifts suggest potential implications for mucosal immunity and gut barrier function. Collectively, these findings demonstrate that repeated mRNA vaccination significantly alters the gut microbiome composition and diversity in NOD mice, potentially impacting intestinal health and immune responses associated with autoimmune conditions. These microbiome-related changes highlight the importance of further research into vaccine-microbiome interactions and their implications for autoimmune disease management.

3088 C — Functional analysis of bile acids and the gut microbiome as important mediators of gut-liver interactions

PRESENTING AUTHOR: ***Ulrike Rolle-Kampczyk, Helmholtz Centre for Environmental Research, Germany***
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SUB THEME: ***Microbiome research***

The gut microbiota plays a pivotal role in host health by supporting nutrient metabolism, regulating immune responses, and maintaining epithelial integrity. Dysregulation of these microbial communities is linked to diseases such as inflammatory bowel disease, obesity, and metabolic syndrome. Among microbial functions, lipid metabolism—particularly involving bile acids—emerges as a key axis in host-microbe interactions. Bile acids aid lipid solubilization and act as signaling molecules via receptors like FXR and TGR-5, thereby influencing systemic metabolism, gut barrier function, and microbial composition. However, the mechanistic understanding of species-specific bile acid transformations and microbe-microbe interactions remains limited due to microbiome complexity and cultivation challenges.

To address this, we utilized a gut microbiome model consortia (SIHUMIx), comprising eight bacterial species representative of the human gut. We integrated Eggerthella lenta (E. lenta), a known bile acid-transforming bacterium, to investigate its role in modulating bile acid stress and microbial interactions. In batch co-cultivation experiments, E. lenta transformed deoxycholic acid (DCA) into less toxic oxidized and epimerized forms, alleviating DCA-induced stress in gram-positive SIHUMIx members, notably Thomasclavelia ramosa. Proteomic analyses indicated reduced expression of stress-related proteins and decreased membrane permeability in these strains.

Bioreactor co-cultures showed no DCA transformation, attributed to low E. lenta abundances. Still, its presence influenced the community by modulating nucleotide metabolism and membrane stability through its metabolic byproducts. Notably, Bacteroides thetaiotaomicron exhibited increased guanine nucleotide synthesis, while Lactiplantibacillus plantarum showed enhanced growth, correlating with the availability of arginine-derived metabolites.

Additionally, microbial bile salt conjugates (MBSCs) were detected, suggesting broader roles for microbial bile acid modification. These findings underscore the significance of species-specific metabolic interactions in shaping microbial community dynamics and stress responses. Our study highlights the utility of defined microbial consortia like SIHUMIx for dissecting complex host-microbe interactions with high resolution and translational relevance.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3089 C — Enhanced Sensitivity and Specificity for Merged Untargeted Profiling and Targeted Quantification of Short-Chain Fatty Acids Using A Novel Dual Ionization GC-HRMS

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CO-AUTHORS: ***Steffen Bräkling, Sonja Klee, Tony Teav, Julijana Ivanisevic, Hector Gallart-Ayala, Arnd Ingendoh, Matthew Lewis***

SUB THEME: ***Microbiome research***

Short-chain fatty acids (SCFAs) are key microbial metabolites produced in the large intestine by bacterial fermentation of dietary fibers, playing key roles in metabolic signaling, inflammation, and immune regulation. Profiling a broad panel of SCFAs is crucial for understanding their molecular mechanisms in host-microbiome interactions. Beyond targeted quantification, detecting diverse SCFAs and identifying unknown metabolites adds significant value. While LC-MS is well-established for untargeted analysis, GC-MS applications have been limited by inefficient ionization and analyzer performance. This study evaluates a novel GC-HRMS system enabling simultaneous electron ionization (EI) and chemical ionization (CI) for targeted and untargeted SCFA and related compound analysis.

Here, we present the unique benefit of a dual ionization mode analysis of SCFAs using a last-generation ecTOF mass spectrometer (ecTOF, Bruker, Bremen). The system's dual ionization provided complementary fragmentation and molecular ion data within a single GC-MS run. This is improving the NIST spectral library matching efficiency by refining match lists using additional CI molecular ion information.

The novel GC-HRMS system was successfully applied for merged targeted quantification of 13 SCFAs and untargeted profiling and identification of additional SCFAs in plasma, mouse feces, and human breast milk. CI-generated [M+H]+ signals exhibited similar or improved signal-to-noise (S/N) levels compared to EI quantifier ions, enhancing SCFA detection sensitivity. Several examples of untargeted SCFA identification are presented, demonstrating the system's ability to expand the chemical space for untargeted metabolite discovery.

This study highlights a significant advancement in comprehensive SCFA analysis, showcasing the dual-ionization GC-HRMS potential for broad metabolomics applications. This approach enhances both targeted quantification and untargeted discovery, supporting improved characterization of SCFA-related metabolites.

3090 C — Probiotic-Induced Modulation of Pathogens Abundance, Resistome, and Metabolome in the Infant Gut

PRESENTING AUTHOR: ***Gaute Hovde Bø, Uit The Arctic University Of Norway, Norway***

CO-AUTHORS: ***Veronika K. Pettersen***

SUB THEME: ***Microbiome research***

Background

Antibiotic resistance in early life is a major global health concern, especially in low- and middle-income countries, where pathogens such as extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) are highly prevalent. Supplementation of probiotic bacteria has emerged as a strategy to prevent pathogen colonisation; however, its impact on the gut resistome and metabolome in full-term infants remains poorly understood. Probiotics are thought to exert their effects by fermentative end products, such as short-chain fatty acids (SCFAs), and other metabolites including aromatic amino acids and conjugated bile acids, all shown to have immuno-modulatory effects.

Method

A randomized, double-blind, placebo-controlled trial was conducted in Tanzania, enrolling 2000 healthy infants who were assigned to receive either probiotics or a placebo during their first 4 weeks of life. Fecal samples were collected at 6 weeks and 6 months of age. The samples were analyzed by selective cultivation of ESBL-E, shotgun metagenome sequencing, a validated liquid chromatography-mass spectrometry method targeting SCFA and central carbon metabolites, and untargeted metabolomic analysis using hydrophilic interaction chromatography and mass spectrometry.

Results

Using culturomics and metagenomic sequencing, we found that probiotics reduced ESBL-E colonisation and significantly decreased resistome diversity and load at 6 weeks. The results from metabolomics analyses showed that probiotic bacteria significantly altered the fecal metabolome, including several classes of metabolites such as SCFA, bile acids, and aromatic amino acids. Molecular networking analysis further confirmed distinct metabolic patterns in the probiotic group compared to the placebo group.

Conclusion

Probiotic intervention causes measurable alterations of the gut microbiome, as demonstrated by the altered gut carriage of pathogenic bacteria and associated antibiotic resistance genes, as well as fecal metabolite profiles. Our multi-omic approach provides high-resolution insights into the impact of microbiome-targeted interventions on human health, providing clues for future development of precision diagnostics and microbiome-based therapies.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3091 C — A sensitive chiral LC-MS/MS method for quantifying aromatic lactic acid enantiomers in infant faeces

PRESENTING AUTHOR: *Ahmed Gaafar, University Of Copenhagen: The Faculty Of Science, Denmark*
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SUB THEME: *Microbiome research*

Aromatic lactic acids (Phenyllactic acid (PLA), 4-hydroxyphenyllactic acid (4OH-PLA) and indolelactic acid (ILA)) are microbial-derived metabolites that modulate immune function via Hydroxycarboxylic acid 3 (HCA3) and Aryl Hydrocarbon (Ah) receptors. Bifidobacterium, predominant in infant gut microbiome, produce racemic mixtures of D- and L-enantiomers with varying production capacities among species. D-enantiomers have been found to exhibit higher receptor affinity and biological activity, suggesting strain-specific contributions to early-life immune modulation. Quantifying D and L aromatic lactic acids in infant feces is essential to demonstrate correlations with Bifidobacterium species, and to evaluate their interaction with HCA3 and Ah receptors. However, no methods to separate and quantify these enantiomers in faeces exist. For this purpose, we developed and validated an LC-MS/MS method on a H-class UPLC coupled to a Vion qTOF. Different mobile phases were tested on a CHIRALPAK QD-AX column for enantioseparation, and chromatographic separation was evaluated by resolution, tailing factor and retention factor. Chromatographic optimization achieved resolutions of 2.47, 1.54, and 1.92 for PLA, 4OH-PLA, and ILA enantiomers, respectively. Sample preparation was optimized by maximizing recovery and minimizing matrix effect. A final method including solid phase extraction with an Oasis HLB cartridge achieved optimal recovery (80% - 107%) and matrix effects (75% - 116%) at low and high concentration ranges. LODs ranged from 3.3 to 4.2 ng/mL-1, LOQs from 10.0 to 12.8 ng/mL-1, with $R^2 > 0.99$ and relative residuals < 15% from the respective linear regressions. Intra-day repeatability was 1.7–10.9%. The method was applied to fecal water extracted from stool of healthy infants for quantifying aromatic lactic acid enantiomers using standard addition, uncovering significant differences in enantiomer concentrations. The described method enables precise and accurate quantification of aromatic lactic acid enantiomers in infant faeces, which may enable the study of enantiomer-specific effects on immune development and host-microbe interactions during infancy.

3092 C — A targeted metabolomic approach to explore the impact on gut-brain axis of prebiotics and postbiotics in Rett syndrome patients.

PRESENTING AUTHOR: *Tawanda Chipurura, Università del Piemonte Orientale, Italy*
CO-AUTHORS: *Silvia Leoncini, Lidia Boasiako, Marco Montecucco, Elettra Barberis, Claudio De Felice, Marcello Manfredi*
SUB THEME: *Neurological disorders*

Rett syndrome is among the most prevalent developmental and intellectual impairment disorders in females. Current treatment options are primarily focused on symptom relief. Previous studies have reported that patients with Rett syndrome exhibit lower microbiota α-diversity compared to healthy individuals. In this study, the effects of prebiotic and/or postbiotic products on the levels of neurotransmitters, neuromodulators, and short-chain fatty acids (SCFAs) in patients with Rett syndrome patients were investigated. A randomized crossover clinical trial was conducted using two products, and fecal and plasma samples were collected before and after treatments. Neurotransmitters levels and their precursors were measured using targeted liquid chromatography-mass spectrometry, and SCFAs were analyzed using gas chromatography.

Clinical data indicated that both the treatments were associated with reductions in symptom severity. Metabolomic analysis identified changes in neurotransmitters and precursors following treatment. Specifically, after one treatment, circulating levels choline, L-arginine, 2-pyrrolidinone, L-phenylalanine, 2-phenethylamine, indole-3-acrylic acid, L-lysine, L-carnitine, and L-tryptophan were decreased, while Nα-acetyl-L-glutamine levels increased. Fecal levels of indole-3-acrylic acid and L-phenylalanine were also decreased. Following the second treatment, circulating levels 2-pyrrolidinone, nudifloramide, L-phenylalanine, 2-phenethylamine, and L-tryptophan decreased, along with fecal levels of indole-3-acrylic acid, L-methionine, L-glutamic acid, nudifloramide, and indole-3-carboxyaldehyde. Circulating levels of acetic acid and propanoic acid levels increased after both treatments, while levels of 2-methylpropanoic acid decreased only after the first one.

In conclusion, metabolomics provided insights into microbiota-related metabolic changes associated with treatment. Here, both treatments modulated neurotransmitters and SCFAs, warranting further investigation into their potential impact on symptom management in Rett syndrome.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3093 C — Monitoring Neurotransmitter Pathways: A Metabolomics Approach to Neurochemical Profiling

PRESENTING AUTHOR: *Roland Pálkovács, Universität Heidelberg, Germany*
CO-AUTHORS: *Tamara Prentzell, Nina Kunze-Rohrbach, Michael Schultz, Christiane Opitz, Gernot Poschet*
SUB THEME: *Neurological disorders*

Neurotransmitters such as serotonin, dopamine, norepinephrine, γ-aminobutyric acid (GABA), and glutamate play a pivotal role in regulating cognition, memory, mood, and social behaviors. Disruptions in their synthesis or degradation are linked to neurological and psychiatric disorders, including Alzheimer's disease, Parkinson's disease, schizophrenia, and depression.

Accurate quantification of these neurotransmitters is essential for understanding disease mechanisms and evaluating therapeutic interventions. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) stands as the gold standard for such analyses, offering high sensitivity and specificity.

At the Metabolomics Core Technology Platform (MCTP) of Heidelberg University, we have developed and validated a robust LC-MS/MS assay capable of simultaneously quantifying 16 neurotransmitters and related metabolites. This assay has been rigorously tested across various biological matrices, including brain tissue, liver, plasma, and induced pluripotent stem cells (iPSC), ensuring its versatility for diverse research applications. Validation parameters encompass selectivity, sensitivity, linearity, accuracy, precision, recovery, matrix effects, stability, and carryover, adhering to stringent analytical standards.

In our poster, we present data demonstrating the application of the assay in detecting alterations in neurotransmitter metabolism between wild-type and mutant mouse brain samples. These findings underscore the utility of the method in elucidating neurochemical alterations associated with genetic modifications.

Our validated LC-MS/MS platform offers a reliable and comprehensive tool for researchers investigating the complex landscape of neurotransmitter dynamics in health and disease.

3094 C — Lipid Metabolism in Traumatic Brain Injury – Increased identification confidence whilst extending coverage with multi-reflecting ToF technology

PRESENTING AUTHOR: *Lee Gethings, Waters Corp, United Kingdom*
CO-AUTHORS: *Eszter Ujvari, Rita Campros-Pires, Lee Gethings, Elizabeth Want, Robert Dickinson*
SUB THEME: *Neurological disorders*

A rat model of TBI was utilized, consisting of a sham cohort (no injury) and TBI cohort (treated with control gas or xenon). Treatment began 30 min after injury over a 3-hr duration to model the scenario of treatment by first responders. Samples of brain tissue were taken at 3 and 24 hrs in addition to a sham at the same timepoints with control gas only. Lipids were extracted from plasma using a folsch procedure and diluted appropriately for LC-MS analysis. Lipids were separated over a 6-min gradient using a U(H)PLC configured for reversed-phase (RP) chromatography. The LC was interfaced with a multi-reflecting time-of-flight mass spectrometer and data collected with a data independent (DIA) and data dependent (DDA) mode of acquisition prior to being processed and database searched. Features with a mass accuracy of sub 1 ppm (precursor and fragment ions) and CV of <20% (based on study QC reproducibility) were used for further multivariate statistical analysis (MVA) to establish differences between groups. The data inherently showed that the lipidome is significantly affected after TBI, with clear differences between xenon and control gas. A variety of lipid classes were shown to change in their levels of expression over time (e.g., ceramides and cholesterol esters). In particular, Cer (36:2) and cholesterol ester (16:0) indicated under expression with xenon treatment, whilst in contrast PC (22:6) and (36:0) were up-regulated. Ceramides are thought to be altered in TBI due to their association with neuronal bodies and are linked to oxidative stress (as are cholesterol esters) with induced abnormalities. The link with choline-based phospholipids is related to their involvement with maintaining integrity of neuronal glial cell membranes and involved in pathways, such as cholinergic neuronal transmission.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3095 C — Nutrimetabolomics Approach to Unravel Diet-Depression Association

PRESENTING AUTHOR: *Ivy Hing Yee Sy, Hong Kong Metropolitan University, Hong Kong*

SUB THEME: *Nutrimetabolomics & dietary biomarkers*

Depression is a leading cause of disability worldwide, with growing evidence linking dietary patterns, particularly fruit and vegetable intake (FVI), to mental health outcomes. Metabolomics offers a powerful tool to uncover biological mechanisms underlying this relationship by identifying metabolic pathways influenced by diet and associated with depressive symptoms. However, comprehensive metabolomic profiling in the context of depression and FVI remains underexplored.

This study aims to (1) characterise metabolomic signatures associated with depression, (2) identify metabolites modulated by FVI, and (3) explore potential metabolic pathways linking diet to depressive symptoms.

A cross-sectional analysis was conducted among the young adult population. Untargeted metabolomic profiling from faecal samples was performed using ultra-high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC-qTOF-MS). Depression severity was assessed using Patient Health Questionnaire-9 (PHQ-9), while FVI was estimated by food frequency questionnaire (FFQ).

Herein, we report the preliminary findings that may elucidate novel biomarkers and mechanistic insights into how dietary interventions could mitigate depressive symptoms. This study will contribute to the growing body of metabolomic research in nutritional psychiatry, with potential implications for personalised dietary recommendations in mental health management.

3097 C — Untargeted Lipidomic Profiling of Mild Cognitive Impairment in Thai Elderly with and without Major Depressive Disorder

PRESENTING AUTHOR: *Pannipa Janta, Chulalongkorn University, Thailand*
CO-AUTHORS: *Chavit Tunvirachaisakul, Michael Maes, Rossarin Tansawat*

SUB THEME: *Other*

Major Depressive Disorder (MDD) is a psychiatric condition characterized by persistent sadness, loss of interest, and cognitive and physical symptoms that impair daily functioning. It is associated with cognitive and neurobiological changes, increasing the risk of Mild Cognitive Impairment (MCI) and Alzheimer's disease (AD). Identifying biomarkers that differ between MCI patients with and without MDD may uncover the biological pathways through which depression accelerates cognitive decline. These biomarkers could facilitate early risk assessment, inform personalized treatment strategies, and provide potential therapeutic targets for preventing or delaying the progression from MCI to Alzheimer's disease. Changes in lipid metabolism have a significant impact on neurodegeneration, neuroinflammation, and brain function, making lipids key candidates for biomarker discovery. This study aimed to evaluate plasma lipid profiles and identify candidate lipid biomarkers among Thai elderly (aged 60-75 years) with MCI, with and without MDD. Plasma samples from two groups, MCI with MDD (MCI-MDD, n=61) and MCI without MDD (MCI-Non-MDD, n=60) were extracted via monophasic extraction. The extracted samples were analyzed using LC-MS/MS with a ZenoTOF system, employing both collision-induced dissociation (CID) and electron-activated dissociation (EAD) fragmentation. Fifty-five lipids were identified, including triacylglycerol, diacylglycerol, ceramide, sphingomyelin, and glycosphingolipids. Significantly altered lipids were selected based on VIP scores > 1 and $p < 0.05$. Receiver operating characteristic (ROC) curve analysis identified Cer 50:1;O2|Cer 12:0;O2/38:1 as a potential biomarker, with an area under the curve (AUC) value of 0.666 (95% confidence interval = 0.581-0.754). Elevated ceramide levels were observed in the MCI-MDD group compared to MCI-Non-MDD, with the highest VIP score (2.906) and a fold change of 0.571. Ceramides, involved in sphingolipid metabolism, play key roles in neuroinflammation, mood regulation, and neurodegeneration, highlighting their potential as biomarkers in MCI-MDD patients. These findings underscore ceramides as promising candidates for distinguishing MCI in individuals with and without MDD.

3098 C — Reactivity-Based Metabolomics Reveal Cysteine has Glyoxalase Activity

PRESENTING AUTHOR: *Mogens Johannsen, Dept. Forensic Medicine, Aarhus University, Denmark*

SUB THEME: *Other*

Methylglyoxal (MG) is a reactive metabolite involved in diabetes and aging, via formation of protein adducts. Less is known about the extent MG, and its metabolic product S,D-lactoylglutathione (LGSH), form adducts with cell metabolites. Using a "symmetric" isotope labeled and reactivity-based metabolomics approach in living cells, we find over 200 adducts and, surprisingly, discover that 10 of the most abundant are lactoylated amino acids mainly derived from LGSH. The most abundant adduct D-Lac-Cys is formed rapidly between LGSH and cysteine, whereas the diastereoisomer L-Lac-Cys is formed directly from MG and cysteine, assigning cysteine with both glyoxalase 1 and 2 like activity. Cellular cysteine and MG dynamically regulate D- and L-Lac-Cys levels and the adducts are increased in diabetes suggesting use as novel biomarkers. Finally, cysteine amides, as proxies for protein cysteines, also undergo lactoylation by MG and LGSH, suggesting the existence of two additional pathways for non-enzymatic lactoylation of proteins.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3099 C — Heat stress alters milk metabolome of lactating Holstein cows

PRESENTING AUTHOR: *Tibor Czabany, DSM-Firmenich, Austria*
CO-AUTHORS: *S. Rodriguez-Jimenez, L.H. Baumgard, N. Reisinger*
SUB THEME: *Other*

This study investigated the impact of heat stress (HS) on the milk metabolome of Holstein cows using liquid chromatography–high-resolution mass spectrometry (LC-HRMS). After an acclimation phase (baseline, 13 days), eighteen lactating Holstein cows (156 ± 5 days in milk) were assigned to one of three treatments for seven days: (1) thermoneutral (TN; n=6), (2) HS (n=6), and (3) pair-fed (PF; n=6) to their HS counterparts. Except for the PF group, all cows had ad libitum access to feed. Heat stress was induced using electric heat blankets (Thermotex Therapy Systems Ltd., Calgary, AB, Canada). As expected, HS increased rectal temperature and reduced milk yield and dry matter intake ($P < 0.05$). Milk samples were collected on day 3 of baseline and days 1, and 7 of the experimental phase. Samples were stored at -80 °C and analyzed via LC-HRMS to compare metabolomic profiles across groups. Principal component analysis of 2,933 detected features showed no clustering at baseline, separation of HS and PF from TN on day 1, and distinct clustering of all three groups on day 7. Metabolites were identified by matching MS2 fragmentation spectra (available for 754 features) to the mzCloud database. More than 50 features were annotated at level 2. Further analysis revealed alterations in the milk metabolome of HS cows compared to TN cows. Concentrations of lactose and other carbohydrates were significantly altered ($P < 0.05$). Additionally, hydroxybutyric acid and glutamine levels increased, whereas cAMP decreased ($P < 0.05$). These findings suggest that short-term HS induces metabolic changes in milk, primarily altering carbohydrate metabolism.

3100 C — Lipidomics in Immune-Mediated Diseases: Exploring Disease Mechanisms Through Advanced Mass Spectrometry Techniques

PRESENTING AUTHOR: *Lisa Hahnefeld, Fraunhofer ITMP, Germany*
CO-AUTHORS: *Dominique Thomas, Robert Gurke*
SUB THEME: *Other*

Immune-mediated diseases present significant challenges due to the limited efficacy of existing symptomatic immunosuppressive therapies, exemplified by the fact that only 20% of inflammatory bowel disease (IBD) patients achieve medium-term disease control. There is a pressing need to better understand the pathophysiology of these diseases to develop more effective treatments. This study explores the potential of LC-MS-based lipidomics, particularly in conjunction with mass spectrometry imaging (MSI), for analyzing tissue biopsies to elucidate disease mechanisms.

We focus on robust pre-analytical protocols for plasma sample collection and analysis to investigate systemic lipid changes associated with IBDs. We present various blood sampling strategies that are feasible across different clinical settings. By implementing these protocols, we conducted cohort studies utilizing LC-HRMS and LC-MS/MS to identify key lipid biomarkers, such as ceramides and endocannabinoids, which serve as a foundation for understanding disease mechanisms. Building on these findings, we employed Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) to investigate lipid profiles in human colon biopsies, aiming for a consistent spatial resolution of 10 x 10 µm. This sequential approach allows us to correlate systemic lipid changes with localized tissue distributions, ultimately enhancing our understanding of the pathophysiology of IBDs.

Our findings underscore the critical factors influencing pre-analytical practices and demonstrate that DESI-MS can provide valuable insights into the spatial distribution of lipids, potentially verifying hypotheses generated from blood-based studies. Importantly, the tissue investigations have led to new insights regarding fatty acids and their metabolites, which were not well characterized before. We also discuss the challenges of sample preparation and optimizing MS parameters for reliable high-resolution imaging.

In conclusion, while LC-MS-based lipidomics combined with DESI-MS offers promising avenues for advancing our understanding of IBDs, overcoming these challenges is essential for fully leveraging lipidomic analyses as diagnostic and prognostic tools.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3101 C — Sex discrimination by multi-site spectrometric analyzes (LC-MS & NMR)

PRESENTING AUTHOR: *Celine Dalle, French Armed Forces Biomedical Res. Inst., France*
CO-AUTHORS: *Baptiste Panthu, Nicolas Taudon*
SUB THEME: *Other*

Metabolomics, for human studies, is a powerful tool to highlight early metabolic disturbances before the first clinical signs. However, these differences can be hide by confounding factors as BMI or age. Sexual dimorphism is also a potential factor of variation in metabolomics studies. Although the urinary metabolic profiles of men and women contain identical metabolites, approximately 30% of them differ quantitatively between the sexes. This study aims to determine the metabolic parameters explaining the 30% difference observed between men and women and identify metabolites common to the main LC-MS and NMR analytical spectrometers.

Urine samples were collected from four healthy donors (2 men and 2 women, aged 29 to 49 years). Osmolality was measured before diluting the samples to 150 mOsm/kg. Ten technical replicates of each dilution were analyzed by LC-MS and NMR. Replicates were randomized and quality control samples were added. LC-MS analyses were performed on five QToF mass spectrometers, and NMR analyses were performed by the standardized Bruker Avance platform, using proton NMR. Data preprocessing was performed on the Galaxy-Workflow4metabolomics platform, and multivariate analyses were conducted with SIMCA (Umetrics).

Multivariate models built from each five LC-MS data matrix discriminate samples according the sex with 30% of ions that are significant and among them only five ions are in common (two carnitines, two exposome metabolites and a glucuronide metabolite). Sex differentiation is also observed with a matrix composed of the 50 most abundant ions, common between the five spectrometers. The pre-dilution step of the samples allowed optimal acquisition on all five LC-MS models, but led to suboptimal acquisition in NMR, without altering the latter's discriminatory capacity. The final identification of discriminating metabolites revealed that pantothenate, creatinine, and creatine are relevant markers for sex discrimination, compatible between LC-MS and NMR.

3102 C — Exercise intensity determines circulating levels of Lac-Phe and other exerkines: a randomized crossover trial

PRESENTING AUTHOR: *Dirk Weber, Karlsruhe Institute of Technology (KIT), Germany*
CO-AUTHORS: *Paola G. Ferrario, Achim Bub*
SUB THEME: *Other*

Introduction

Exercise metabolomics research has revealed significant exercise-induced metabolic changes and identified several exerkines as mediators of physiological adaptations to exercise. However, the effect of exercise intensity on metabolic changes and circulating exerkine levels remains to be examined.

Objectives

This study compared the metabolic responses to moderate-intensity and vigorous-intensity aerobic exercise.

Methods

A two-period crossover trial was conducted under controlled conditions at the Max Rubner-Institute in Karlsruhe, Germany. Seventeen young, healthy, and physically active men performed 30 min moderate-intensity (50% VO2peak) and vigorous-intensity (75% VO2peak) aerobic exercise using two bicycle ergometer protocols in a randomized sequence. Blood samples obtained immediately before exercise and at four time points after exercise were analyzed in an untargeted metabolomics approach, and separate mixed linear models were applied to over 1000 metabolites.

Results

Vigorous-intensity exercise induced a greater metabolic response than moderate-intensity exercise. Several intensity-dependent metabolites were identified, primarily involved in amino acid metabolism and energy conversion pathways, including N-lactoyl-amino acids, TCA cycle intermediates, N acetylated amino acids, and acylcholines. The exerkines N-lactoyl-phenylalanine, lactate, and succinate were among the most intensity-dependent metabolites. N-acetylated amino acids and acylcholines were systematically altered by exercise intensity, indicating potential physiological functions.

Conclusion

Exercise intensity is a key determinant of exercise-induced metabolic alterations and changes in exerkine levels. Our results expand the knowledge about exerkine dynamics and emphasize the role of exercise intensity in promoting physiological adaptations to exercise.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3103 C — Metabolomic profiling reveals early biomarkers of gestational diabetes mellitus and associated hepatic steatosis

PRESENTING AUTHOR: *Youngae Jung, Korea Basic Science Institute, South Korea*
CO-AUTHORS: *Geum-Sook Hwang*
SUB THEME: *Other*

This study aims to identify early metabolomic biomarkers of gestational diabetes mellitus (GDM) and evaluate their association with hepatic steatosis.

We compared maternal serum metabolomic profiles between women who developed GDM (n=118) and matched controls (n=118) during the first (10–14 gestational weeks) and second (24–28 gestational weeks) trimesters using ultra-performance liquid chromatography coupled with mass spectrometry. Mediation analysis was performed to evaluate the mediating role of metabolic dysfunction-associated steatotic liver disease (MASLD) in the relationship between metabolites and subsequent development of GDM. A refined prediction model was developed to predict GDM using established clinical factors and selected metabolites.

Significant alterations in circulating metabolites, including amino acids, bile acids, and phospholipids, were observed in the GDM group compared to controls during early pregnancy. Mediation analysis revealed that several metabolites, including glycocholic acid (proportion mediated (PM)=31.9%), butanoyl carnitine (PM=25.7%), and uric acid (PM=22.4%), had significant indirect effects on GDM incidence mediated by hepatic steatosis. The refined prediction model composed of clinical factors and selected metabolites in the first trimester demonstrated higher performance in predicting GDM development than the established prediction model composed solely of clinical factors (AUC, 0.85 vs. 0.63, $p<0.001$).

Women who developed GDM exhibited altered metabolomic profiles from early pregnancy, which showed a significant correlation with GDM, with MASLD as a mediator. Selected metabolomic biomarkers may serve as predictive markers and potential targets for early risk assessment and intervention in GDM.

3104 C — Metabolic Reprogramming by Mitochondrial Complex I Dysfunction in Colorectal Cancer

PRESENTING AUTHOR: *Min-Sun Kim, Korea Food Research Institute, South Korea*
CO-AUTHORS: *Miso Nam*
SUB THEME: *Other*

Mitochondrial Complex I plays a pivotal role in electron transport and cellular energy production, and its dysfunction is increasingly recognized as a key factor in metabolic reprogramming in cancer. NUBPL (Nucleotide Binding Protein-Like) is an essential assembly factor for Complex I, but its metabolic impact in cancer cells remains unexplored.

In this study, we investigated the metabolic effects of NUBPL knockdown in HCT116 colorectal cancer cells using untargeted metabolite profiling with UPLC-Orbitrap mass spectrometry.

Untargeted metabolomics analysis showed distinct changes in central carbon metabolism upon NUBPL knockdown. In NUBPL knockdown cells, intracellular and extracellular glucose levels were elevated, while glycolytic intermediates, including glucose-6-phosphate and fructose-1,6-bisphosphate, were decreased. Lactate levels were also reduced. These changes are consistent with a reduction in glycolytic flux. In the TCA cycle, α-ketoglutarate and succinate levels were increased, suggesting a possible slowdown or disruption in TCA cycle turnover. Additionally, the NAD^+/NADH ratio was elevated in NUBPL knockdown cells, which may reflect reduced NADH production or an altered redox state associated with disrupted mitochondrial electron transport.

These findings indicate that NUBPL knockdown alters central carbon metabolism in colorectal cancer cells, characterized by reduced glycolytic flux, accumulation of TCA cycle intermediates, and changes in redox balance. These metabolic alterations reflect the cellular response to mitochondrial Complex I dysfunction.

3105 C — Biomarker Identification for Depressive Disorders via Untargeted Metabolomics

PRESENTING AUTHOR: *Mansha Soni, Max Planck Institute of Molecular Physiology, Germany*
CO-AUTHORS: *Georg Juckel, Frank Schulz*
SUB THEME: *Other*

Major depressive disorder (MDD) is a complex and multifactorial condition with significant global health impact. Despite ongoing research, its underlying metabolic mechanisms remain poorly understood, and reliable diagnostic biomarkers are still lacking. Small-molecule biomarkers could improve early detection and clinical decision-making, but existing studies often suffer from limited sample sizes, focus on narrow metabolomic subsets and face challenges in reproducibility and specificity.

We present an untargeted metabolomic workflow for biomarker discovery in MDD based on the analysis of 430 human serum samples using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Our extraction protocol leverages the differential polarity of metabolites, employing reverse-phase chromatography for lipids and HILIC for polar compounds. The methods are standardized and highly reproducible across batches.

In addition to metabolomic data, we incorporate detailed patient metadata to account for the multifactorial nature of the disease. Group differentiation between patients and healthy controls is achieved through supervised machine learning, specifically using the random forest algorithm. Here, we report our preliminary findings and demonstrate the robustness of our analytical pipeline for biomarker discovery.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3106 C — Metabolomics Profiling of NAFLD Cells Treated with Aurantiochytrium sp. Hot Water Extract

PRESENTING AUTHOR: *Jen Kit Tan, National University of Malaysia, Malaysia*
CO-AUTHORS: *Kartthigeen Tamel Selvan, Jo Aan Goon, Suzana Makpol*
SUB THEME: *Other*

Non-alcoholic fatty liver disease (NAFLD) is a prevalent metabolic disorder characterized by lipid accumulation and oxidative stress in hepatocytes. Recent studies suggest that microalgae-based natural products may offer therapeutic potential for metabolic disorders. Our ongoing study found that Aurantiochytrium sp. hot water extract (HWE) demonstrates lipid-lowering properties. This study investigates the metabolomic alterations in NAFLD cells pre-treated with Aurantiochytrium sp. HWE, providing insights into its potential mechanisms of action. HepG2 cells were pre-treated with Aurantiochytrium sp. HWE for 24 hours before being induced into an NAFLD-like state using free fatty acids. Intracellular metabolites were extracted using the Folch method, and the aqueous phase was collected for subsequent analysis. Untargeted metabolomics analysis was performed using ultra-high-performance liquid chromatography–tandem mass spectrometry. Data processing and annotation were conducted using MS-DIAL, followed by statistical and pathway analyses with MetaboAnalyst. Metabolomic profiling revealed significant alterations in amino acid metabolism, including arginine biosynthesis, and alanine, aspartate and glutamate metabolism, which involved changes in aspartic acid and glutamic acid in NAFLD cells pre-treated with Aurantiochytrium sp. HWE, suggesting a link between amino acid homeostasis and disease pathogenesis. Metabolites such as pantothenic acid, glutamic acid, and pyroglutamic acid were restored, suggesting their potential as biomarkers for monitoring disease progression following treatment. Additionally, Aurantiochytrium sp. HWE increased intermediates of purine metabolism, including inosine, hypoxanthine, and xanthine, suggesting its role in the lipid-lowering mechanism via this pathway. These findings highlight the potential of Aurantiochytrium sp. HWE as a metabolic modulator in NAFLD. The observed shifts in amino acid and purine metabolism provide mechanistic insights into its lipid-lowering effects. Further studies are warranted to elucidate specific bioactive components responsible for these effects and to explore their therapeutic potential in vivo.

3107 C — Aspartate role in Nitric oxide production:

PRESENTING AUTHOR: *Mohamad Alalloush, Tu-braschweig, Germany*
SUB THEME: *Other*

Macrophages play a critical role in innate immunity by producing nitric oxide (NO) to combat pathogens. During activation, proinflammatory macrophages undergo metabolic reprogramming, shifting from oxidative phosphorylation (OXPHOS) to aerobic glycolysis to meet increased energy demands. However, the interaction between mitochondrial respiration and NO production during macrophage activation remains poorly understood. In this study, using bone marrow-derived macrophages (BMDMs), we showed that inhibition of mitochondrial complex III using antimycin A significantly reduced NO production as measured by the Griess assay, demonstrating the essential role of electron transport chain activity in sustaining NO synthesis. Importantly, supplementation with aspartate restored NO levels following ETC inhibition, via elevating intracellular arginine quantified by HPLC-based amino acid analysis. HPLC data further revealed that extracellular arginine is depleted after 24 hours of macrophage activation, while citrulline levels increase, implying that macrophages rely more on intracellular arginine regeneration through the citrulline-arginine recycling pathway to maintain NO production. These findings highlight the metabolic flexibility of macrophages in sustaining antimicrobial functions under nutrient-deprived and respiratory-compromised conditions. Ongoing experiments aim to further elucidate the precise mechanisms by which aspartate supports mitochondrial metabolism and to explore the synergistic effects of aspartate and citrulline supplementation on NO production. Understanding these pathways might uncover novel therapeutic targets to enhance host defense during microbial infection.

3108 C — Metabolomic and Lipidomic Insights into Fabry Disease: An Untargeted UPLC-Orbitrap Exploris 240 LC-MS Study

PRESENTING AUTHOR: *Hannadi Alamri, University of Liverpool, United Kingdom*
CO-AUTHORS: *Subadra Wanninayake, Warwick Dunn*
SUB THEME: *Other*

Introduction: Metabolomics and lipidomics are analytical approaches that facilitate comprehensive profiling of metabolites and lipids in biological systems. These methodologies are fundamental in understanding metabolic alterations associated with different diseases, e.g Fabry disease. Fabry disease is a rare X-linked lysosomal storage disorder caused by mutations in the GLA gene, resulting in deficient α-galactosidase A enzyme activity and the accumulation of glycosphingolipids, particularly globotriaosylceramide (Gb3) and its derivatives, in various tissues. This metabolic disruption contributes to progressive multi-organ dysfunction, including renal, cardiac, and neurological complications.

This study aims to enhance the understanding of metabolic alterations in Fabry disease (FD) by applying an untargeted metabolomics approach to investigate global metabolic changes across different disease phenotypes, including classical and non-classical forms. The primary objective is to explore correlations between GLA enzyme activity, MRI-derived left ventricular mass, troponin levels, and T2 relaxation times with the metabolic profiles of Fabry disease patients.

Method: The samples were analyzed using a Vanquish binary pump H system coupled with a heated electrospray Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific, MA, USA). Using a Hypersil GOLD C18 column (100 x 2.1mm, 1.9 µm; Thermo Fisher Scientific, MA, USA).

Results: This study demonstrated correlations between specific metabolic variables and metabolites using statistical analyses, with a focus on the relationships between GLA enzyme activity, MRI-derived left ventricular mass, troponin levels, and T2 relaxation times and the metabolic profiles of both classical and non-classical Fabry disease patients. The identified metabolites and their associations will be presented in the poster.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3109 C — Constructing a GC-MS EI-RI Library Using Kit-Based Standards: "An Optimized Approach for Level One Metabolite Identification"

PRESENTING AUTHOR: ***Shiva Jalili, University Of Liverpool, United Kingdom***

SUB THEME: ***Other***

Accurate metabolite identification and annotation in complex biological samples remains a critical challenge in metabolomics studies. Existing commercial spectral libraries often lack adequate coverage of biologically relevant metabolites or have limitations such as computationally derived spectra, absence of retention index (RI) data, and inclusion of non-biological compounds. To address these challenges, and to achieve level one of metabolites identification, we constructed a biologically focused GC-MS spectral library for low-molecular-weight compounds (<450 Da), using commercially available chemicals standard kits covering key metabolic pathways— including glycolysis, the TCA and urea cycles, sugar alcohols, microbiome derived metabolites kit, organic acids, etc— that are easier to source and more cost-effective than individual standards.

A total of 1,429 standards were dissolved in (MeOH): (ACN): (H2O) solvent in 3:3:2 ratio with a final concentration of 0.17 mM, dried down, derivatized by methoxyamine and MSTFA, and analysed and processed by LECO ChromaTOF (v5.58.07) software.

In total, 840 compounds (~59%) were successfully detected and included in the library with n-alkane-based RI and electron ionization fragmentation spectra. Of these, 281 metabolites exhibited multiple peaks due to derivatization at multiple reactive sites.

Comparison with public libraries (GOLM and Fiehn) using InChIKey matching showed that approximately 46% of the metabolites in our library overlapped with those in the public databases, while the remaining 54% were uniquely represented in our dataset—highlighting its expanded coverage and complementary value for GC-MS-based metabolite identification. Furthermore, subclass distribution analysis confirmed the strong biological focus of the library, with broad coverage of amino acids, microbiome-derived metabolites, carnitines, and sugars. Notably, 21 subclasses—including aminophenyl ethers, minoxides, anisoles, and anthracenecarboxylic acids—were uniquely covered by our library. This study demonstrates a reproducible and cost-effective strategy for constructing a high-quality GC-MS EI-RI library using kits, reducing the dependency on sourcing hundreds of individual chemical standards.

3110 C — Urinary metabolomics and lipidomics reveal the effects of particulate matter on maternal health

PRESENTING AUTHOR: ***Minki Shim, College Of Pharmacy, Chung-ang University, South Korea***

CO-AUTHORS: ***Sunwha Park, Young Ju Kim, Dong-Kyu Lee***

SUB THEME: ***Pregnancy, maternal and neonatal health***

Particulate matter 2.5 ($PM_{2.5}$, particles ≤2.5 μm in aerodynamic diameter) is considered as a critical air pollutant associated with numerous adverse health outcomes, and maternal exposure to elevated $PM_{2.5}$ levels during gestation has been implicated in metabolic disruptions. In this study, metabolomics and lipidomics strategy was employed to maternal urine, to elucidate alterations in metabolic pathways and lipid metabolism associated with $PM_{2.5}$ exposure during pregnancy. Urine specimens were collected from seven hospitals in South Korea (2021-2023). A case-control cohort was randomly established, comprising 30 individuals each in the high (>15 $\mu g/m^3$) and low (<15 $\mu g/m^3$) $PM_{2.5}$ exposure groups. Seven outliers were excluded by principal component analysis (PCA), resulting in a final dataset of 53 individuals for statistical analysis. Lipidomic profiling quantified 117 lipid species, of which 52 exhibited significant changes, including 26 triacylglycerols, indicating disruption of glycerolipid metabolism. 45 urinary metabolites were identified via untargeted metabolomics, and potential metabolic biomarkers were selected by supervised multivariate analysis using orthogonal partial least squares-discriminant analysis (OPLS-DA), along with t-test, fold change (FC) analysis, and receiver operating characteristic (ROC) analysis. 15 metabolites were confirmed as biomarkers, predominantly associated with pentose and glucuronate interconversions. Subsequent pathway enrichment analysis based on these metabolites revealed 11 significantly altered metabolic pathways, and metabolic network mapping with these pathways further characterized the specific alterations induced by $PM_{2.5}$ during pregnancy. This study demonstrated that $PM_{2.5}$ exposure affects maternal metabolic pathways and identified urinary pentose metabolites and triacylglycerols as potential biomarkers. These findings suggest that these urinary metabolites and lipids may serve important diagnostic and prognostic roles, and provide a basis for expanding research into the detailed biological mechanisms underlying $PM_{2.5}$-induced metabolic disruptions and their associations with pregnancy-related complications. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2023-00212534).

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3111 C — Identifying biomarkers of disease progression from gestational to type 2 diabetes for machine learning-based prediction and deeper metabolic characterization

PRESENTING AUTHOR: ***Emily Hashimoto-roth, University Of Toronto, Canada***
CO-AUTHORS: ***Julie A. D. Van, Erica P. Gunderson, Michael B. Wheeler, Hannes L. Röst***
SUB THEME: ***Pregnancy, maternal and neonatal health***

As one of the most common metabolic disorders during pregnancy, gestational diabetes mellitus (GDM) afflicts up to 14% of pregnancies worldwide and 35-50% of these women develop type 2 diabetes (T2D) within 10 years postpartum. The underlying defect may involve pancreatic beta cells, liver, or other tissues, but the exact mechanisms remain unclear and hard to predict. Therein, identifying GDM patients at high risk of developing T2D remains a critical, yet unresolved, question. To investigate future T2D onset, we applied mass spectrometry-based metabolomics and lipidomics profiling to fasting plasma samples collected from 660 subjects, 6-9 weeks postpartum. These subjects were recruited to the SWIFT cohort following a GDM diagnosis and, over the course of ~13 years, 260 developed T2D. We identified 38 differentially abundant metabolites in T2D-progressors. Subsequently, we applied robust computational methods and identified a 10-metabolite signature, of which all 10 metabolites are differentially abundant. We constructed several classification models for predicting future T2D during early postpartum. Our best performing SVM model performed with a ROCAUC of 0.81, largely outperforming relevant clinical variables as predictors, such as a random forest model using clinically-measured fasting plasma glucose (ROCAUC=0.58). This collection of metabolites additionally revealed seven statistically enriched and perturbed KEGG pathways in T2D-progressors, primarily dysregulated amino acid metabolism pathways. We also identified a subset of 255 subjects who were prediabetic 6-9 weeks postpartum, 155 of whom developed T2D. In these subjects, we identified a unique down-regulation of serotonin (adjusted p-value=0.01), independent of postpartum depression and anti-depressant usage. Our recent findings highlight the early detectable changes in amino acid, biogenic amine, and lipid metabolism putatively contributing to the onset of T2D. Altogether, our study shows that understanding the transition from GDM to T2D and predicting future T2D requires a holistic approach, moving away from a glucose-centric view.

3112 C — In vitro metabolomics as a New Approach Methodology for toxicological mode of action identification and point of departure determination

PRESENTING AUTHOR: ***Philipp Ternes, BASF Metabolome Solutions GmbH, Germany***
CO-AUTHORS: ***Andreas Verlohner, Nicole Rocker, Hennicke Kamp, Franziska Maria Zickgraf***
SUB THEME: ***Toxicology***

In vitro metabolomics is a New Approach Methodology (NAM) for the safety assessment of chemicals. As an in vitro test system, it contributes to the 3Rs by enabling the replacement of animal testing. In combination with other in vitro and in silico methods, it has the potential to not only replace animal testing, but also provide data that is more mechanism-based and human-relevant than data from animal testing.

We tested a range of substances with different modes of action on HepG2 cells. Our metabolomics in vitro ("µMIV") platform is based on a broad targeted metabolomics approach because of its high efficiency, data quality, and reproducibility. Our data show that in vitro metabolomics can distinguish between substances with different modes of action and provides a sensitive method for point of departure determination.

In vitro metabolomics is thus a valuable tool in a future NAM toolbox on which a Next Generation Risk Assessment (NGRA) strategy can be based.

3113 C — Untargeted Metabolomics in Early Drug Development at Boehringer Ingelheim

PRESENTING AUTHOR: ***Federica Fiorini, Boehringer Ingelheim, Germany***
CO-AUTHORS: ***Joerg Thomas Hannich, Martin Pauers, Svenja Mayer-Wrangowski, Stefan Blech***
SUB THEME: ***Toxicology***

The Metabolomics Team at Boehringer Ingelheim supports pre-clinical toxicological drug development studies with metabolomics and lipidomics analyses. Understanding drug induced changes of the metabolome and lipidome helps to detect adverse effects of new active pharmacological ingredients or to elucidate molecular mechanisms underlying pathological effects.

We utilize untargeted metabolomics, based on tandem liquid chromatography coupled with high resolution mass spectrometry, alongside untargeted and semi-targeted lipidomics approaches, to characterize the toxicological effects of new chemical entities in animal models.

The analysis of untargeted metabolomics data generates large and complex datasets, requiring multiple stages of data cleaning, statistical analysis, and data mining. Given the absence of off-the-shelf solutions that meet our data analysis needs, we have developed custom automated workflows for efficient data processing and reporting. This streamlined process has increased efficiency, reduced the risk of human error, and significantly improved the quality of our data outcomes. Ultimately, our approach supports R&D projects and contributes to the early selection of the most promising drug candidates for further development, with the potential to transform patients' lives.

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METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3114 C	**Multi-Omics Profiling Reveals Biomarkers of polyhexamethylene guanidine phosphate (PHMG-p)-Induced Toxicity in human alveolar epithelial A549 cells**

PRESENTING AUTHOR: ***Jung Dae Lee, Center For Human Risk Assessment, South Korea***

CO-AUTHORS: ***Hyang Yeon Kim, Suhkmann Kim, Kyu-Bong Kim***

SUB THEME: ***Toxicology***

Polyhexamethylene guanidine phosphate (PHMG-p) induces severe pulmonary fibrosis. In a previous study using Wistar rats, ARG1, LCN2, glutamate, and choline were identified as key biomarkers of PHMG-p-induced pulmonary damage. To confirm candidate biomarkers, transcriptomics, proteomics, and metabolomics analyses were conducted using A549 cells. The IC50 of PHMG-p was determined to be 7.56 μg/mL, and cells were treated with 0, 5, or 10 μg/mL for 24 h. RNA-seq identified 2,815 differentially expressed genes (DEGs), with 582 commonly upregulated and 495 downregulated in both dose groups. Proteomic profiling revealed 297 differentially expressed proteins (DEPs). Enrichment analysis of Gene Ontology biological processes (GOBPs) highlighted pathways such as PPAR, AMPK, phosphatidylinositol, autophagy, Rap1 signaling, and transcriptional misregulation in cancer. Metabolomics identified 40 key metabolites, including glutamate, choline, glycerol, glutathione, and taurine. The affected metabolic pathways included aspartate metabolism, arginine and proline metabolism, glutathione metabolism, the urea cycle, and purine metabolism. DEGs and DEPs associated with lung diseases (COPD, pulmonary fibrosis, asthma, cystic fibrosis) included NFE2L2, HMGA2, ITGAE, GNAQ, SCD, and FADS2, with NFE2L2 being the only shared transcriptomic and proteomic biomarker. Furthermore, four DEGs (TLR3, IL13, EGR1, and Arg2) and five metabolites (glutamate, choline, glycerol, glutathione, and taurine) were identified as key biomarkers. While glutamate and choline were confirmed as biomarkers in both in vivo and in vitro studies, ARG1 and LCN2 from in vivo studies were not detected in PHMG-p-treated A549 cells. In conclusion, these findings suggest key molecular signatures for PHMG-p-induced pulmonary toxicity in A549 cells, providing insights into potential biomarkers and mechanisms of damage.



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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3150 C — Strength of ^{1}H NMR in Plant Science and Biotic Stress Research

PRESENTING AUTHOR: *Anna Mascellani Bergo, Czech University Of Life Sciences Prague, Czech Republic*
CO-AUTHORS: *Kirsten Leiss, Estuardo Hernandez Olesinski, Jaroslav Havlik*
SUB THEME: *Crop quality research*

Metabolomics has become an important tool in elucidating the complex relationship between a plant genotype and phenotype. For over 20 years, nuclear magnetic resonance (NMR) spectroscopy has been known for its robustness, quantitative capabilities, simplicity, and cost-efficiency. ^{1}H NMR is the method of choice for analysing a broad range of relatively abundant metabolites, which can be used to capture the plant chemical profile at one point in time and understand the pathways underpinning plant defence. We aim to highlight the challenges and advantages of using ^{1}H NMR in plant metabolomics, interpret common trends observed in metabolite responses to biotic stress, suggest guidelines for method development and establish standard procedures.

In our research, we explored the utility of ^{1}H NMR in plant stress metabolomics, integrating bioassay results and plant resistance measurements to identify underlying key metabolic processes. Through the multivariate statistical analysis, we screened resistant and susceptible gerbera varieties, identifying polyketides as important contributors to resistance against powdery mildew and leading to a metabolome-based prediction model for mildew resistance for gerbera breeders. Reducing nitrate fertilisation levels in gerbera increased resistance to powdery mildew by enhancing polyketide and phenylpropanoid concentrations. Induction with methyl jasmonate of tomato plants grown in different substrates increased trip defence, stimulating an increase in phenylpropanoid pathway metabolites.

Our results highlight the crucial role of secondary metabolites in plant defence. At the same time, our findings underscore the strengths and limitations of ^{1}H NMR in studying plant defence pathways, highlighting when it outperforms liquid chromatography coupled with mass spectrometry (LC-MS) and when LC-MS is preferable. The proposed methodological guidelines and standardised procedures enhance reproducibility, cross-study comparability, and the broader adoption of ^{1}H NMR in plant metabolomics.

3151 C — Metabolomic Profiling of Guadua Bamboo Species: Altitudinal Influence, Cytotoxic Potential, and Chemical Diversity

PRESENTING AUTHOR: *Ian Castro-Gamboa, IQ-UNESP, Brazil*
CO-AUTHORS: *Luis Carlos Chitiva, Geison Modesti Costa*
SUB THEME: *Environmental exposures*

Bamboo species of the genus Guadua have been widely recognized for their structural applications and ecological importance. However, their metabolomic diversity and bioactive potential remain largely unexplored, particularly in relation to environmental factors such as altitude. This study employs untargeted metabolomics, molecular networking, and cytotoxicity assays to assess the influence of altitude on the chemical profile of Guadua species and their biological effects on colon cancer cells.

Using LC-QTOF-MS and multivariate statistical analyses, we identified 89 altitude-dependent metabolites, predominantly flavonoids and cinnamic acid derivatives. The cytotoxic evaluation of Guadua incana leaf extract (BLEGI) against HCT-116 colon cancer cells demonstrated significant metabolic alterations, particularly in glutathione metabolism and the tricarboxylic acid cycle. The molecular characterization of 30 Guadua species revealed the presence of 40 flavonoids and 9 hydroxycinnamic acid derivatives, which were correlated with antioxidant and cytotoxic activities.

These findings underscore the metabolic plasticity of Guadua species and their potential as sources of bioactive compounds with pharmaceutical relevance. The study also highlights the impact of environmental factors on secondary metabolism, reinforcing the importance of exploring Latin American biodiversity for natural product discovery.

3152 C — Distinct and Interactive Metabolic Dynamics of Single-Strain Lactic Acid Bacteria and Raw Materials in Kimchi Fermentation

PRESENTING AUTHOR: *Yumi Kim, Seoul National University, South Korea*
CO-AUTHORS: *Hak-Jong Choi, Do Yup Lee*
SUB THEME: *Fermented foods*

Lactic acid bacteria (LAB), including Lactobacillus, Weissella, and Leuconostoc, play central roles in kimchi fermentation. While previous studies have predominantly focused on microbial chracteristics, limited attention has been given to how bacterial inoculation and ingredient-derived factors collectively shape metabolomic dynamics. In this study, we aimed to elucidate the combined effects of raw materials (red pepper powder and glutinous rice paste) and single inoculation of representative LAB strains on metabolite alterations during fermentation. Kimchi was separately fermented for six weeks with Lactobacillus sakei, Leuconostoc mesenteroides, or Weissella confusa. Metabolic profiling of kimchi was conducted using gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) and liquid chromatography orbitrap mass spectrometry (LC-orbitrap-MS), resulting in the annotation of 242 metabolites, including amino acids, carbohydrates, and fatty acids. We identified both strain-specific metabolic signatures, raw materials-specific metabolic signatures and common fermentation-derived patterns. Our findings demonstrate the unique and interactive metabolic contributions of bacterial strains and raw materials in fermentation process, thereby providing new insights into the metabolic mechanisms underlying mixed-culture kimchi fermentation.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3153 C — Metabolomic and Proteomic Insights into Plasma-Activated Water-Induced Functional Changes in Radish

PRESENTING AUTHOR: *Ravi Gupta, Kookmin University, South Korea*
CO-AUTHORS: *Neha Kaushik, Manorma Negi, Nagendra Kumar Kaushik, Eun Ha Choi*
SUB THEME: *Foodomics and food security*

Nitrogen is a critical macronutrient for plant growth and a major component of commercial fertilizers. To reduce fertilizer dependency and promote sustainable agriculture, nonthermal plasma technologies have emerged as promising alternatives. Plasma-activated water (PAW), known to alter physicochemical properties such as pH, conductivity, and reactive species content, has shown beneficial effects on plant development. However, its impact at the molecular level remains underexplored. In this study, we investigated the effects of PAW on Raphanus sativus (radish) roots using an integrated proteomics and metabolomics approach. Plants treated with PAW exhibited enhanced accumulation of reactive oxygen and nitrogen species, along with increased antioxidant enzyme activities. Proteomic analysis identified 6,054 proteins, with 1,845 significantly modulated by PAW, primarily involved in energy metabolism, ROS detoxification, hormone signaling, and glucosinolate biosynthesis. Metabolomic profiling revealed 314 metabolites, of which 194 responded significantly to PAW. Notably, there was a marked increase in health-beneficial compounds such as vitamin C, vitamin B5, glutathione, and glucosinolates. The integration of multi-omics data uncovered key molecular pathways underlying plasma-induced growth promotion and metabolic enhancement. These findings highlight the potential of PAW as an eco-friendly strategy to boost crop quality and nutritional value through targeted metabolic reprogramming.

3154 C — Honey fraud detection through UHPLC-HRMS-based untargeted metabolomics

PRESENTING AUTHOR: *María Monge, CIBION - CONICET, Argentina*
CO-AUTHORS: *Nicolás Zabalegui, Eduardo Diez*
SUB THEME: *Foodomics and food security*

Honey adulteration leads to nutritional and health risks to consumers and causes a severe impact on local and international markets. The most frequent type of adulteration is the addition of inexpensive sugar-based products [1]. Additional known fraud practices are mislabeling or feeding bee colonies with syrups after the onset of nectar flow. Almost half (46%) of honey samples imported into the European Union are suspected of being adulterated [1]. Due to the complexity of honey fraud, there is no single method or analytical technique that can detect the different adulteration strategies. Moreover, given the high price and limited production of this product, novel and sophisticated methods of adulteration challenge established detection methods. The present work aimed at identifying novel adulteration markers using a UHPLC-QTOF-MS-based untargeted metabolomics approach. Given the high complexity of the honey matrix, composed of carbohydrates in very high concentration, proteins, enzymes, organic acids, phenols, minerals, and amino acids [1], different sample preparation protocols were evaluated as well as different chromatographic methods and UHPLC columns. The selected hydrophilic interaction chromatography method yielded the highest peak capacity for saccharide separation. Honey samples (n=37) collected from different geographical regions of Argentina, which are available in the local market, were analyzed to search for known fraud markers as well as novel adulteration fingerprints. Initial findings revealed that certain oligosaccharides suspicious of being adulteration markers were detected, and their identification was verified with authentic chemical standards. The developed analytical method enabled the detection of endogenous honey compounds as well as potential adulteration markers, highlighting the capabilities of HRMS-based metabolomics as a novel analytical strategy for honey authentication.

[1]Paiano, V.; Breidbach, A.; Lörchner, C.; Ždiniaková, T.; De Rudder, O.; Maquet, A.; Alvarellos, L.; Ulberth, F. Separations 2025, 12 (2), 47. https://doi.org/10.3390/separations12020047.

3155 C — A metabolomics approach to developing a flavor enhancer for a fat substitute

PRESENTING AUTHOR: *Minkyung Park, Korea Food Research Institute, South Korea*
CO-AUTHORS: *Ji Yoon Char, Yun-Sang Choi*
SUB THEME: *Foodomics and food security*

Alternative foods are an important research area for ensuring a sustainable food supply, particularly in response to environmental concerns food shortages, and health-related issues. This study employed an omics-based approach for preparing animal fat substitutes using plant-based oils. For animal fats, fats directly extracted from meat were used, while for vegetable oils, commercially available products were used. Through specific thermal treatment, volatile metabolites in animal fats (e.g. beef tallow, pork lard) and vegetable oils (e.g. soybean, sunflower oils) using GC-MS. Some volatile metabolites contributing to meaty, roasted, and off- flavor notes were identified as main volatile metabolites of each sample. The Results showed that animal fats, rich in saturated and monounsaturated fatty acids, predominantly generated aldehydes, ketones, and sulfur-containing volatiles associated with savory aromas. In contrast, vegetable oils, containing higher levels of polyunsaturated fatty acids and phytosterols, formed more lipid oxidation products, such as hexanal and nonanal, related to grassy or rancid notes. These findings provide insight into the flavor formation mechanisms across fat types, providing guidance for designing customized flavors in processed foods.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3156 C — Assessing the Nutri-Score's limitations through a metabolomic approach: The case of soluble cocoa powders

PRESENTING AUTHOR: ***Marta Palma Morales, University of Granada, Spain***
CO-AUTHORS: ***Oscar Daniel Rangel Huerta, Estela Castilla Ortega, Celia Rodríguez Pérez***
SUB THEME: ***Foodomics and food security***

Introduction

Despite the well-documented benefits of cocoa, its nutritional value is often misrepresented by simplified labelling systems such as Nutri-Score. This score primarily considers factors such as energy, sugar, saturated fat, protein, fibre, sodium, and the presence of fruits, vegetables, legumes, nuts, and specific oils (rapeseed, walnut, and olive), but overlook bioactive compounds like flavonoids and the degree of processing. The aim of this work was to use a metabolomics approach to assess whether Nutri-Score captures the health-promoting compounds in commercial soluble cocoa powders.

Methodology

A total of 54 soluble cocoa products available in Spain, covering 19 different brands and four Nutri-Score categories (A–D) were included. Metabolites were extracted using ultrasound-assisted techniques and analyzed by high-performance liquid chromatography coupled with time-of-flight mass spectrometry. The resulting data were processed with MS-DIAL and further analyzed using multivariate statistical methods, including Principal Component Analysis (PCA) and Partial Least Squares regression (PLS2), to investigate associations between metabolite profiles, nutritional composition, and Nutri-Score ratings.

Results

PCA showed no clear clustering of products based on Nutri-Score category. In contrast, PLS2 analysis revealed more distinct clusters associated with cocoa content and the presence of bioactive compounds. Key discriminant metabolites included certain flavonoids, small peptides, phenolic acids, terpenoids, fatty acids and glycerophospholipids, all linked to higher antioxidant activity and elevated levels of cocoa, fat, fibre, and protein. Notably, products rich in these beneficial compounds and macronutrients often received lower Nutri-Score ratings, while more highly processed products with reduced cocoa and bioactive content were rated more favourably.

Conclusions

Metabolomics highlighted significant discrepancies between actual nutritional quality and Nutri-Score classification and represents a promising tool to complement current labelling systems by providing more accurate and health-relevant information to guide consumer choices.

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3157 C — MS2LDA 2: Advancing Substructure Discovery to improve Food Safety based on Untargeted Metabolomics Profiling

PRESENTING AUTHOR: ***Jonas Dietrich, Wageningen University and Research, Netherlands***
CO-AUTHORS: ***Rosina Torres Ortega, Hans Mol, Justin Joris Johannes van der Hooft***
SUB THEME: ***Foodomics and food security***

Food matrices are complex metabolite mixtures and monitoring pesticides therein, especially novel types or variants, represent a main challenge. Untargeted metabolomics linked to tandem mass spectrometry generates profiles that aid in structural annotation. Classical library matching compares spectra in these profiles to reference libraries, limiting identification to already known compounds. Although advancements in the field—such as molecular networking with GNPS, analogue searching by MS2Query, and fragmentation trees via SIRIUS—enhance and complement library matching, low identification rates (around 10%) remain the biggest challenge in untargeted metabolomics, and thus in food safety applications.

We present MS2LDA 2.0, a complete re-implementation of the decade-old MS2LDA unsupervised substructure discovery tool, designed to improve scalability for large datasets, integrate with common Python frameworks, and implement automated annotation guidance to facilitate substructure identification.

MS2LDA 2.0 links fragmentation patterns to substructures, enabling the identification of novel compounds that share substructures with known compounds or allowing manual annotation of entirely new substructures. Here, we highlight a case study where 216 pesticides were spiked in tomato samples as a model of a complex biological matrix. MS2DLA 2.0 discovered 8 Mass2Motifs connected to spiked pesticide substructures. Among other findings, all six spiked dimethoxylated sulfonylurea pesticide analogues, and all four spiked triazole-dichlorobenzene-containing compounds, could be traced based on a common Mass2Motif. Hence, MS2LDA 2.0 effectively identifies substructures and complements existing identification tools such as SIRIUS and GNPS.

In the field of food safety, MS2LDA 2.0 could be used to mine spectral alerts—fragmentation patterns associated with potentially hazardous substructures, and they could be correlated with specific toxicological endpoints. Such spectral alerts can be stored in a database, queried, and leveraged for compound screening in untargeted food toxicant screening. This capability enhances food safety by enabling the detection of compounds beyond known spectral libraries of hazardous substances.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3158 C — Untargeted lipidomic approach reveals coral health risk from exposure to a plastic additive

PRESENTING AUTHOR: *Chuan-Ho Tang, National Museum of Marine Biology and Aquarium, Taiwan*
CO-AUTHORS: *Ting-Ju Liu, Ching-Yu Lin*
SUB THEME: *Marine metabolomics*

The bioaccessibility of hexabromocyclododecane (HBCD as a plastic additive) to particle feeders (such as corals) substantially increases with the occurrence of microplastics in the ocean. It is very limited to know what ecotoxicological effects can be induced by HBCD on the organisms. An untargeted lipidomic profiling of coral (Pocillopora acuta) exposed to HBCD was thus performed for exploring the effect on coral health in this study. A dose-dependent lipidomic change was observed in coral with a reduction in lipid oxygenation and peroxidation after HBCD exposure. Based on physiological functions of the changed lipids, particularly vinyl ether glycerophosphoethanoamines and ether glycerophosphocholines, a compromise of the physiological fitness was implied in coral cells. Therefore, HBCD pollution should be considered as a potential threat to coral health in the ocean.

3160 C — Freeze-Drying–Induced Intracellular Metabolites Changes: Considerations for Yeast Drying

PRESENTING AUTHOR: *Jang-Eun Lee, Korea Food Research Institute, South Korea*
CO-AUTHORS: *Eunjung Lee*
SUB THEME: *Microbial metabolomics*

This study investigates the effects of yeast drying processes on microbial metabolite profiles, focusing on both extracellular and intracellular metabolic alterations. Extracellular metabolites were analyzed using LC-QTOF/MS and LC-Orbitrap-MS/MS following four different drying methods, including freeze-drying. Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) revealed no significant differences in extracellular metabolite profiles across the drying methods. In contrast, intracellular metabolite analysis showed distinct alterations induced by freeze-drying, as demonstrated by PCA and validated through PLS-DA models. Notable changes were observed in amino acids, sugars, organic acids, and other metabolites. A t-test conducted on 29 key metabolites identified significant differences between control and freeze-dried samples, with 15 metabolites more abundant in the control group and 14 elevated in the freeze-dried group. These findings suggest that while extracellular metabolite profiles remain largely stable, freeze-drying markedly impacts intracellular metabolism in yeast, affecting key metabolic pathways. This study underscores the importance of metabolite-level monitoring for optimizing preservation strategies in industrial yeast applications.

3161 C — Differences between S. cinnamonensis wild-type and industrial strains and their influence on the production of secondary metabolites

PRESENTING AUTHOR: *Sofia Camila Bravo, Ruhr University Bochum & Max Planck Institute, Germany*
CO-AUTHORS: *Daniel Petras, Frank Schulz*
SUB THEME: *Microbial metabolomics*

Streptomyces species are famous for their ability to produce numerous bioactive compounds. S. cinnamonensis is known for producing monensin, which is used both as an antiparasitic agent and as a growth promoter. To enhance monensin production, an industrial strain was acquired and compared to the wild-type (WT) strain. Fermentations in optimized medium showed no significant differences in monensin production between the two strains at laboratory scale. However, strain differentiation based on growth characteristics and culture color suggested potential metabolomic differences.

Our goal was to identify these metabolomic differences and evaluate their relevance for production, strain maintenance, or other applications. Sequential extractions using different solvents, followed by chromatographic analysis with high-resolution mass spectrometry, demonstrated good reproducibility. Preliminary findings indicated a significant correlation between metabolomic profiles and phenotypic characteristics, with variations in culture color linked to differences in endophenazine production.

Metabolomic analysis identified several secondary metabolites, including monensin, siderophores such as coelichelin and mirubactin, and various bioactive furaquinocins. The corresponding biosynthetic gene clusters were also detected in the WT genome using antiSMASH. Preliminary results revealed a difference in enzyme specificity: the WT strain produced more variants of each compound, while the industrial strain either did not produce variants or produced fewer congeners. Additionally, the industrial strain was found to produce lipopeptides and antioxidants not detected in the WT.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3162 C — Comparison of Microbial Extracellular Vesicle Isolation Methods for Metabolomic Profiling

PRESENTING AUTHOR: ***Myeongwon Lim, Seoul National University, South Korea***
CO-AUTHORS: ***Swan Kim, Do Yup Lee***
SUB THEME: ***Microbial metabolomics***

Microbial metabolites play essential roles in regulating host physiology and host-microbial interactions, significantly influencing health and disease states. Extracellular vesicles (EVs) are nano-sized particles secreted by microbes which mediate these interactions by transporting bioactive metabolites. However, studying EV metabolites remains challenging due to its low yield and difficulty in obtaining sufficient purity for metabolite analysis. Developing and optimizing high-throughput EV isolation methods for metabolomics is needed to enhance our understanding of host-microbial interactions.

This study aimed to compare metabolomic profiles of microbial EVs isolated through three different methods using liquid chromatography-Orbitrap mass spectrometry (LC-Orbitrap MS). Particle number, protein concentration, and zeta-potential were measured with nanoparticle tracking analysis (NTA), bicinchoninic acid (BCA) assay, and dynamic light scattering (DLS) to validate the quality and stability of EV samples. Peak picking and compound annotation were performed using MS-DIAL, and all statistical analyses were conducted using R.

Our experiments showed the differences in the EV cargo metabolite composition isolated with three different methods and confirmed the suitability of each isolation method for EV metabolite analysis. This study serves as a foundation for developing optimized EV isolation protocols for metabolomics, enabling deeper exploration of functional role of EVs in host-microbial interactions.

3163 C — LC–MS/MS based investigation on the biotransformation of phytochemicals by skin bacteria

PRESENTING AUTHOR: ***Kyungha Lee, Sookmyung Women's University, South Korea***
CO-AUTHORS: ***Yeji Kim, Kyo Bin Kang***
SUB THEME: ***Microbial metabolomics***

The skin functions as a primary barrier and interacts with diverse xenobiotics. Although skin microorganisms are expected to metabolize a wide range of compounds, studies on their metabolic roles remain limited. We aimed to investigate the biotransformation of phytochemicals by skin bacteria and to elucidate the underlying mechanisms, including the enzymes involved. To achieve this, six bacterial strains were obtained from KCTC and cultured with 17 phytochemicals. The acquired LC–MS/MS dataset was analyzed using molecular networking to annotate metabolites based on MS/MS fragmentation similarity. Moreover, the metabolic ability of each strain was evaluated by measuring the proportion of remaining standard compounds. Among 17 compounds, the metabolites of eupatilin, naringenin, licochalcone A, and isoliquiritigenin were annotated with fragmentation patterns indicating hexosylation and hydrogenation. To our knowledge, the derivatives of licochalcone A have not been reported from plants. Therefore, licochalcone A was prioritized for further large-scale purification, and compound 1 was identified as its hydrogenated form. We expect that our study design will contribute to a deeper understanding of the biotransformation of plant specialized metabolites by skin bacteria.

3164 C — Plant-growth promoting rhizobacteria to improve the phytochemistry of South African medicinal plants: Plectranthus species.

PRESENTING AUTHOR: ***Akhona Myoli, University Of Johannesburg, South Africa***
CO-AUTHORS: ***Justin van der Hooft, Ntakadzeni Madala, Fidele Tugizimana***
SUB THEME: ***Natural products***

Recently, pharmaceutical companies have shown increased interest in plant natural products with the aim of either finding novel compounds or developing plant-derived drugs. Consequently, the demand for the cultivation of medicinal plants is increasing in both developing and developed countries. However, during domestic cultivation of these plants, variability in yield and quality of the phytochemical profiles due to cultivation practices and environmental factors are considerable drawbacks. Hence, the sustainable use of biostimulants, particularly microbial biostimulants such as plant-growth-promoting-rhizobacteria (PGPR), has been proposed with the aim of improving their yield and phytochemical content. In the context of the phytochemical content, it is currently not clear to what extent PGPR affects the phytochemical profiles of South African (SA) medicinal plants. Thus, here, a metabolomics approach was applied to comprehensively characterize the phytochemical space of PGPR-treated and untreated Plectranthus species- SA medicinal plants. A digitized liquid chromatography-tandem mass spectrometry (LC-MS/MS) computational metabolomics analysis workflow was used to characterize the metabolome of the two species. The chemometric models pointed to underlying differential metabolic profiles of the untreated and PGPR-treated plants. Additionally, molecular networking revealed that the chemical space of the two species comprises a wide spectrum of bioactive metabolite classes such as phenolic acids, flavonoids, and terpenoids. Relative quantification of the detected metabolites showed that PGPR increases the levels of metabolites such as glycosylated flavonoids and phenolic acids in Plectranthus species. Notably, Salviaflaside – a phenolic acid with antioxidant activities- was found in higher levels (log2 fold change > 1, p-value < 0.05) in the PGPR-treated plants compared to the untreated plants. Thus, the generated knowledge will positively impact both agricultural practices for medicinal plant cultivation and the natural product/drug discovery pipeline. These insights will also contribute to the current efforts in sustainable agricultural practices by reducing environmental degradation.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3165 C — Metabolomic and Genomic Exploration of Cameroonian Actinomycetes for Novel Antimicrobials Leads Discovery (MINED)

PRESENTING AUTHOR: *Rufin Marie Toghueo Kouipou, Institute Of Microbiology, Czech Republic*
CO-AUTHORS: *Zdenek Kamenik*
SUB THEME: *Natural products*

Natural products from actinomycetes have historically yielded the most potent antibiotics, yet traditional discovery methods and the frequent rediscovery of known compounds have stagnated the field. Fortunately, recent advances in genomic and metabolomic technologies have revealed the untapped potential of microbes, including well-studied actinomycetes. Therefore, revisiting these microbial resources with modern multi-omics technologies offers a promising path to uncover the next generation of antibiotics. The MINED project will leverage metabolomics, and genomics approaches to investigate actinomycetes from underexplored Cameroonian diverse environments, including forests, mountains, rivers, landfills, ant and termite nests, zoological parks, swamps, and ponds. We will screen culture broths of 100s actinomycetes isolates against our in-house collection of ESKAPE pathogens that includes clinical isolates from Czech hospitals to identify strains producing bioactive metabolites. Selected isolates will undergo LC-MS/MS metabolomic profiling, with spectral data analyzed and dereplicated against our in-house library (10,000 compounds), commercial MetaboBase (Bruker), and open-source GNPS libraries. StreptomeDB, and Molecular networking using and MicrobeMASST will be used to visualize the chemical space in the context of metadata and for taxon-specific annotations. For compounds classification and molecular substructure identification and annotation, the computational tools SIRIUS will be used to predict molecular formulas, while MS2LDA will decode substructure motifs. Strains producing bioactive and novel chemical motifs will be sequenced to high depth, and their genomes mined for biosynthetic gene clusters (BGCs) using antiSMASH, PRISM, CluSeek, and BiG-SCAPE to map BGC diversity and evolutionary relationships. For the preliminary investigation, selected isolates were characterized using 16S rDNA sequencing, and their culture extracts were analyzed with our in-house metabolomics workflow. By integrating ecological sampling, bioactivity-guided screening, and multi-omics technologies, MINED aims to contribute to the antibiotic discovery efforts while contributing a curated dataset of Cameroonian actinomycetes genomes and metabolites to public repositories to accelerate antibiotic discovery and combat antimicrobial resistance.

3166 C — Metabolite profiling of serum and feces to elucidate the anti-obesity effects of Curcuma longa rhizome extract in high-fat diet-fed mice model

PRESENTING AUTHOR: *Yu Ra Lee, Korea Food Research Institute, South Korea*
SUB THEME: *Natural products*

Obesity is associated with metabolic disorders, and the gut microbiota plays a crucial role in modulating host metabolism. In this study, we performed metabolomic profiling using LC-MS/MS to investigate the anti-obesity effects of Curcuma longa rhizome extract in a high-fat diet-fed mouse model. Metabolite analysis of serum and fecal samples revealed more significant metabolic alterations in fecal samples than in serum. Notably, significant differences were observed in amino acids, deoxycholic acid, and 3-indolepropionic acid levels in fecal samples. Correlation analysis between identified metabolites and metabolic disorder parameters demonstrated a significant negative correlation between fecal metabolites and colon length, as well as tight junction protein expression levels. These findings suggest a potential relationship between metabolites and metabolic disorder biomarkers, highlighting the impact of gut-derived metabolites on obesity-related metabolic dysfunction. However, further studies are required to fully elucidate these associations and their underlying mechanisms.

3167 C — Rapid Screening and Metabolomic Investigation of Essential Oil Components Targeting Acetylcholinesterase

PRESENTING AUTHOR: *Man-Hsiu Chu, Taipei Medical University, Taiwan*
CO-AUTHORS: *Ching-Kuo Lee*
SUB THEME: *Natural products*

Many plants and herbs produce essential oils and are widely grown for their economic value. However, because of the large number of species and their complex chemical makeup, it is difficult to focus on specific components that may be useful for medical purposes. In this study, we used a 96-well ELISA assay with essential oils and Tween 80 to screen for activity. The initial results showed that some essential oils could inhibit acetylcholinesterase, with Canarium luzonicum showing the strongest effect. To quickly find the active compounds responsible for this effect, we used both laboratory experiments and computer-based methods, including ELISA plate readings, enzyme-assisted GC-MS analysis, and molecular docking. These approaches helped identify several compounds as possible active compounds. This study offers a faster way to discover useful substances from natural sources and provides helpful direction for future drug development, especially in treating Alzheimer's disease.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3168 C — Investigation of MicroRNAs in Curcuma longa-Derived Exosomes Using Tandem Mass Spectrometry

PRESENTING AUTHOR: *Pei Hsuan Chen, Taipei Medical University, Taiwan*
CO-AUTHORS: *Ching Kuo Lee*
SUB THEME: *Natural products*

Exosomes are extracellular vesicles, approximately 30-150 nm in diameter, secreted by various cell types. They play a crucial role in intercellular communication by transporting proteins, nucleic acids (such as miRNAs and mRNAs), and lipids to recipient cells, thereby modulating diverse physiological functions.

MicroRNAs (miRNAs) are a class of non-coding RNAs, typically 20-24 nucleotides in length, that regulate gene expression at the post-transcriptional level and participate in a wide range of cellular processes. Traditionally, the qualitative and quantitative analysis of miRNAs has relied on techniques such as quantitative PCR (qPCR), Northern blotting, and RNA sequencing (RNA-seq).

In this study, we applied liquid chromatography-high resolution mass spectrometry (LC-HRMS) to analyze miRNA samples isolated from Curcuma longa-derived exosomes. By employing optimized sample purification procedures and removing excess proteins, background noise was effectively minimized, thereby improving the sensitivity and specificity of miRNA detection.

Our results demonstrate the potential of LC-MS/MS for structural identification and molecular weight confirmation of miRNAs, establishing it as a valuable complementary technique to conventional RNA sequencing. Using next-generation sequencing (NGS), we successfully identified 146 miRNA sequences. Subsequent validation of major miRNAs was performed through LC-MS/MS, confirming their molecular weights. This integrative analytical strategy highlights the practical utility and reliability of LC-MS/MS in the study of small RNA biomarkers, offering essential technical support for miRNA functional analysis and prospective clinical applications.

3169 C — Glycoalkaloids: toxic secondary metabolites in potato-derived products

PRESENTING AUTHOR: *Ondrej Brabenec, Uct Prague, Czech Republic*
CO-AUTHORS: *Tereza Jablonska, Ondrej Rubas, Jana Hajslova*
SUB THEME: *Other*

This study focused on a monitoring toxic glycoalkaloids (GAs) and products of their hydrolysis in potato crisps collected from the Czech retail market. Using the validated UPLC-HRMS method, 2 parent GAs, α-solanine, α-chaconine, together with γ-chaconine and their aglycone solanidine were quantified. In addition, also β-solanine, β-chaconine and γ-solanine were screened. The sums of concentrations of α-solanine and α-chaconine in crisps were in the range of 16 to 177 mg/kg with 9 samples exceeding the indicative level (100 mg/kg) set in the EU Commission Recommendation (2022/561/EU). When recalculating the determined concentrations of degradation products, even 14 samples would exceed the mentioned indicative level. In the next phase, the dynamics of hydrolysis after potatoes disintegration was studies; 50% decrease of parent GA was observed after 120 min at room temperature. At the same time, products of hydrolysis which are also of toxicological concern, were accumulated. As GAs exhibit, besides of other effects, also anti-cholinesterase properties, rapid assay for screening of inhibitory potential of various potato product was developed, thus enabling effective estimation of GAs content.

3170 C — Digging Deep into a Catena Sequence: New Extraction Approaches for Soil Metabolomics

PRESENTING AUTHOR: *Adam Burke, University Of Liverpool, United Kingdom*
CO-AUTHORS: *Robert Brown, Royston Goodacre*
SUB THEME: *Other*

The Centre for Metabolomics Research (CMR) is a member of the NERC Environmental Omics Facility (NEOF). CMR has developed and applied versatile mass spectrometry methods across a wide range of different sample types, to support a variety of projects.

Soil is a principal component of many ecosystems with a range of critical functions, including carbon storage and providing food. However, soil is a complex medium with highly dynamic characteristics. The soil metabolome provides insight into microbial communities, nutrient cycles, environmental conditions, potential contamination sources and biochemical pathways. Commonly presented metabolomics extraction methods for soil samples are time consuming, require large solvent volumes and high sample mass. Here, we present a simplified, greener and rapid extraction method, requiring a relatively small volume of extraction solvent and soil mass. The method was applied to five soil types across a catena sequence (from 4 to 550 m) at Henfaes Agricultural Research Station and compared to a more traditional large volume extraction method.

Samples were analysed with gas chromatography mass spectrometry (GC-MS) to capture small, polar molecules, and a reversed phase liquid chromatography mass spectrometry (LC-MS) method for non-polar and lipophilic molecules. The two extraction methods were compared for extraction yield, sensitivity, reproducibility and ability to discriminate between the different soil types following multivariate data analysis.

Results show a comparable and consistent extraction yield using 50% less solvent, 97% less time and similar discrimination of soil types through principal component analysis (PCA). The overlap of detected metabolites is presented, along with those unique to each method.

This new extraction method may be used to reduce solvent consumption, environmental impact and labour required, as well as improving time efficiency and throughput.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3172 C — Optimizing PFAS Identification: A Comparative Study of HPLC-MS/MS Workflows and Data Analysis Software

PRESENTING AUTHOR: *Jenny Fischer, BASF Metabolome Solutions GmbH, Germany*
CO-AUTHORS: *Rodion Demin, Philipp Ternes*
SUB THEME: *PFAS*

The rapid and reliable identification of per- and polyfluorinated alkyl substances (PFAS) has become crucial as PFAS are under scrutiny. Suitable analytical methods for the identification and (semi-) quantification still need to be developed. High-performance liquid chromatography combined with tandem mass spectrometry (HPLC-MS/MS) is a highly effective technique for detecting and analyzing both polar and long-chain PFAS. In this presentation, we outline an optimized workflow for PFAS analysis, which includes sample extraction, high-resolution HPLC-MS/MS, and data analysis. We compare a generic non-target workflow using MZmine with specialized PFAS software such as FluoroMatch and PFΔScreen to assess their effectiveness. Additionally, we delve into the advantages and limitations of each software package in relation to specific experimental requirements.

3173 C — Metabolomic insights into cross-feeding interactions between Priestia megaterium PM and Pseudomonas fluorescens NO4: Unveiling microbial communication in plant growth-promoting rhizobacteria

PRESENTING AUTHOR: *Nompumelelo Sibanyoni, University Of Johannesburg, South Africa*
CO-AUTHORS: *Lizelle Anne Piater, Pavel Kerchev, Msizi Innocent Mhlongo*
SUB THEME: *Plant and soil microbiome*

Plant growth-promoting rhizobacteria (PGPR) have gained increasing attention due to their ability to enhance plant growth and protect against pathogens. Among these, Bacillus megaterium BM and Pseudomonas fluorescens N04 have demonstrated promising plant growth-promoting properties. However, the influence of microbial interactions and chemical communication between these PGPR strains and the pathogenic P. syringae DC3000 Pst remains largely unexplored. This study employs an LC-MS/MS-based metabolomics approach in combination with multivariate statistics to investigate how microbial cross-feeding between the PGPR strains B. megaterium BM and P. fluorescens NO4 influences chemical communication and metabolite production and its impact on the pathogenic bacterium P. syringae DC3000 Pst. Cultures of B. megaterium BM, P. fluorescens NO4, and P. syringae DC3000 Pst were grown in minimal media until an optical density of 1. Subsequently, 0.1 (OD) of the respective microbes (receiver) were cross-fed into a filter-sterilised media (donor). Cell turbidity was measured every 6 hours, and samples were collected at 6-hour intervals, followed by extraction of metabolites. An LC-MS-based metabolomics approach and statistical analysis were used to investigate the metabolic perturbation caused by cross-feeding. Growth curves indicated that PGPR-PGPR cross-feeding maintained a similar growth pattern to controls with a slight decrease in growth, whereas PGPR-Pathogen interactions significantly decreased pathogen growth. Conversely, Pathogen-PGPR interactions significantly reduced NO4 growth. Metabolic analysis showed time-dependent perturbations in the cross-fed organisms, indicating metabolic re-adjustment to the donor media. MVA highlighted the metabolite perturbations between the cross-fed and controls throughout the time points. The annotated metabolites were from the following classes: amino acids, organic acids, sugars, cyclic lipopeptides, flavonoids, macrolides, and benzenoids, indicating significant metabolic perturbations. Metabolite analysis emphasised the diverse metabolite classes involved in microbial cross-culture interactions. This study enhances understanding of complex interactions between PGPR strains and pathogenic bacteria in the rhizosphere.

3174 C — Assessment of the plasticity of flower chemistry in response to herbivory and florivory

PRESENTING AUTHOR: *Selina Gaar, Bielefeld University, Germany*
CO-AUTHORS: *Thomas Dussarrat, Caroline Müller*
SUB THEME: *Plant defense, pathogens and interactions*

Plants face a wide range of biotic stresses, with herbivory and florivory being among the most important challenges. A major defence strategy is the production of specialised metabolites, where the response strength depends on the individual's plasticity, i.e. the ability to adjust the phenotype depending on the type and intensity of the stress. The aromatic plant species Tanacetum vulgare (Asteraceae) shows high intraspecific variation in leaf and flower terpenoid profiles, forming so-called chemotypes. These chemotypes influence the preferences of herbivores, such as aphids of Macrosiphoniella tanacetaria, which feed on the flower stems of T. vulgare, as well as florivorous Olibrus beetles. Attack by M. tanacetaria has been shown to alter the chemical composition of leaves and phloem sap. However, little is known about the effects of feeding by these aphids or florivorous beetles on flower chemistry and whether these responses differ between chemotypes. Here, we investigated chemotype-dependent floral responses of T. vulgare to M. tanacetaria or Olibrus aeneus infestation in a field setting. Once flowering, each plant was infested with aphids and beetles on different shoots, while one shoot remained herbivore-free. After the infestation period, the flowers were harvested for metabolic fingerprint analysis using UHPLC-QTOF-MS/MS. Differences were found not only in flower fingerprints of plants exposed to insects compared to the control, but also in the type of herbivory. Aphids had a more pronounced effect on altering flower chemistry than florivorous beetles. Although several compounds responded significantly to herbivory regardless of chemotype, the results showed a highly chemotype-dependent response. Such changes in specialised metabolites in flowers may have implications not only for florivores but also for pollinator interactions. Furthermore, these differences in plasticity among chemotypes may lead to high intraspecific variation in plant-insect relationships.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3176 C — Regional Metabolomic Profiling of Onions (Allium cepa) Cultivated in South Korea: Chemical Diversity and Potential for Geographical Differentiation

PRESENTING AUTHOR: *Hyemi Jang, Korea Basic Science Institute, South Korea*
CO-AUTHORS: *Mira Choi, Yeon-Sik Bong, Kyoung-Soon Jang*
SUB THEME: *Plant metabolomics*

Onions (Allium cepa) exhibit diverse chemical compositions depending on cultivation conditions, yet comprehensive metabolomic studies on regionally grown onions in Korea remain limited. This study applied untargeted metabolomic analysis using UPLC-TIMS-QTOF MS to investigate the chemical diversity of ethanol extracts from onions collected nationwide. A total of 2,339 and 1,350 molecular features were detected in positive and negative ion modes, respectively. Among these, spectral library matching annotated 243 compounds (<10%), providing limited insights into chemical classification. Based on the annotated compounds, classification revealed that lipids and lipid-like molecules (37.0%) were the most abundant, followed by phenylpropanoids/polyketides (15.2%) and organic acids/derivatives (14.8%). To enhance the understanding of regional differences, accurate mass-based molecular formula determination identified 2,055 molecular formulas, enabling broader metabolic characterization. Using these molecular formulas, unsupervised clustering of metabolite profiles distinguished two major regions: the Central-Southwestern (CSW) region (Chungcheongnam-do, Jeolla-do) and the Southeastern (SE) region (Gyeongsangnam-do, Busan, Ulsan). Key molecular indices (H/C, O/C, DBE, AI) indicated that CSW onions had a higher H/C ratio, reflecting a predominance of aliphatic compounds, whereas SE onions exhibited a lower H/C ratio, suggesting a greater abundance of aromatic structures. Metabolic network analysis further demonstrated distinct geographical differentiation, with CSW onions enriched in lipid-like molecules, including linoleic acid derivatives with antioxidant properties, while SE onions contained more phenylpropanoids and polyketides, such as flavonoids with anti-hyperglycemic effects. This study identifies region-specific metabolic signatures in Korean onions by integrating spectral analysis and annotation, chemical classification of annotated compounds, molecular formula-based chemical property analysis, and clustering. These findings provide valuable insights into their chemical diversity, geographical differentiation, and nutritional and pharmacological potential, supporting the development of region-specific functional cultivars.

3177 C — Metabolome and Hormone Analysis to Elucidate Sweet Potato (Ipomoea batatas L.) Root Responses to Chilling Temperature

PRESENTING AUTHOR: *Sangjung Kim, Seoul National University, South Korea*
CO-AUTHORS: *Joonggon Kim, Eun Jin Lee*
SUB THEME: *Plant metabolomics*

Sweet potatoes (Ipomoea batatas L.) are subtropical crops highly sensitive to chilling temperatures. Exposure to low temperatures can cause chilling injury (CI), such as internal browning, significantly reducing their commercial value. This study investigates the metabolic and molecular mechanisms underlying the chilling response in sweet potato roots by analyzing metabolic and hormonal changes. Sweet potato roots (cv. 'Beni Haruka') were stored at 13°C (recommended storage temperature) and 4°C (chilling temperature) for 28 days, and CI symptoms were assessed. Phytohormone quantification was conducted using LC-MS, while RT-qPCR and phenolic compound profiling were performed to examine gene expression and metabolite accumulation. Polar phase metabolites and volatile organic compounds were analyzed using GC-MS. CI symptoms appeared after 21 and 28 days of storage. Abscisic acid (ABA), salicylic acid (SA), and jasmonic acid were detected, whereas other phytohormones were not. ABA content sharply decreased at 4°C due to the upregulation of IbCYP707A1, whereas SA content markedly increased due to the upregulation of IbPAL. Among phenolic compounds, chlorogenic acid and vanillic acid accumulated at 4°C. Additionally, reducing sugars such as glucose and fructose, along with amino acids including leucine and serine, increased under chilling conditions. However, terpene accumulation was not observed at 4°C. These findings suggest that ABA and SA play crucial roles in regulating the chilling response in sweet potato roots through diverse metabolic changes. This research was supported by the Rural Development Administration (RS-2023-00229042) and the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) through the Agriculture and Food Convergence Technologies Program for Research Manpower Development, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (No. RS-2024-00398300).

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3178 C — Metabolite Profiling of Tissue-Specific Herbivore Responses in Capsella bursa-pastoris

PRESENTING AUTHOR: *Yukyung Choi, Sookmyung Women's University, South Korea*
CO-AUTHORS: *Bo Eun Nam, Youngsung Joo, Kyo Bin Kang*
SUB THEME: *Plant metabolomics*

Plant-insect interactions significantly influence phytochemical diversity. Upon insect attack, plants produce tissue-specific specialized metabolites involved in defense. Capsella bursa-pastoris is attacked by various herbivorous insects with distinct organ-specific feeding preferences, including three leaf-chewers (Phyllotreta striolata, Plutella xylostella, and Spodoptera litura), three sap feeders (Lipaphis erysimi, Aphis craccivor, and Myzus persicae), a root-feeder (Phyllotreta striolata), a seed-predator (Ceutorhynchus albosuturalis), and a stem borer (Ceutorhynchus rapae). Untargeted LC-MS/MS-based metabolomics combined with multivariate analyses (PCA, PLS-DA) was used to identify tissue- and treatment-specific metabolic changes in response to herbivory. Significant metabolite features were further annotated using in silico tools (GNPS, ChemWalker, SIRIUS). In leaves, phenylpropanoids levels increased in response to chewers but decreased with sap-feeders, indicating feeding mode-dependent regulation. In roots, glycoside-containing metabolites increased, while fatty amides decreased following root-feeder. In seeds and pericarps, both glucosinolates and isocyanates decreased upon herbivore exposure. In stem, glucosinolates levels decreased while isothiocyanates increased, implying myrosinase-mediated activation. Notably, carbamothioate-class metabolites were specifically induced in herbivory, indicating a highly inducible stem-specific responses. This study demonstrates the utility of metabolomics in uncovering complex, tissue-specific plant responses to insect herbivory.

3179 C — Increasing plant metabolome coverage using Enhanced Dynamic Range (eDR) scan mode on a modified Orbitrap Hybrid mass spectrometer

PRESENTING AUTHOR: *Michał Kaczmarek, Thermo Fisher Scientific, Germany*
CO-AUTHORS: *Christian Klass, Bashar Amer, Catharina Crone, Claire Dauly*
SUB THEME: *Plant metabolomics*

High-confidence detection and identification of low to medium abundant metabolites in complex matrices pose significant challenges in LC-MS-based metabolomics. Segmenting the mass range into narrower windows, known as the BoxCar or spectral stitching approach, has shown to improve signal-to-noise ratios and increase the quality of detection of low-abundance molecules. We advanced these concepts by implementing intelligent MS1 multiplexing strategies with optimized injection times. This resulted in the Enhanced Dynamic Range (eDR) scanning mode on the modified Orbitrap Hybrid MS to increase metabolome coverage.

This is achieved by dividing the mass range into two separate Orbitrap MS1 subscans, each encompassing alternating mass range windows. For each subscan, quadrupole automatically isolates different m/z windows based on the user-defined settings, which are then transferred to Orbitrap in a single injection.

In this study, we analyzed tea extract samples on MS1 level to assess the increase in detected metabolome coverage resulting from eDR, and on MS2 level with DDA AcquireX Deep Scan mode to evaluate its impact on fragment-based annotation. The MS1 analysis, after background subtraction in eDR mode, resulted in more than a threefold increase in detected compounds (CVs ≤ 20%) compared to the legacy full scan. We also noticed an increase in spectral signal-to-noise ratios as well as lower CVs for selected low and medium intensity signals, some of which were not detected in the legacy full scan. Results align with the principle of the eDR scan mode, where the signal-to-noise ratio in windows with low total ion current is enhanced by longer ion injection times. Moreover, when comparing the number of full mzCloud library matches, eDR increased the identification rate by approximately 50%. This improvement is linked to generating a more comprehensive inclusion list, which enabled a thorough MS2 investigation in the Deep Scan mode.

3180 C — Comprehensive Metabolomic Profiling of Cannabis Using Gas Chromatography–High-Resolution Mass Spectrometry

PRESENTING AUTHOR: *Michal Stupák, UCT Prague, Czech Republic*
SUB THEME: *Plant metabolomics*

Cannabis (Cannabis sativa L.) is a chemically diverse and polymorphic plant species that has recently attracted renewed scientific attention, particularly due to its applications in medicine and the pharmaceutical industry. Its complex chemical composition, comprising thousands of bioactive compounds, underlies the plant's unique biological effects. While targeted analyses of major compound classes such as cannabinoids and terpenoids are common, a more holistic approach is required to elucidate the full metabolomic landscape of cannabis. In this study, we developed and optimized a comprehensive method for untargeted metabolomic profiling of cannabis using gas chromatography coupled with high-resolution mass spectrometry (GC–HRMS). Special attention was paid to optimizing the sample preparation, including extraction and derivatization steps. Various derivatizing agents were evaluated to improve the detectability of polar, thermolabile, and high-boiling-point compounds, thereby enhancing overall metabolome coverage. The optimized method was applied to 20 cannabis samples representing different varieties. Both non-derivatized and derivatized extracts were analyzed. The resulting data were processed using chemometric techniques, enabling classification of the samples based on their metabolomic fingerprints. Partial least squares discriminant analysis (PLS-DA) was employed to identify variety-specific marker compounds, which were subsequently annotated or identified. This study demonstrates the potential of GC–HRMS-based metabolomics in the comprehensive chemical characterization and differentiation of cannabis varieties.

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PLANTS, FOOD, ENVIRONMENT AND MICROBES

3181 C — Light intensity alters the contribution of the isocitrate pathway to oxalate accumulation in Rumex obtusifolius L.

PRESENTING AUTHOR: *Atsuko Miyagi, Yamagata University, Japan*
CO-AUTHORS: *Wakana Sakuma, Hideki Murayama*
SUB THEME: *Plant metabolomics*

Oxalate, a simplest dicarboxylate, is mainly synthesized in the leaves of various plant species. In plants, oxalate plays essential roles in defense against predators, detoxification of aluminum ions in acidic soils, regulation of calcium ion concentration in plant cells, and production of hydrogen peroxide during maturation, aging, or wounding. On the other hand, excessive consumption of oxalate-rich crops such as spinach and rice straw causes mineral deficiencies and urinary stone disease in humans and livestock. Therefore, reducing oxalate levels in crops has become an important agricultural issue. Three pathways of oxalate synthesis have been reported in plants. The first pathway is the isocitrate pathway, which is derived from the glyoxylate cycle. The second is the glycolate pathway, which is derived from photorespiration. The third is the ascorbate pathway, in which oxalate is synthesized via multiple reactions after ascorbate oxidation. However, it remains unclear which pathway contributes to the oxalate accumulation. To elucidate the mechanisms of oxalate synthesis in plants, we analyzed oxalate accumulation in the leaves of Rumex obtusifolius L., an oxalate-rich plant of the Polygonaceae family, focusing on light intensity. Metabolomic analysis using CE-MS/MS showed that itaconate treatment, which acts as an inhibitor of isocitrate lyase involved in the isocitrate pathway, resulted in a decrease in oxalate content in leaves grown in the dark, but not in the light. Multivariate analyses based on the metabolite datasets revealed that ascorbate levels tended to be negatively correlated with oxalate accumulation, suggesting that the ascorbate pathway rather than the isocitrate pathway will contribute to oxalate accumulation in the light. Furthermore, in the presence of itaconate, the oxalate content in plants grown under the low light was lower than that in plants grown under normal light. It indicates that the isocitrate pathway probably functions inversely proportional to light intensity.

3182 C — Graph theory algorithms, a novel way of exploring red wine 1H-NMR data.

PRESENTING AUTHOR: *Augusto Nozella, BfR, Germany*
CO-AUTHORS: *Daria Savvateeva, Andrew Davis, Janet Riedl, Carsten Fauhl-Hassek, Fredd Vergara*
SUB THEME: *Viticulture & wine growing*

Wine is a highly appreciated product consumed worldwide. It is a beverage with an extensive aroma and taste diversity, stemming from a wide natural variation in its chemical composition. Nuclear magnetic resonance (NMR) is a powerful analytical technique that enables precise analysis of complex chemical compositions offering both qualitative and quantitative capabilities generating a chemical fingerprint of a wine sample. This technique is often combined with multivariate statistical modelling or chemometrics to process a large amount of data for both classification of samples and detection of adulterations. In metabolomics, traditional algorithms based on Principal Component Analysis (PCA) have been employed for data exploration and investigation of trends. However, these algorithms rely on the assumption that the data is normally distributed to perform the dimension reduction, which, for NMR data is not always the case. The same issue is present in other fields, such as genomics and proteomics where new algorithms based in graph theory such as Uniform Manifold Approximation (UMAP) have been employed with great success. This approach allows to both avoid the previously mentioned assumption, while comparing each point of the dataset individually, which might provide a better representation of the NMR data. At the Research Unit “Product Identity, Supply Chains and Traceability” of the German Federal Institute for Risk Assessment (BfR) research on wine chemical composition has been conducted for several years, resulting in the accumulation of a database with thousands of wine 1H-NMR spectra from samples worldwide. This work aims to present an in-house developed data exploration tool using algorithms that are yet to be applied for the study of NMR data (SPIMP, UMAP and HDBSCAN). And apply to large library of red wines available at BfR with the goal of producing a workflow capable of classifying red wine based on regionality and grape variety.

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TECHNOLOGY ADVANCEMENTS

3200 C — Therapeutic drug monitoring of immunosuppressive drugs after lung transplant guided by breath analysis

PRESENTING AUTHOR: ***Pablo Sinues, University of Basel, Switzerland***
CO-AUTHORS: ***Britta Brugger, Noriane Sievi, Malcolm Kohler, Pablo Sinues***
SUB THEME: ***Breath analysis/Volatolomics***

Introduction:

Therapeutic drug monitoring (TDM) of immunosuppressive drugs is essential after lung transplantation to prevent long-term complications such as acute and chronic rejection, infections, drug toxicity, and malignancies. In 2024, 1,336 lung transplants were performed in Europe. Cyclosporin levels are closely monitored, especially in the initial weeks, due to significant interpatient variability and its long elimination half-life.

Currently, TDM relies on invasive techniques. A non-invasive, faster, and more comfortable method, such as exhaled breath analysis, could enhance patient well-being. Future use of an off-line bag method could decentralize measurements to local hospitals or primary care physicians.

Methods:

We conducted a longitudinal study with 38 Cyclosporine-treated patients at the University Hospital Zurich (2022-2023). Patients underwent exhaled breath measurements and blood sampling before and after drug intake to assess trough levels. A total of N = 70 paired breath-blood samples were collected. Breath analysis was performed using SESI-HRMS coupled to a Q-exactive Orbitrap MS. Relevant features were extracted and various feature selection methods applied to identify the most informative features. Different regression models were tested for predictive accuracy, evaluated using Lin's Concordance Correlation Coefficient (CCC).

Results:

A subset of 15 mass spectral features predicted Cyclosporine levels with a CCC of 0.3.

Conclusion:

Breath analysis for TDM of immunosuppressants is feasible, offering a non-invasive method to monitor systemic drug levels. Further development could extend its use beyond transplant hospitals, improving clinical practice.

3201 C — 13C isotope tracer metabolomics lights up the metabolic activity of a quiescent fission yeast mutant

PRESENTING AUTHOR: ***Teo Hebra, IOCB Prague, Czech Republic***
CO-AUTHORS: ***Cecilia Berges, Floriant Bellvert, Tomas Pluskal***
SUB THEME: ***Flux analysis***

Two-stage processes may become the gold standard for fermentation-based bioproduction of goods and chemicals. However, designing a microbial chassis that uncouples its growth and metabolite production is the current bottleneck. We uncovered a fission yeast—Schizosaccharomyces pombe—mutant that arrests growth and proliferation under nutrient abundance by simply increasing the cultivation temperature from 30°C to 36°C.

Our preliminary experiments with this S. pombe strain indicated that the quiescent state can be induced and maintained at any cell density and that the full cell population can stay alive for at least 21 days, if provided with fresh nutrients. Moreover, S. pombe strains engineered to produce specific specialized metabolites (i.e. 6-methylxanthine and 6-methylsalicylic acid) were actively producing the compounds of interest even after 15 days under quiescence. However, the metabolic activity of the mutant under quiescence remained unclear. We decided to pursue ^{13}C isotope tracer experiments to determine to what extent the quiescent cells were metabolically active—were they recycling macromolecules or incorporating fresh nutrients to sustain the biosynthesis?

We conducted a long-term quiescence experiment and changed the medium for fully labeled ^{13}C glucose either after 3 days of cultivation or after 10 days of quiescence. Then, we investigated the consumption and propagation of the isotope tracer within central metabolism, protein, RNA, and 6-methylsalicylic acid from a genetically modified strain using ion and liquid chromatography targeted mass spectrometry and NMR metabolomics. Our results indicate that the quiescent cells fully consume the glucose from their medium in less than 3 days and that they synthesize de novo RNA, proteins, and 6-methylsalicylic acid. We are now investigating the efficiency of the S. pombe quiescent cells in terms of production of gram of 6-methylsalicylic acid per gram of glucose to propose our quiescent strain as a potential chassis for industrial biocatalysis.

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TECHNOLOGY ADVANCEMENTS

3202 C — Investigating Cellular Carbon and Nitrogen Co-Metabolism on a modified Orbitrap Hybrid Mass Spectrometer by the simultaneous use of differently labeled elemental isotope substrates

PRESENTING AUTHOR: *Claire Dauly, Thermo Fisher Scientific, Poland*

CO-AUTHORS: *Rahul Ravi Deshpande, Ayush Midha, Bashar Amer, Isha Jain, Susan Bird*

SUB THEME: *Flux analysis*

Targeted approaches have been used extensively for isotopologue determination in fluxomics. However, these methods either lack the sensitivity to detect low abundant metabolites or the resolution for dual tracer experiments (for e.g., using 13C and 15N). Here we evaluate a modified Orbitrap Hybrid mass spectrometer (MS) with extended sensitivity and enhanced dynamic range for these applications.

HEK cells were grown under different oxygen tensions (50%, 21% and 1.5%). Cells were unlabeled or labeled with 13C6 Glucose, 15N2 Glutamine or both 13C6 Glucose and 15N2 Glutamine. Metabolite separation was carried out on a Mixed Mode HILIC (HILIC-AEX) column and a nano-flow ion pairing chromatography.

Initial experiments were done using 12C and U-13C labeled E.Coli cell extracts. The use of mass resolution above 120K allows one to differentiate between the 15N and 13C isotopologues. The instrument was operated using both the normal as well as using the enhanced dynamic range mode on the instrument. The data quality, reliability, and robustness of measurements were evaluated through selected endogenous metabolites, using metrics such as resolution, mass accuracy, signal response and isotopic fidelity. Significantly reduced un-intentional MS1 fragmentation of the compounds was observed. Utilizing the enhanced dynamic range mode enabled the detection of low abundant compounds and increased compound annotation by more than 20%. The optimized parameters were used for data acquisition of the HEK cell metabolite extracts. The use of nano-flow increases the sensitivity of detected compounds by one to three orders of magnitude depending on the metabolite. It also achieves a significant reduction in the use of solvent and other resources. The stable isotope workflow of Compound Discoverer was used for data processing. Differences in the nitrogen and carbon labeling of the metabolites in three different oxygen tension were quantified and correlated to metabolic pathways.

3203 C — High Throughput Profiling of NPH-Derivatized Central Carbon Metabolites Using prm-PASEF

PRESENTING AUTHOR: *Sofie Weinkouff, Bruker Daltonics, Germany*

CO-AUTHORS: *Matthias Anagho-Mattanovich, Ondrej Hodek, Nikolas Kessler, Sofie Weinkouff, Aiko Barsch, Cristian De Gobba, Matthew R. Lewis, Thomas Moritz*

SUB THEME: *High-throughput metabolomics*

Central carbon metabolism (CCM) is a key metabolic pathway, focusing on carboxylic acids. Derivatization with 3-nitrophenylhydrazine facilitates separation in reversed-phase liquid chromatography (RPLC) and electrospray ionization (ESI). Rapid analytical methods are essential for large-scale biological studies, but similar masses and structures of metabolites complicate analysis. Recent advancements in trapped ion mobility (TIMS) combined with high resolution mass spectrometry (HRMS) aim to accelerate analysis and enhance selectivity. We developed a high throughput method combining fast RPLC with parallel-reaction-monitoring, parallel accumulation serial fragmentation (prm-PASEF) for two-minute analysis of CCM carboxylic acids.

Plasma/serum, fecal, and brown adipocyte samples were extracted with 90% methanol containing stable isotope labelled carboxylic acids. Dried extracts and calibrants were reconstituted in 50% methanol and derivatized with EDC and 3-NPH. After 60 minutes of room temperature incubation, BHT was added. RPLC analysis was performed using an Agilent 1290 II UHPLC coupled to a Bruker timsTOF Pro2 with a two-minute gradient separation on a Bruker Bio-AQ high throughput column. The MS operated in either 4D-MS1 or prm-PASEF mode, enabling metabolite targeting by ion mobility and fragmentation.

LC-TIMS-PASEF analysis was initially conducted on 3-NPH-derivatized standard mixtures. Data were processed using MetaboScape's untargeted feature finding and annotated with its in-silico derivatization algorithm. Identified derivatized analytes were used to set prm-PASEF parameters. The methodology was tested on various sample types, showing high quantitative reproducibility (CV % 8-23) and CCS values (<0.2-4%) in 96 replicate serum samples. The method was also applied to murine brown adipocytes in response to thermogenic lipolysis induction. Results indicated that fast chromatography combined with trapped ion mobility produced data comparable to conventional LC-MS analysis. The method allows routine quantification of over 15 CCM compounds with a throughput of 300-400 samples per 24 hours. Automation of sample preparation and data preprocessing is the next challenge to avoid workflow bottlenecks.

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TECHNOLOGY ADVANCEMENTS

3204 C — Investigating Metabolite Detectability in Mouse Organs Using MALDI Imaging Mass Spectrometry

PRESENTING AUTHOR: *Veronika Saharuka, DKFZ, Germany*
CO-AUTHORS: *Gernot Poschet, Carten Hopf, Christiane Opitz*
SUB THEME: *Imaging MS*

Spatial metabolomics using MALDI imaging mass spectrometry (MS) enables untargeted, label-free analysis of small molecule distributions in tissue. However, designing experiments for untargeted detection of small metabolites remains challenging due to limited empirical data on detectability across protocols, particularly concerning MALDI matrix selection.

We recently systematically evaluate spatial metabolomics protocols, testing 12 MALDI matrices in both polarities using arrays of 172 pure metabolite standards on glass slides.[1] Here, we assess how these reference standard data translate to tissue-based spatial metabolomics. Selected protocols were applied to mouse spleen and brain — organs with defined histology and metabolic function — and analysed using a QTOF-based MALDI imaging system with a custom data pipeline. To broaden the comparison, we also examined detectability patterns across over 4,700 publicly available animal imaging datasets, spanning diverse tissues, instruments, and protocols.

We focused on compound classes including amino acids, carbohydrates, nucleotides, vitamins, cofactors, and their derivatives. Initial comparisons show agreement in detectability trends between reference and tissue data, including broader consistency across public datasets. This study links controlled benchmarking with in situ biological analysis and provides a basis for more informed protocol selection in spatial metabolomics.

[1] Saharuka et. al. bioRxiv doi:10.1101/2024.01.29.577354

3205 C — MALDI-MS imaging via DDTC chelation for assessing metallodrug penetration: A case study of oxaliplatin by peritoneal aerosol chemotherapy

PRESENTING AUTHOR: *Jiyeon Kim, Chung-ang University, South Korea*
CO-AUTHORS: *Hyung-Ho Kim, Dong-Kyu Lee*
SUB THEME: *Imaging MS*

The treatment of peritoneal carcinomatosis (PC) involves platinum-based chemotherapeutic reagents such as oxaliplatin, which are administered via aerosol chemotherapy systems to enhance drug delivery to tumor cells. In recent years, with the advent of novel drug administration techniques, there has been an increasing need to establish methods for evaluating the extent of drug penetration into the peritoneum. Specifically, we employed a matrix-assisted laser desorption ionization (MALDI)-mass spectrometry imaging method based on diethyldithiocarbamate (DDTC) chelation to validate the selective dosing mode in the peritoneum and enhance the sensitivity of the metallodrug. 1% DDTC in water (pH 7) produced $Pt(DDTC)_3$ (M.W. 639) and $Pt(DDTC)_2$ (M.W. 491), achieving a 100 times greater sensitivity than unchelated oxaliplatin. The most stable form, $Pt(DDTC)_3$, exhibited the highest signal intensity with matrix solution consisted of α-cyano-4-hydroxycinnamic acid (CHCA) in 90:10 (v/v) acetonitrile: 0.1% trifluoroacetic acid. The newly proposed method was applied to the peritoneal quadrants of pigs treated with oxaliplatin via intravenous and aerosol methods. The findings indicated that the pharmaceutical agent exhibited enhanced selective delivery to the peritoneum when administered via aerosol compared to intravenous delivery. These findings can be applied to other platinum or metal drugs, thereby expanding the scope of drug permeability analysis. This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT*) (RS-2023-00212534).

3206 C — Development of a semi-quantitative targeted DESI-QqQ approach to visualize analyte distribution in biological samples.

PRESENTING AUTHOR: *Camille Dupuy, Inserm Dr Grand Ouest, France*
CO-AUTHORS: *Antoine Lefèvre, Sylvie Bodard, Jérémy Monteiro, Adeline Oury, Lydie Nadal-Desbarats, Patrick Emond, Laurent Galineau*
SUB THEME: *Imaging MS*

Mass spectrometry imaging (MSI) is an analytical technique that provides spatial molecule distribution information of a sample. The combination of a DESI (Desorption ElectroSpray Ionization) source with a triple-quadrupole mass spectrometer (QqQ) is a recent improvement, enabling targeted metabolite imaging using Multiple Reaction Monitoring (MRM) transitions to reach higher sensitivity. However, the lack of TIC disables signal normalization and so, the comparison between section analysis as well as the availability to follow a dysregulation over time.

Our objective is to develop a semi-quantitative DESI-QqQ imaging method to visualize the distribution of a set of molecules. In order to compare the sections, we added a solution of internal standards at known concentrations on the tissue section by spraying it before ionization (DESI). This approach allows the calculation of intensity ratios between the targeted metabolites and their corresponding internal standards, enabling comparative analysis across different tissue sections or experimental conditions.

The proof-of-concept application of this approach to rat brain sections is a first step toward robust relative quantification of targeted metabolites by DESI-MSI, with promising perspectives for comparative and longitudinal studies.

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TECHNOLOGY ADVANCEMENTS

3207 C — How to increase the reliability of ultra-high performance liquid chromatography analysis of small molecules

PRESENTING AUTHOR: ***Elnur Garayev, Aix-Marseille University/IMBE, France***
CO-AUTHORS: ***Maximilien Fil, Célia Breaud, Béatrice Baghdikian, Sandrine Alibert, Eldar Garaev, Sok-Siya Bun-Llopet, Elnur Garayev***
SUB THEME: ***New developments in instrumentation***

In recent years, liquid chromatography techniques, particularly high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC), have become essential analytical tools in a wide range of fields, including pharmaceuticals, environmental sciences, food safety, and natural product discovery. These techniques offer high resolution, reproducibility, and sensitivity, making them ideal for separating and/or analyzing complex mixtures.

The main challenges in HPLC analysis are problems related to the apparatus or chromatography columns, such as distorted peak shape or micro-leaks, and the variation of retention time. Retention time variation can be instrument and column dependent and can also appear over time or during analysis realized with a long-time interval.

In this research work we present a standardized mix of compounds that can replace the retention time parameter by retention index and align retention times for time-shifted analysis. The standardized mix allows the complete coverage of the chromatogram on reverse phase column with consistent peak elution. The developed in-house python script allows the alignment of time-shifted analysis.

3208 C — On global profiling accuracy and the effect of instrument sensitivity

PRESENTING AUTHOR: ***Matthew Lewis, Bruker Life Sciences Mass Spectrometry, United Kingdom***
CO-AUTHORS: ***Aiko Barsch, Sven Meyer, Claudia Martelli, Cristian De Gobba, Nikolas Kessler, Matthew Lewis***
SUB THEME: ***New developments in instrumentation***

The observed quantitation of a given analyte introduced for mass spectrometry via electrospray ionization (ESI) is commonly influenced (suppressed or enhanced) by the simultaneous presence of other, potentially biologically unrelated, analytes. This so-called “matrix effect” may be measured and corrected in targeted analyses using isotopically labelled internal standards. However, application of the same principles to discovery-oriented global profiling studies is impractical owing to both the broad scope of analytes measured and a lack of a priori knowledge of their identity. Consequently, the accuracy of metabolite measurement is easily compromised. Inaccurate measurement of the metabolic profiles across samples can confound statistical analysis, obscuring (or erroneously creating) biomarker signatures depending on how random or correlated to the study design the effects are. In global profiling studies, conventional assessment of data quality is focused on the precision of replicated measurement in an unchanging matrix (i.e. a “pooled QC” sample), and therefore is not able to see or account for matrix effect, representing a substantial underappreciated risk in the metabolomics workflow. Here, greater urine sample dilution was associated with reduced matrix effect (most commonly, with reduced ionization suppression), indicating that measurement accuracy is proportional to sample dilution. The extent of the observed effect was dependent on the local matrix environment (based on LC co-elution), with the most heavily suppressed areas of the chromatogram being the early column breakthrough. However, working at high sample dilution also has a negative effect on profile depth and richness (amount of the observed metabolome) that can be compensated by increasing instrument sensitivity. In this manner, mass spectrometry sensitivity is translated directly into profile accuracy, thereby enhancing global profiling studies and enabling better biomarker discovery.

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TECHNOLOGY ADVANCEMENTS

3209 C — Evaluating the performance of a dielectric barrier discharge ion source for lipidomics: A comparison with ESI in LC-MS

PRESENTING AUTHOR: *Víctor González-Ruiz, Centre for Metabolomics and Bioanalysis (CEMBIO), Spain*

CO-AUTHORS: *Luz Alonso-Dasques, Isabel Fernández-Prades, Coral Barbas, Miguel Fernández-García, Víctor González-Ruiz*

SUB THEME: *New developments in instrumentation*

Electrospray ionization (ESI) remains the dominant ionization technique in lipidomics due to its high sensitivity and versatility. However, its coverage is limited in low-polarity analytes and its propensity to form adducts and multimers can obfuscate data analysis. By using a different ionization mechanism, the SICRIT (Soft Ionization by Chemical Reaction in Transfer) dielectric barrier discharge ion source becomes an alternative to ESI (1). A comprehensive assessment of its performance is necessary to determine its potential advantages for such applications, particularly in complex biological matrices.

In this study, we conducted a systematic comparison of SICRIT to ESI on an LC-MS QToF platform (Agilent 6545), focusing on lipidomic analyses of biological samples (blood plasma, seminal plasma, and bacterial lipid extracts). The ionization sources were evaluated based on their production of adducts containing lipid monomers and multimers, with adduct intensities relativized against the base peak to determine semiquantitative differences in ion formation and assess their impact on metabolite annotation.

Our results show that SICRIT generates a larger number of monomer-containing ions, –including novel adducts– but significantly fewer multimer-containing ones compared to ESI. When normalizing ion signals, we found that multimer-containing adducts were 2–3 times less abundant in SICRIT than in ESI. These findings suggest that SICRIT has a characteristic ion production profile which reduces complex, multimer-related ion formations. However, on the other side, additional adduct species can be present in the ionization process. Further investigations are warranted to explore its broader applicability, particularly in terms of overall and low-polarity metabolite coverage.

Acknowledgements

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References:

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3210 C — Live Cell FTIR for Drug Discovery and Development through metabolomics

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CO-AUTHORS: *Andrew Chan*

SUB THEME: *New developments in instrumentation*

Drug discovery and development process is a high-cost, high-risk activity. Current state-of-the-art in in-silico modelling can screen thousands of compounds for their potential activities at the drug-target but the quality of hit remains varied with ~95% of them eliminated in the hit to lead selection stage. The lack of consideration of cell membrane permeation in in-silico screening is one of the main reasons for the high attrition rate. Moreover, alternative to the in-silico screening - conventional in vitro study, often involves the destruction of the cells and it is a costly and laborious process which cannot monitor changes as a function of time. On other hand, metabolomics can be a powerful tool for drug discovery but requires sophisticated instruments with high running cost. Recently, we have developed a novel, non-destructive, label-free, sensitive and low-cost live-cell Fourier-transform infrared (FTIR) approach that can directly measure biochemical compositional changes within living cells in situ with highly specific spectral changes to the drug applied. In this work, we have developed a drug screening approach where different permeable and non-permeable drugs were tested to living cells considering their IC50 level. The changes to their spectra after the addition of drugs were monitored at different time points. Chemometric analysis of the spectral data shows that drugs with different cell permeabilities are clearly identified. In conclusion, live-cell FTIR has shown to be able to distinguish and group spectral signatures of cells treated with drugs according to their permeabilities. Further collection of live-cell data would enable the machine-learning algorithm to be developed for the prediction of the permeability of novel drugs for drug screening.

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TECHNOLOGY ADVANCEMENTS

3211 C — Comprehensive data acquisition workflow on a Modified Orbitrap Astral MS to achieve deep lipidome coverage with high confidence annotations

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CO-AUTHORS: *Rahul Deshpande, Giorgis Isaac, Alan Baik, Robitaille Aaron, Ralf Tautenhahn, Bashar Amer, Thomas Moehring, Isha Jain, Susan Bird*
SUB THEME: *New developments in instrumentation*

Lipids play a crucial role in cellular processes and disease mechanisms. The use of high-resolution mass spectrometry (HRMS) is essential for lipid identification and characterisation. High-confidence lipid annotation and identification requires both high-resolution MS1 and comprehensive MS2 fragmentation data for structural elucidation and isomer differentiation. Having fragmentation for most of the lipids detected in a sample hence is highly desirable. This requires fast and sensitive instrumentation. Obtaining extensive MS2 coverage within a single LC-MS run remains challenging due to scan speed and sensitivity limitations. Here, we evaluate a modified Orbitrap Astral mass spectrometer featuring two high-resolution detectors, a scanning rate exceeding 200Hz, and a high-sensitivity detector to enhance lipid annotation.

Lipids extracted from plasma samples of mice fed a high-fat or normal diet were analysed using a C30 column at analytical flow rates. The data quality, reliability, and robustness of measurements on the new instrument were evaluated through selected spiked standards (Avanti Lipid Mix) and endogenous lipids, using metrics such as resolution, mass accuracy, signal response and isotopic fidelity. MS1 data was acquired at 120K resolution, while MS2 fragmentation occurred at top-speed with a 0.6-second cycle time, generating ~250,000 MS2 events per run. This led to >90% fragmentation of detected compounds, significantly improving lipid identification. High-quality MS2 spectral data was obtained for low-abundance lipids, facilitating confident annotation.

Comparative analyses demonstrated a >10% increase in lipid identification compared to the original Orbitrap Astral mass spectrometer and >50% improvement over traditional MS platforms. Additionally, nano-flow LC increased sensitivity by 1- to 3-fold, improving low-abundance lipid detection further. The workflow enabled differentiation of lipid profiles between dietary groups, with notable changes observed in triglycerides and cholesterol esters. The combination of high-speed scanning and enhanced MS2 fragmentation provides a robust platform for lipidomics, supporting metabolic and disease research.

3212 C — Towards Optimized Multiomics Sample Preparation: Comparison of Extraction Strategies

PRESENTING AUTHOR: *Tilman Arnst, Saarland University, Germany*
CO-AUTHORS: *Selina Hemmer, Claudia Fecher-Trost, Markus R. Meyer*
SUB THEME: *New multi-omics workflows*

Multi-omics approaches seek to provide a comprehensive understanding of complex biological systems by exploring the interconnectedness of various biological processes. Achieving this aim requires the generation of multiple omics datasets, ideally from the same sample within a single experiment, to account for inherent biological variability and ensure consistency across different omics layers. This is crucial for effective downstream data analysis. While several workflows have been proposed, it is essential to carefully (re)evaluate and potentially modify them based on the specific experimental conditions and instrumental setups involved.

This study aimed to compare and evaluate two established sample preparation procedures for the direct and simultaneous extraction of metabolites, lipids, and proteins from plated HepG2 cells, considering the specific analytical setup. For the extraction of polar and non-polar small molecules, a fractionated biphasic extraction using methyl-tert-butylether:methanol:water (10:3:2.5) was compared to a monophasic extraction using n-butanol:acetonitrile:water (3:1:1). In the biphasic method, proteins from an interphase pellet were resolubilized using urea, followed by an overnight tryptic digestion. In contrast, the monophasic extraction employed a paramagnetic bead-based procedure, which was further investigated in terms of beads sizes and digestion conditions.

Untargeted analysis of proteins and non-polar small molecules was performed using reverse-phase nanoflow LC-IMS-HRMS/MS, employing data-independent acquisition for proteins and data-dependent acquisition for non-polar small molecules. Polar small molecules were analyzed through HILIC chromatography coupled to data-dependent Orbitrap-based mass spectrometry. Both sample preparation techniques were systematically evaluated based on total feature count, number of identified biomolecules, and reproducibility of selected features.

The biphasic workflow yielded better outcomes regarding the number of proteins and non-polar small molecules. In contrast, polar small molecules were extracted in higher quantity and with lower variability using the monophasic method. Additionally, the latter provided significant advantages in ease of handling and time efficiency, making it the preferred method for future experiments.

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TECHNOLOGY ADVANCEMENTS

3213 C — Metabolomic Insights into Honey Bees: How Season, Caste, and Body Region Define Biochemical Profiles

PRESENTING AUTHOR: *Saetbyeol Lee, Czech University of Life Sciences Prague, Czech Republic*
CO-AUTHORS: *Jaroslav Havlik, Martin Kamler, Dalibor Titera*
SUB THEME: *NMR metabolomics*

As eusocial insects, honey bees (Apis mellifera) exhibit a lifespan that varies by season of emergence and caste. Summer worker bees live approximately 30 days, whereas winter bees can survive up to 300 days, similar to queens. Despite their critical role in global food security, honey bee metabolic adaptations to environmental and physiological factors remain underexplored. NMR-based metabolomics provides a powerful tool for investigating biochemical variations across seasons, castes, and anatomical regions.

This study employed ^{1}H NMR spectroscopy to analyze seasonal metabolic shifts, caste-specific metabolites, and body region-specific metabolic fingerprints. A total of 330 worker bee bodies were analyzed over one year to assess seasonal trends, while 40 workers (20 nurses, 20 foragers) and 15 queens were examined for caste- and body part-specific metabolomics (head, thorax, abdomen, gut, hemolymph). Samples were extracted in methanol and analyzed using a Bruker Avance III 500 MHz spectrometer, identifying approximately 70 metabolites via Chenomx NMR Suite 9.0.

Seasonal analysis revealed a 13-fold increase in S-Methyl-L-cysteine sulfoxide (SMCSO) in summer bees, likely derived from rapeseed nectar and pollen, along with a notable decrease in essential amino acids compared to autumn and winter season (Kruskal-Wallis test, $q < 0.05$). Hierarchical clustering analysis revealed clear metabolite distribution patterns across body parts and castes, supporting their functional specialization. In the caste comparison, workers and queens exhibited high 10-hydroxy-2-decenoic acid (a royal jelly acid) in their heads, while workers had elevated 3-hydroxykynurenine, and queens had higher (2E)-9-oxodecenoic acid (a queen pheromone). Among workers, nurses' heads contained significantly more glucose and fructose than foragers' heads (Kruskal-Wallis test, $q < 0.05$).

These findings provide novel insights into honey bee metabolic changes, indicating various physiological states, emphasizing the potential of NMR metabolomics in pollinator research.

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3214 C — The challenge of a multi-year NMR metabolomics project – The case of 1H-NMR wine spectra classification and quantification

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SUB THEME: *NMR metabolomics*

Wine is a valuable commodity with an annual world trade valued in billions of USD; quality, authenticity, and safety are therefore of utmost importance for buyers and sellers along the entire supply chain. Nuclear magnetic resonance (NMR) spectroscopy excels at qualitatively and quantitatively characterizing the complexity of wine chemical composition enabling identification and classification, measurement of compound concentrations, as well as detecting contaminants and ensuring authenticity. As such, private and public research institutions, including governmental regulators, have adopted NMR as an important tool for the surveillance of wine.

Here, we report a systematic, multi-year study on wine authentication using 1H-NMR. The main goal was the acquisition and processing of NMR spectra from thousands of wines with diverse botanical and geographical origins as well as vintages, specifically for: (i) non-targeted, multivariate statistical descriptions of wine chemical diversity, and (ii) targeted compound quantification. However, to assure comparability among spectra acquired over such a long period, a single, multi-compound reference was regularly measured. In addition to serving as a normalization spectra for comparative analysis and quantification, this periodic measurement allows the monitoring of the spectrometer performance over time.

3215 C — Comparative metabolomics study on 30 East Asian traditional herbal medicines using ^{1}H NMR

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CO-AUTHORS: *Thi Huong Pham, Kyo Bin Kang*
SUB THEME: *NMR metabolomics*

East Asian traditional medicines are composed of multiple herbs, each potentially containing hundreds of bioactive metabolites with expected synergistic effects. The variations in the combinations of different herbs further lead to challenges in ensuring consistent quality. Although chromatographic methods are widely used, advanced techniques such as NMR, which enables quantitative analysis with minimal sample preparation, offer improved reproducibility despite their sensitivity limitations. In this study, we investigate the potential of 1H NMR spectroscopy for quality assessment by analyzing 30 traditional medicine formulations. Batch-to-batch and formulation-to-formulation consistency were evaluated using Pearson coefficient analysis. While batch-to-batch comparisons exhibited strong correlations, formulation-to-formulation consistency was influenced by saccharide abundance. Additionally, we identified three key specialized metabolites—paeoniflorin, naringin, and baicalin—by targeting specific signals linked to herbal compositions. This approach enhances formulation consistency and offers valuable insights into metabolite variability, contributing to improved quality control strategies for complex herbal formulations.

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TECHNOLOGY ADVANCEMENTS

3216 C — Parameters Optimization for Molecular Networking-Based Untargeted Profiling of Neutral Lipids and Phospholipids

PRESENTING AUTHOR: *Subin Bae, Kyungpook National University, South Korea*

CO-AUTHORS: *Kwang-Hyeon Liu*

SUB THEME: *Non-targeted and semi-targeted methods*

Lipidomics enables the systematic analysis of lipid molecules to elucidate their structures, functions, and metabolism, playing a crucial role in biomarker discovery for predicting metabolic diseases and drug responses through targeted approaches. However, untargeted lipidomics is essential for the comprehensive characterization of the entire lipidome. To address this need, we developed a molecular networking-based untargeted lipidomics approach to enhance lipid identification. In this study, we optimized mass spectrometry and Global Natural Products Social Molecular Networking (GNPS) conditions to construct molecular networking for neutral lipids and phospholipids. Standard mixtures of neutral lipids and phospholipids were used for optimization, and applicability to biological samples was validated using NIST SRM 1950. Samples were analyzed in data-dependent acquisition (DDA) mode using a Q-Exactive (QE) Orbitrap mass spectrometer. Our results demonstrated that for neutral lipids, clustering was observed at normalized collision energy (NCE) 30 and stepped NCE, with a cosine score of 0.7 and a minimum of six matched fragment ions. Further analysis across six scan ranges using NIST SRM 1950 confirmed that more neutral lipids were detected at NCE 30. For phospholipids, clustering was consistent across various NCE values, with a cosine score of 0.7 and a minimum of six matched fragment ions. These findings provide valuable insights into optimizing molecular networking-based lipidomics workflows. Future studies will refine collision energy selection using NIST SRM 1950 to identify a more suitable collision energy value for forming molecular networking of phospholipids, aiming to further enhance the robustness and reproducibility of untargeted lipidomics. This approach has the potential to improve lipid identification strategies, advancing biomarker discovery and expanding the scope of lipidomics research.

3217 C — SQUAD metabolomics: uniting targeted precision and untargeted discovery for in-depth fecal metabolite profiling on Orbitrap Astral MS

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SUB THEME: *Non-targeted and semi-targeted methods*

Introduction

Untargeted metabolomics often struggles with accurate metabolite identification and quantification due to the lack of internal standards and comprehensive libraries. This limitation can hinder study design and data interpretation, leading researchers to focus on a small subset of analytes, potentially overlooking biologically relevant compounds.

To address this, the SQUAD (Single-injection Simultaneous Quantitation and Discovery) workflow combines accurate targeted quantitation with untargeted allowing the discovery of biologically unknown compounds.

The faster MS2 scanning of the high-resolution MS Orbitrap Astral™ enables sensitive quantitation of numerous metabolites through Astral PRM, simultaneously, ensuring robust unknown annotation and comprehensive sample coverage.

Methods

We utilized a Fecal Metabolites Library (MetaSci) to develop a library and analyzed fecal samples from mice exposed to different oxygen levels (21% or 8% FiO_2) and fed diets varying in fat composition (standard (soybean oil), SFA-rich (+ cocoa butter), and MUFA-rich (+olive oil). After 29 days, samples were extracted using 80% methanol, separated by RP and HILIC chromatography, and analyzed using Thermo Scientific™ Orbitrap Astral™.

Preliminary Data

The SQUAD workflow provided reliable quantitation of a broad range of targeted components in mice feces with heightened sensitivity. Calibration curves for xanthosine showed precise quantitation across a dynamic range of four orders with LLOQ of 2.85 femtomoles and LLOD of 0.71 femtomoles on column.

The workflow achieves over 80 % of compound fragmentation allowing confident annotation of unknown low-abundance metabolites in complex matrix.

The instrument showed excellent performance, delivering high reliability, robustness, low %CV across replicates, and consistent sub-ppm mass accuracy throughout the analysis.

This study enabled the evaluation of metabolic profile variations, helping to better understand how diet and oxygen exposure influence metabolic responses in the mice model.

Novel Aspect

The SQUAD workflow combines targeted quantitation with extensive untargeted coverage for confident annotation of fecal metabolites using the Orbitrap Astral™ MS.

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TECHNOLOGY ADVANCEMENTS

3218 C — Comparison of different reversed-phase liquid chromatography stationary phases for untargeted and semi-targeted metabolomics assays

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SUB THEME: *Non-targeted and semi-targeted methods*

Introduction: Reversed-phase is the most frequently applied stationary phase in liquid chromatography-mass spectrometry assays used in metabolomics studies to investigate water-soluble metabolites. C18 stationary phases dominate in reported metabolomic studies. However, other potentially complementary reversed phase stationary phases are available (e.g. phenyl, biphenyl and PFP) and could provide complementary or improved chromatographic resolution and separation. The research presented compares different reversed-phase stationary phases and assess similarities and differences between reversed-phase and HILIC stationary phases for water-soluble metabolites.

Methods: Ten-component mixtures of metabolite standards (>800 metabolites) as well as mammalian biological samples were analysed applying four different stationary phases (C18, biphenyl, PFP and HILIC-amide) using a Vanquish UHPLC system coupled to an electrospray Exploris 240 mass spectrometer operating in positive and negative ion modes. Raw data processing was performed using Compound Discoverer v3.3. Chromatographic resolution and selectivity were assessed to compare the four stationary phases.

Results and conclusion: All four stationary phases provided some complementarity in either/and elution series, retention times or chromatographic resolution. No single stationary phase retained all metabolites. The choice of stationary phase should be based on the classes of metabolites to be investigated.

3219 C — Three-phase extraction for enhancement of lipidomic analysis

PRESENTING AUTHOR: *Vladimír Vrkoslav, IOCB Prague, Czech Republic*
CO-AUTHORS: *Mária Mellová, Štěpán Strnad, Jiří Šantrůček, Josef Cvačka*
SUB THEME: *Novel approaches in lipidomics*

Due to the large diversity of lipids in different biological materials, extraction is a critical step in the sample preparation process. Currently used two-phase systems extract all lipids in one phase, which often leads to a loss of the number of annotated lipid species. We optimized and evaluated a three-phase extraction system. The optimized three-phase extraction system was compared with the commonly used two-phase methyl-tert-butyl ether (MTBE) extraction. Untargeted lipidomics was performed using LC-MS (UltiMate 3000 UHPLC coupled with an Orbitrap Fusion Lumos Tribrid mass spectrometer) with heated electrospray ionization. The Waters Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm) was operated at 45 °C with a flow rate of 180 µL/min.

A total of 72 different solvent mixtures were tested to create three-phase systems. Suitable three-phase systems were formed from 21 mixtures. The ability of 12 of these mixtures to separate lipid standards was investigated in detail using a commercially available mixture of lipid standards. Lipidomic analysis showed how selected three-phase systems separate lipid classes into individual phases of the extraction system. Finally, two solvent systems were selected for the lipidomic analysis of blood plasma. The extraction systems IO:MTBE:ACN:H_2O (2:2:2:1, v/v) and IO:EtOAc:ACN:H_2O (4:3:4:4, v/v) resulted in the lowest lipid loss to the aqueous phase. Moreover, they enabled the identification of a higher number of lipid species compared to the traditionally used two-phase MTBE extraction.

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3220 C — Accurate Determination of Circulatory Lipids Using a Combination of HILIC-MRM and RPLC-PRM

PRESENTING AUTHOR: *Jae-Seung Lee, Seoul National University, South Korea*
SUB THEME: *Novel approaches in lipidomics*

Circulatory lipids are important markers for characterizing disease phenotypes, but accurately determining lipid species remains a significant challenge in lipidomic analysis. Here, we present a novel analytical workflow for accurate lipidome characterization in human plasma, using mass spectrometry (MS) through the integration of hydrophilic interaction liquid chromatography (HILIC) and reversed-phase liquid chromatography (RPLC).

This workflow enables rapid screening of 1,966 lipid species across 18 lipid classes using HILIC-multiple reaction monitoring (MRM), which enables facile identification of lipid species by lipid class-based separations. In the NIST® Standard Reference Material for Human Plasma (SRM 1950), 489 lipid species were identified using HILIC-MRM and subsequently analyzed with RPLC-parallel reaction monitoring (PRM) to resolve potential lipid isobars within the same lipid class. Notably, RPLC-PRM identified 70 additional lipidomic features in SRM 1950 that were not detectable with HILIC-MRM. Furthermore, a high correlation (Pearson correlation coefficient = 0.81) was observed regarding the concentrations of lipid species that not carrying isobaric interferences in between HILIC-MRM and RPLC-PRM, indicating that the individual lipid concentrations measured by each platform can be integrated. The workflow was further applied to a cohort of 284 human plasma samples from chronic kidney disease (CKD) patients, successfully profiling lipidomic phenotypes across CKD subtypes. These findings demonstrate that combining HILIC-MRM and RPLC-PRM as complementary platforms enhances the accuracy and comprehensiveness of lipidomic analysis.

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TECHNOLOGY ADVANCEMENTS

3221 C — Towards a chiral dimension in lipidomics: Enantioselective profiling of monoacylglycerols, diacylglycerols, and FAHFAs in biological samples by SFC-MS

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SUB THEME: *Novel approaches in lipidomics*

The stereochemistry of lipids plays a crucial role in their biological functions, yet remains underexplored in lipidomics. In this work, we present a chiral lipid analysis strategy for the enantioselective profiling of monoacylglycerols (MGs), diacylglycerols (DGs), and fatty acid esters of hydroxy fatty acids (FAHFAs) in biological samples using supercritical fluid chromatography-mass spectrometry (SFC-MS). We developed a column-coupling approach combining an amylose-based chiral column and an achiral octadecyl column, enabling effective separation of MGs and DGs from highly abundant triacylglycerols (TGs) in total lipid extracts without the need for time-consuming sample fractionation. The method revealed distinct enantiomeric profiles for selected MGs and symmetric DGs in biological samples, suggesting sample-specific lipid stereochemistry.

To address the complex isomerism of FAHFAs, we introduced an offline two-dimensional separation strategy, combining reversed-phase liquid chromatography for the isolation of FAHFA regioisomers with subsequent chiral SFC-MS analysis of collected fractions. This approach allowed for the characterization of FAHFA enantiomers in complex biological samples, such as white adipose tissue and rice, revealing the presence of both R- and S-FAHFAs, with one enantiomer being predominant in each case.

These methodologies provide an innovative tool for investigating lipid stereochemistry and its implications in metabolic regulation.

This work was supported by the Czech Science Foundation (project no. 24–12234S).

3222 C — Fecal filter paper – an alternative sample collection for targeted and untargeted metabolomic cohort

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SUB THEME: *Novel samples/matrices*

Fecal metabolomics provide valuable insight into gut health, microbiome interactions, nutrient and drug metabolism as well as disease biomarkers. However, fecal sample collection and storage of freshly frozen material present a high obstacle in cohort or intervention studies. Sample collection timepoint is difficult to predict and large sample tubes require substantial storage facilities. Storing fecal samples on filter paper offers several benefits, including the possibility of sample collection outside of clinics, reduced space, higher temperature storage, easier transport, and minimal sample volume and material required for analysis.

To evaluate the feasibility of this novel storage method for targeted and untargeted metabolomic analysis, fecal sample material was collected, simultaneously freshly frozen and spread on Whatman filter paper. Metabolites were extracted using an aqueous buffer. Amino acids, tryptophan metabolites and citric acid cycle metabolites were quantified by GC-MS. Short chain fatty acids quantification and untargeted metabolite analysis were performed using UHPLC-MSMS.

Replicates prepared from freshly frozen material and filter paper cards showed a comparable reproducibility suggesting a comparable homogeneity with the biological variation exceeding the variation between replicates. While the recovery of metabolites as well as contaminants varies between the collection types, a comparison between individuals shows similar ratios/profiles of metabolites with sufficient number of metabolites.

While freshly frozen feces sample will remain the gold standard in metabolomics analysis collecting sample using filter paper can confidently be used for screening cohorts or indicative analysis.

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TECHNOLOGY ADVANCEMENTS

3224 C — Metabolomics in Core Facilities: Navigating Challenges to Deliver High-Quality Services

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CO-AUTHORS: *David Ruso, Bernhard Seidl, Rainer Schuhmacher*
SUB THEME: *Other*

Core facilities (CFs) are increasingly introduced in academia to host infrastructure and maintain advanced equipment. While providing access to high-resolution mass spectrometry (HRMS) systems and other sophisticated instruments is a primary task, users also expect CF staff to offer extensive knowledge and scientific advice from using instrumentation to method development and data interpretation. Many users seek full-service support—from sample delivery to receiving evaluated results—requiring comprehensive expertise from the CF team.

Metabolomics, the study of metabolites in biological systems, has become essential in fields like medicine, agriculture, and environmental science. There is a growing demand for metabolomics services via CFs due to their specialized support (e.g. stable isotope assisted workflows) and advanced technology. However, providing these services presents several challenges impacting service quality and efficiency.

Effective communication and collaboration among interdisciplinary teams are crucial yet challenging. Ensuring that methods are standardized and quality control is rigorous is essential for reproducibility and reliability of generated results. Data management and evaluation demand specialized software and skilled personnel for accurate interpretation of large datasets. Moreover, customization and flexibility in services to be offered are necessary to meet diverse user needs, while user training and support are vital for maximizing the utility of CF services. Cost management is critical to balance operational expenses with competitive pricing. Finally, keeping up with technical advances requires continuous updates to equipment and methodologies, necessitating ongoing staff training and substantial financial resources.

This poster will highlight these challenges and showcase how our CF tackles them to provide high-quality, reliable, and efficient metabolomics services.

3225 C — MetaboHUB (French national infrastructure in metabolomics and fluxomics for life sciences), towards next generation metabolomics and fluxomics: from population to single cells.

PRESENTING AUTHOR: *Anne-Emanuelle Hay, INRAE, France*
CO-AUTHORS: *MetaboHUBconsortium*
SUB THEME: *Other*

Since 2013, French National Infrastructure MetaboHUB (MTH) funded by the French National Research Agency (ANR) addresses key critical priorities in metabolomics, fluxomics and bioinformatics to i) develop new means to measure metabolism more quantitatively, dynamically and broadly, ii) contribute to answer questions in applied research (health, plants, biotechnologies and environment), (iii) provide access to high added-value services to national/international scientific community and industry players and (iv) attract and train a new generation of scientists and users through promotion of metabolomics in higher education and continuing education. The ambition of MTH has been to draw on its unique analytical (68 Mass spectrometers, 16 NMR and 7 robotic platforms), digital (online open services, >3000 users worldwide) and human capacity (6 national leading facilities, 150 scientists, 70 FTEs) in order to develop a technological offer through complementary skills in analytical chemistry, robotics, bioinformatics, biostatistics and biochemistry toward a wide range of academic and non-academic scientific communities in France and worldwide.

MTH is engaged in ambitious projects for deep metabolic phenotyping of human and plant cohorts (notably via international projects with Canada and Europe). In the frame of health application, it is of paramount importance to reach high throughput and quantitative data production enabling the analysis of large medical and/or epidemiological cohorts. For plants, deep phenotyping will be key for example in the context of agricultural adaptation to climate changes. Another key aspect is the integration of Artificial Intelligence (AI) in several steps of metabolomics studies. Finally, stable isotopic studies are a strong originality of MetaboHUB with many applications: metabolite quantification, metabolite tracing to decipher metabolic pathways and fluxomics (both in vitro and in vivo) to measure reaction dynamics. All these aspects will be developed from the population to the single cell level.

3226 C — Chiral SFC(Super Critical Fluid Chromatography) Separation of Bioactive Indole-Containing Triarylmethanes

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CO-AUTHORS: *Jin Won Lee, Hee Seo Jung, Seunghun Kim*
SUB THEME: *Other*

Novel indole-containing triarylmethane compounds with promising bioactivity were synthesized and separated by chiral supercritical fluid chromatography. Their indole, and phenol groups induce strong hydrogen bonding and aromatic interactions, limiting conventional HPLC separation. Optimized SFC conditions with supercritical CO_2 and organic modifiers achieved rapid, high-resolution enantiomeric separation, demonstrating SFC as a highly efficient alternative for challenging chiral metabolites.

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TECHNOLOGY ADVANCEMENTS

3227 C — Absolute Quantification in MS-based Metabolomics: Matrix Effect Compensation by Post-Column Infusion of Standards

PRESENTING AUTHOR: *Mengle Zhu, Leiden University, Netherlands*
CO-AUTHORS: *Anne-Charlotte Dubbelman, Lieke Lamont, Pascal Maas, Amy Harms, Thomas Hankemeier*
SUB THEME: *Quantitation in metabolomics*

Matrix effects (ME) present a major challenge for absolute quantification in liquid chromatography–mass spectrometry (LC-MS) analysis, particularly in the identification and quantification of polar metabolites. ME can lead to ionization suppression or enhancement due to co-eluting components. The golden standard for ME correction is the use of stable-isotope-labeled (SIL) standards; however, their high cost and limited availability pose significant constraints.

Here, we introduce a post-column infusion of standards (PCIS) approach coupled with LC-MS to achieve absolute quantification of polar metabolites. Sixteen SIL standards and four PCIS standards were used for ME correction when analyzing thirty polar metabolites, including amino acids, sugars, organic acids, nucleosides, and nucleotides. Calibration lines were constructed using nine calibration levels, and relative ME between plasma samples was assessed using three distinct plasma samples. To ensure more reliable results, a total of three batches were analyzed. Slope differences between neat and plasma calibration curves were compared for the assessment of ME.

To evaluate the effectiveness of ME correction, we compared slope differences across three conditions: no correction, SIL correction, and PCIS correction for all standards. Furthermore, a systematic strategy was developed based on linearity, ME, precision, and accuracy to identify the most suitable PCIS standard for each metabolite. The comparison of neat and plasma calibration lines resulted in twenty-four targets that benefit from PCIS correction. In particular, those targets without an available SIL standard in this study. Quantification accuracy was further validated using NIST samples. In conclusion, the PCIS approach offers a strong potential for ME compensation and provides a more cost-effective solution for the absolute quantification of a large number of metabolites. This study emphasizes PCIS as a promising and reliable approach for absolute metabolite quantification.

3228 C — From Match to Multiplex: alternative SIL Calibrants for Broad-Scope LC-MS/MS quantitative analysis

PRESENTING AUTHOR: *Oriane Strassel, University Of Geneva, Switzerland*
CO-AUTHORS: *Julien Boccard, Serge Rudaz*
SUB THEME: *Quantitation in metabolomics*

Absolute quantification plays an essential role in biomarker assessment by characterizing the concentration of metabolites that act as indicators of physiological states or diseases, with potential applications in clinical research and diagnostics.

Multi-targeted internal calibration (IC) is an attractive and simple approach for the quantification of endogenous analytes, as it avoids the need for in-depth assessment of matrix similarity in the absence of a blank matrix. The principle is to use a stable isotope labelled standard (SIL) as a one-point calibrant. This approach enables direct quantification of the analyte within the sample. However, this methodology has certain limitations: the commercial availability of SILs is restricted to the most common compounds in each chemical family, their cost can be high, and the purity limited.

For the analysis of multiple analytes, it is therefore advantageous to explore the potential of employing a generic calibrant to quantify several analytes. The effectiveness of this approach, which involves the use of alternative SILs for an extended quantification, is based on the determination of response factors between the target metabolites and the SILs in the studied matrix.

Internal calibration was applied to plasma samples collected as part of a clinical study of chronic kidney disease. This approach was used to quantify 20 analytes of biological interest by LC-MS/MS, already quantify. These metabolites are mainly involved in the tryptophan metabolic pathway. A complete study was then carried out to evaluate the quantification performance obtained for each endogenous compound using alternative calibrants. This study assessed the suitability of various SIL-analyte combinations based on result accuracy, revealing that alternative SILs could be effectively used to quantify multiple endogenous analytes.

3229 C — Single-Cell Imaging of Lipid Unsaturation via $Pd(QS)_2$-Catalyzed Deuterium Labeling and Nanoscale Secondary Ion Mass Spectrometry

PRESENTING AUTHOR: *Seungjin Jung, College Of Pharmacy, Chung-Ang University, South Korea*
CO-AUTHORS: *Yooseong Jeong, Dong-Kyu Lee*
SUB THEME: *Single-cell metabolomics and lipidomics*

Recent findings have established that unsaturated fatty acids are critical not only for regulating membrane fluidity, but also for performing essential functions at the subcellular organelle level. However, techniques for the quantitative imaging of lipid unsaturation at the single cell and organelle resolution have been limited. In this study, we developed a nano scale secondary ion mass spectrometry (Nano-SIMS) based methodology to quantitative measure lipid unsaturation within individual intracellular organelles. The palladium-based catalyst $Pd(QS)_2$ was utilized in the selective targeting and incorporation of deuterium at the sites of unsaturation in fatty acid chains. Confirmation at the level of fatty acids demonstrated the specificity of deuterium incorporation, while experiments at the cellular level demonstrated that labelling predominantly occurred in unsaturated membrane lipids, such as phospholipids. Nano-SIMS analysis enabled precise visualization and quantification of deuterium distribution in cells, accessed through alterations in the $^{12}C^{2}H/^{12}C^{1}H$ ion ratio. Our results revealed that the distribution of unsaturated fatty acid across cellular organelles is highly heterogeneous. This catalyst-based isotope labeling approach establishes a robust methodology for imaging the distribution of lipid unsaturation at the single-cell levels. This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2023-00212534).

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TECHNOLOGY ADVANCEMENTS

3230 C — Optimizing microdissection sample sizes for reliable microscale global metabolomics of host-microbiota metabolites in chicken gut samples

PRESENTING AUTHOR: *Olli Kärkkäinen, Afekta Technologies Ltd, Finland*

CO-AUTHORS: *Soile Turunen, Topi Meuronen, Bryan Wang, Kati Hanhineva, Victoria Drauch, Michael Hess, Claudia Hess, Urvish Trivedi, Olli Kärkkäinen*

SUB THEME: *Small volume metabolomics technologies*

The 3D'omics project aims to generate spatially resolved multi-omics information by processing samples in ways that preserve their 3D relationship, structure and biomolecular integrity. Organs are flash frozen, subsegmented, embedded, cryosectioned, and further microdissected using a laser-microdissection microscope. Analyzing the metabolome of gut microsections enables the investigation of host-microbiota interactions at a microscopic level especially when combined with other omics data. In this context, metabolites play a key role in how microbiota influences host health.

We aimed to determine the sizes of microdissection samples that can be analyzed using non-targeted LC-MS metabolomics approach. We evaluated five sizes of 10 µm thick carboxymethylcellulose-embedded laser microdissected chicken caecum samples, ranging from 2.5e5 to 10e6 µm2 to identify the balance between sample size and metabolite detection limits. Smaller samples provide detailed locational data but might lack detectable metabolite concentrations. Metabolite extraction was carried out with 15 µl of 80 % (v:v) methanol and supernatants were analyzed using both RP and HILIC chromatography coupled with QTOF-MS.

Our results show that the percentage of identified metabolites fulfilling predefined quality criteria of S/N>5 and peak area>10,000 drops from 83% in the 10e6 µm2 samples, to 79% in the 5e6 µm2 samples, to 64% in the 2.5e6 µm2 samples, to 53% in the 1e6 µm2 samples, to 30% in the 5e5 µm2 samples, and finally to 22% in the smallest 2.5e6 µm2 samples.

Our findings indicate that as microdissection samples become smaller, the quality of the signals decreases, limiting usability of the data. However, these results do show that it is possible, for example, to measure metabolite profiles separately from the host tissue and the lumen from chicken gut microsamples. We demonstrated this by analyzing samples from Salmonella Infantis infected chickens revealing specific alterations related to the infection in the host tissue and gut lumen microsection samples.

3231 C — Discovery workflows: is hi-res always necessary?

PRESENTING AUTHOR: *Hannah Florance, Agilent, United Kingdom*

CO-AUTHORS: *Karen Yannell, Cate Simmermaker, Daniel Cuthbertson*

SUB THEME: *Targeted methods*

Discovery research for LCMS based metabolomics studies tend to lean towards high resolution non-targeted approaches. These can prove to be computationally heavy and time consuming. Not all scientists are analytical chemists, and not all labs have access to high resolution instruments, so this is often the bottleneck for a biologist looking to understand the metabolism of their system, with a view to developing or optimizing their next experiments. Targeted screening can go some way to simplifying and speeding this process up.

We discuss how triple quadrupole technology, offering targeted workflows using standardized methods helps us gain a functional understanding of our multi-omic studies. With fast dwell times and rapid polarity switching, hundreds of MRMs, covering a wide range of compound classes and metabolic pathways, can be accurately quantified within a single run. We have developed databases curated with retention times and transitions for over 500 metabolites using HILIC chromatography, and 763 lipids from over 44 lipid classes using reverse phase column separation, not far off typical numbers for metabolite identification from more naïve, non-targeted approaches. Automated post-acquisition data extraction provides us with a simple first step of a workflow with enough data points to carry out chemometrics. We now have a 'pseudo discovery' pipeline, where every metabolite is confidently identified.

This is an ideal platform open to both customization regarding specific metabolic pathways, or further optimization for more focused, routine targeted screening assays.

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TECHNOLOGY ADVANCEMENTS

3232 C — A novel UHPLC-MS/MS approach for the sensitive quantification of isoprostanes in oral fluid with minimal solvent consumption

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SUB THEME: *Targeted methods*

In living organisms, a dangerous imbalance in the antioxidant defense system can occur due to the overproduction of reactive oxygen species (ROS) or their missing degradation; ROS exert their toxic action, leading to a condition called oxidative stress (OS). OS has been implicated in pathological events such as cardiovascular and neurodegenerative diseases. Isoprostanes (IsoPs) are recognized as biomarkers of lipid peroxidation and OS in vivo and they demonstrate greater chemical stability compared to biomarkers of oxidative damage to DNA and proteins. IsoPs are formed in situ from the oxidation of arachidonic acid by the action of ROS in the cellular membrane; they are found at low concentrations in biological fluids, so that the need to develop highly sensitive methods for their detection is mandatory. In this study a new robust and sensitive method for the quantification of IsoPs in oral fluid through UHPLC-MS/MS has been developed. With the aim of reducing the volume of organic solvents employed in the extraction step, parallel artificial liquid membrane extraction (PALME) was selected for sample pretreatment. In the PALME set up, target analytes are extracted through a three phases system, exploiting a pH gradient to facilitate mass transfer of uncharged analytes from the original matrix to an aqueous extracting phase, across an organic solvent (3 µL) immobilized within the pores of a membrane. This technique allows a high enrichment factor (40-fold) without an increase of matrix effect (below 10%). Noteworthy, for the first time, a microextraction technique in the 96-well format was employed in target metabolomics for oral fluid. This matrix is favourable because of non-invasive, fast and patient-friendly collection. LODs obtained ranged between 1 and 5 pg mL –1 that allowed the detection of basal levels of five different IsoPs in oral fluid of healthy volunteers.

3233 C — Targeted Lipidomics Method for Qualitative and Quantitative Measurement of Plasma Lipids

PRESENTING AUTHOR: *Markos Leggas, St. Jude Children's Research Hospital, United States*

CO-AUTHORS: *Akash Bhaskar, Megha Garg, Hanfu Shi, Andrew Pappas, Jamie Horn*

SUB THEME: *Targeted methods*

The complexity of biofluid lipidomes, characterized by numerous lipid classes and structural variations, poses significant analytical challenges for accurate identification and quantification. Here, we report a targeted lipidomics method for precise and accurate lipid quantification, which is essential for understanding their biological roles. We present a targeted lipidomics method developed on the SCIEX 7500 QTRAP, capable of identifying and quantifying over 2,100 lipid species across 17 lipid classes in plasma. Lipid extraction of 20 µL of plasma was performed using a modified Bligh and Dyer method. Analytes from 10 µL injections were eluted at 35 °C over 18 min on a Phenomenex LUNA- NH 2 (100mm x 2mm, 3 µm) column with a gradient mobile phase – Mobile phase A [10 mM NH4CH3CO2, pH 8.2: acetonitrile (1:1, v/v)] and Mobile phase B [DCM:ACN (7:93, v/v)]. Dynamically optimized retention time windows and dwell times were employed to maximize the number of multiple reaction monitoring (MRM) transitions. Retention times were validated by aligning the MS/MS spectra of 86 deuterated standards with their respective lipid molecular structures, which represent the 17 classes. The scheduled MRM files were uploaded into Skyline (version 24.1.0.414), and integration of compounds was performed based on results from quality control samples. Lipid quantification relied on class-specific calibration curves created using 1-9 deuterated standards per class, enabling the accurate interpolation of endogenous lipids. Analytical procedures followed FDA bioanalytical method validation guidelines, reporting only analytes that can be interpolated after achieving system suitability criteria. Batch variability was mitigated using the ComBat algorithm. Using the NIST-SRM-1950 plasma reference material, we reliably detected 930 to 1,230 lipid species and quantified 350 to 400 lipids with a coefficient of variation (CV) below 30%. This approach demonstrates robust capabilities for comprehensive lipidome quantitation, providing valuable insights into lipid biology and associated physiological changes.

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TECHNOLOGY ADVANCEMENTS

3234 C **Advancing Bile Acid Quantitation: High-Specificity MS2 and MS3 Analysis Using the Stellar Mass Spectrometer**

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SUB THEME: ***Targeted methods***

Bile acids (BA) are cholesterol-derived molecules synthesized in the liver that play a crucial role in lipid digestion, absorption, and signaling. Beyond their traditional function, they serve as key regulators of gut microbiome interactions while also modified by gut microbiota into secondary and conjugated forms, providing insights into various states of health and disease. Notably, individuals with autism spectrum disorder (ASD) exhibit distinct bile acid profiles, suggesting disruptions in microbial metabolism. These changes may contribute to digestion, immune function, and neurological development differences observed in ASD. Given the growing recognition of bile acid-microbiome interactions, there is an increasing need for analytical tools capable of differentiating structurally similar bile acid isomers with high specificity. The Thermo Scientific™ Stellar™ mass spectrometer, incorporating both MS2 and MS3 capabilities with complementary activation types, provides a powerful solution for enhanced bile acid quantitation and characterization.

Bile acids were extracted from plasma and fecal samples and spiked with stable isotope-labeled standards. A reversed-phase C18 chromatography method ensured effective separation, while a scheduled MS2 and MS3 acquisition approach employing beam-type CID (HCD) and resonance CID enabled improved specificity. Preliminary results demonstrated the Stellar mass spectrometer's ability to achieve a wide dynamic range, quantifying bile acids over five orders of magnitude with femtomole-level sensitivity. The MS3 acquisition provided critical selectivity, resolving co-eluting isomers that MS2 alone could not differentiate. This capability proved particularly valuable in determining bile acid alterations in the ASD population, offering deeper insight into potential metabolic and microbial imbalances. By accurately profiling bile acid species with high specificity, this approach enhances our understanding of ASD-related metabolic differences and broader bile acid-related health implications. This method streamlines bile acid analysis, minimizing the need for extended chromatographic separation while maintaining high analytical performance.



APPENDIX – LATE ENTRY



PLANTS, FOOD, ENVIRONMENT AND MICROBES

1250 A	Exploring the uncharted realm of mitochondrial diversity using complementary analytical tools

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CO-AUTHORS: *Magdalene Reinkensmeier, Viola Jeck, Aiko Barsch, Stefanie Wernisch, Markus Lübeck, Matthew Lewis, Rita Groß-Hardt*

SUB THEME: *Model organisms*

Mitochondria are dynamic organelles essential for numerous cellular processes, and their dysfunction can disrupt growth and survival in animals, humans, and plants. Traditionally viewed as part of homogenous populations, recent studies highlight that mitochondria differ between and even within cells, suggesting a division of labor among mitochondrial subtypes (mitotypes). However, the molecular basis and biological relevance of mitotypes remain largely unexplored.

To explore mitochondrial diversity, we developed mRACE, a method for cell-type-specific mitochondrial isolation using a chimeric protein (MTF) that facilitates targeting, visualization, and biotinylation of mitochondria. By directing MTF and a biotin ligase to specific cells, we enable selective isolation through biotin-streptavidin binding, allowing for cell-type-specific analysis of mitochondria.

We isolated mitochondria from functionally distinct leaf cell types: mesophyll cells, which are specialized for light-energy conversion, and guard cells, which regulate gas exchange.

Isolated mitochondria were subjected to non-targeted proteomics and lipidomics using liquid chromatography (LC)-trapped ion mobility spectrometry (TIMS)-MS/MS with a new high-sensitivity timsTOF platform (Bruker Daltonics, Germany). Lipids were analyzed with C8 chromatography and 4D-TIMS-PASEF acquisition. Data processing included rule-based lipid annotation and statistical evaluation with MetaboScape 2025 (Bruker Daltonics, Germany). Proteomics samples were digested (iST-Kit, PreOmics, Germany) and analyzed in dia-PASEF mode on a timsTOF Ultra 2 using 22 min nanoLC gradients (Bruker Daltonics, Germany). Data were analyzed with Spectronaut-19 (Biognosys, Switzerland) and results correlated using StringDB for functional analysis.

Proteomic and lipidomic profiling revealed differential enrichment of mitochondrial factors, reflecting the metabolic and respiratory specialisation of cells. We analyzed gene activity profiles for five differentially enriched factors, finding that four promoter reporter constructs exhibited differential activity, consistent with mass spectrometry data. These findings uncover a new layer of mitochondrial specialization between mesophyll and guard cells and demonstrate the power of combining mRACE with high-resolution mass spectrometry to explore the largely uncharted realm of mitochondrial diversity.

COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

2038 B	Medicinal Plant-Derived Caffeoylquinic Acids as Inhibitors of Rift Valley Fever Virus: A Computational Study

PRESENTING AUTHOR: *Garland More, University Of South Africa, South Africa*

SUB THEME: *Systems biology*

Rift Valley fever virus (RVFV) poses a significant public health threat, particularly in tropical and subtropical areas, with clinical manifestations ranging from asymptomatic cases to severe outcomes like hemorrhagic fever and renal failure. Currently, there are no approved treatments for RVFV, making the discovery of effective antiviral agents crucial. One promising approach is to target host proteins involved in viral entry.

This study explored six bioactive caffeoylquinic acids from South African medicinal plants: 3-caffeoylquinic acid (1), 4-caffeoylquinic acid (2), 5-caffeoylquinic acid (3), 4,5-dicaffeoylquinic acid (4), 3,4-dicaffeoylquinic acid (5), and 3,5-dicaffeoylquinic acid (6). These compounds were assessed through molecular docking, molecular dynamics (MD) simulations, and density functional theory (DFT) analysis against the RVFV LRP1(CR17) protein, crucial for viral entry.

The DFT and MD simulations indicated the stability of protein-ligand interactions, with molecular docking revealing compounds 6 and 2 as effective inhibitors of LRP1-CR17 (docking scores: -8.117 and -7.969 Kcal/mol, respectively). Notably, compound 3 showed the highest stability and lowest reactivity in DFT calculations. Binding interactions of compounds were identified, with compound 1 engaging various amino acids in the protein, while compound 2 had six key interactions facilitating binding.

Overall, these caffeoylquinic acids present a promising scaffold for further optimization in developing entry inhibitors against RVFV, warranting additional in vitro and in vivo studies.

COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

3313 C | **Strategies for Omic Data Analysis and Integration**

PRESENTING AUTHOR: ***Aneta Sawikowska, Poznan University Of Life Sciences, Poland***

SUB THEME: ***Statistical methods***

The rapid expansion of omic technologies has revolutionized biological research, enabling a more comprehensive understanding of complex molecular interactions. This study presents a systematic approach to omic data analysis and integration, covering metabolomics, proteomics and transcriptomics datasets across diverse experimental conditions and plant species. Advanced statistical and computational techniques are employed to extract meaningful biological insights from high-throughput data generated by various platforms, including LC-MS, GC-MS, UPLC-UV and Illumina sequencing.

The workflow includes preprocessing, normalization, statistical modeling, and network-based approaches for multiomic integration. Data preprocessing and statistical analyses were performed in R, incorporating tools such as Genstat for initial modeling and various R-based packages for specialized analyses. Correlation network analysis using Weighted Gene Co-expression Network Analysis (WGCNA) allowed for the detection of functional modules across datasets. Differential correlation networks were constructed using Fisher's Z transformation with Bonferroni correction, identifying condition-specific interactions. Gaussian graphical modeling with rags2ridges provided a refined approach for mapping trait associations across multiple treatments.

Multiomic datasets from different plant species include barley (Hordeum vulgare), rye (Secale cereale), wheat (Triticum aestivum), Brachypodium distachyon, and basil (Ocimum basilicum), analyzed under various stress conditions such as pathogen infection (Fusarium culmorum, Puccinia recondita), drought, and nutrient limitation.

Proteomic and metabolomic profiling was performed using mass spectrometry (LC-MS, GC-MS), while transcriptomic data were generated through RNA-Seq. Data integration leveraged machine learning approaches and Bayesian inference models to identify key regulatory components and predict functional interactions. Visualization of correlation and differential correlation networks in Cytoscape highlighted key hub traits and their biological significance.

This study demonstrates the power of multiomic integration in revealing complex molecular relationships and provides a scalable framework for analyzing high-throughput omic data from diverse technological platforms. The proposed methodologies can be adapted to various biological systems, facilitating the interpretation of large-scale experimental datasets.

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

3324 C | **Strategies for Omic Data Analysis and Integration**

PRESENTING AUTHOR: ***Muhammad Imran, University of Alberta, Canada***

CO-AUTHORS: ***Rupasri Manda, Sandy Davidge, David Wishart***

SUB THEME: ***Cardiovascular diseases***

Introduction: Cardiovascular diseases (CVDs), responsible for 33% of global mortality, are influenced by advanced maternal age (AMA, pregnancy >35 years), which heightens risks of hypertension and metabolic dysfunction.

Methods: This study employed targeted LC-MS/MS metabolomics to compare plasma metabolites in young (YMA) and aged (AMA) rat dams (n=10/group), identifying biomarkers linked to AMA-associated cardiovascular risks. Metabolites, including amino acids, lipids, and biogenic amines, were quantified via chemical derivatization and mass spectrometry. Statistical analysis (two-way ANOVA, Tukey's test) and bioinformatics tools assessed metabolic disruptions.

Results: Principal Component Analysis revealed distinct metabolic clustering between groups (46% variance). Fold-change analysis (>2-fold) identified 25 differentially expressed metabolites between YMA and AMA groups (6 upregulated, 19 downregulated in AMA vs. YMA). Two-way ANOVA with Tukey's post-hoc test showed significantly ($p<0.05$) elevated metabolites in AMA, including methylhistidine, indole, tryptophan, asymmetric dimethylarginine, indole-3-propionic acid, kynurenine, glucose, and 5-hydroxylysine. Conversely, homoarginine, serine, cis-aconitic acid, citric acid, dimethylglycine, 2-hydroxybutyric acid, N-acetyl-asparagine, 3-indoleacetic acid, and propionic acid were significantly higher in YMA ($p<0.05$).

Lipidomic analysis revealed significantly ($p<0.05$) elevated levels of lipid species containing short-to-long chain fatty acids (C0-C20) in AMA dams, including free carnitine (C0), propionylcarnitine (C3), lysophosphatidylcholine acyl C17:0, lysophosphatidylcholine acyl C18:1, and lysophosphatidylcholine acyl C20:3. In contrast, YMA dams exhibited significantly higher ($p<0.05$) levels of long-to-very long chain fatty acids. Proinflammatory cytokines (IL-1β, KC/GRO) were significantly higher in AMA, while IFN-γ and TNF-α dominated in YMA. Pathway analysis implicated disruptions in hypertension-related metabolic pathways, suggesting AMA-driven biochemical alterations heighten cardiovascular susceptibility.

Conclusions: This study highlights age-specific metabolic dysregulations in AMA pregnancies, including perturbed lipid profiles, altered amino acids, and proinflammatory shifts, which correlate with hypertensive risks. Targeted metabolomics provides novel insights into biomarkers and pathways underlying AMA-associated cardiovascular dysfunction, offering potential avenues for risk prediction and intervention.

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