METABOLOMICS SOCIETY

22nd Annual Conference of the Metabolomics Society

METABOLOMICS 2026

BUENOS AIRES ARGENTINA | JUNE 21-24

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 21

	Salon C1	Salon C2	Salon E	VIP2
7:30 a.m.	REGISTRATION / INFO DESK OPEN			
8:15 a.m. – 10:15 a.m.	**W1:** Cross-repository analysis of public met data w/ MetabolomicsHub	**W2:** Demystifying Stable Isotope Labelling Metabolomics	**W3:** LC-MS Data Processing with mzmine	
10:15 a.m. – 10:30 a.m.	COFFEE BREAK			
10:30 a.m. – 12:30 p.m.	**W4:** Metabolomics and AI Approaches for Precision Health	**W5:** CHESS-Hub and Mapping Community Needs in Metabolite ID	**W6:** MetaboApps in GNPS2	
12:30 p.m. – 1:30 p.m.	LUNCH – TICKET FOR PURCHASE			
1:30 p.m. – 3:30 p.m.	**W7:** EMN Career Development	**W8:** Quality Control in untargeted met using mQACC	**W9:** Introduction to MetExplore 3	**W10:** Multisegment Injection-Capillary Electrophoresis-Mass Spec
3:45 p.m. – 5:15 p.m.	**Opening Ceremony & Plenary Session 1:** Jessica Lasky-Su			
5:15 p.m. – 7:30 p.m.	**Poster Session 1 & Welcome Reception** (Poster Presentations from 5:15pm – 6:15pm)			
6:45 p.m. – 8:00 p.m.	**Career Night Roundtable Discussions**			

MONDAY, JUNE 22

	Salon E	Salon C1	Salon C2
8:00 a.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 9:30 a.m.	**Plenary Session 2** – Marta Cascante		
9:30 a.m. – 10:15 a.m.	COFFEE BREAK		
10:15 a.m.– 12:00 p.m.	**1** Cardiometabolic & Organ-Specific Disease	**2** Metabolite Annotation	**3** Plants
12:00 p.m. – 1:30 p.m.	LUNCH BREAK AND SPONSOR PRESENTATIONS		
12:20 p.m. – 1:20 p.m.		**Sponsor Pres:** SCIEX	**Sponsor Pres:** Shimadzu do Brasil Comercio
1:30 p.m. – 3:00 p.m.	**4** Multi-Omics	**5** Standardization and Multi-Site Validation	**6** Microorganisms
3:00 p.m. – 3:30 p.m.	COFFEE BREAK		
3:30 p.m. – 5:15 p.m.	**7** Neurological Disorders	**8** Analytical Methods and Workflow Innovations	**9** Food
5:15 p.m. – 6:30 p.m.	**Poster Session 2**		
6:30 p.m. – 8:00 p.m.	**EMN Reception**		

TUESDAY, JUNE 23

	Salon E	Salon C1	Salon C2
8:15 a.m.	REGISTRATION / INFO DESK OPEN		
8:30 a.m. – 9:30 a.m.	**Plenary Session 3** – Huiru Tang		
9:30 a.m. – 10:15 a.m.	COFFEE BREAK		
10:15 a.m. – 12:00 p.m.	**10** Microbiota & Infectious Disease	**11** Machine Learning and AI Systems	**12** Ion Mobility and Integrated Workflows
12:00 p.m. – 1:30 p.m.	LUNCH BREAK AND SPONSOR PRESENTATIONS		
12:20 p.m. – 1:20 p.m.	**Sponsor Pres:** Agilent Technologies	**Sponsor Pres:** Bruker	**Sponsor Pres:** Thermo Fisher Scientific
1:30 p.m. – 3:00 p.m.	**13** Immunometabolism and Infectious Disease	**14** Real-Time Analysis	**15** Plants and Food
3:00 p.m. – 3:30 p.m.	COFFEE BREAK		
3:30 p.m. – 5:15 p.m.	**16** Chemical Exposures and Bioactive Metabolites	**17** Data Ecosystems and Knowledge Sharing	**18** Environment I
5:15 p.m. – 6:30 p.m.	**Poster Session 3**		
6:30 p.m. – 8:00 p.m.	**Tango Social & Celebration**		

WEDNESDAY, JUNE 24

	Salon E	Salon C1	Salon C2
9:00 a.m.	INFO DESK OPEN		
9:30 a.m. – 10:30 a.m.	**Plenary Session 4** – Fidele Tugizimana		
10:30 a.m.– 11:30 a.m.	**Poster Session 4**		
11:30 a.m. – 1:15 p.m.	**19** Environment II	**20** Cancer Metabolomics	**21** Nutrimetabolomics
1:15 p.m. – 2:45 p.m.	LUNCH AND TOWN HALL MEETING		
1:35 p.m. – 2:35 p.m.	**Town Hall Meeting**		
2:45 p.m. – 4:30 p.m.	**Plenary Session 5** – Mariana Larraz Ferreira – **Awards and Closing**		

METABOLOMICS SOCIETY

22nd Annual Conference of the Metabolomics Society

METABOLOMICS 2026

BUENOS AIRES ARGENTINA | JUNE 21-24

CONTENTS

CONTENTS

PLENARY, VENDOR TALKS – PARALLEL SESSIONS AND SPONSORS 4

PLENARY SESSIONS 5

VENDOR TALKS – SCIENTIFIC PARALLEL SESSIONS 8

PLATINUM SPONSOR POSTERS 12

SPONSOR LUNCH PRESENTATIONS 13

SPONSORS 16

ORAL ABSTRACTS 18

POSTER ABSTRACTS 97

COMPUTATIONAL METABOLOMICS, STATISTICS AND BIOINFORMATICS 98

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE 111

PLANTS, FOOD, ENVIRONMENT AND MICROBES 167

TECHNOLOGY ADVANCEMENTS 200

SPONSOR ADS

PLATINUM

AGILENT 23
BRUKER 35
SCIEX 46
SHIMADZU 58
THERMO FISHER SCIENTIFIC 69

GOLD

WATERS 83

PLENARY, VENDOR TALKS – PARALLEL SESSIONS AND SPONSORS

PLENARY SESSION 1

From Discovery to Decision: How Integrative Metabolomics is Enabling Precision Medicine

Sunday, June 21
4:15 p.m. – 5:15 p.m.
Salon E

Jessica Lasky-Su, Brigham and Women's Hospital, Harvard Medical School – United States

***Recipient of 2025 Metabolomics Society Honorary Fellowship**

Precision medicine seeks to move beyond one-size-fits-all approaches by identifying the biological mechanisms that drive health and disease in individual patients and translating those insights into clinically actionable decisions. Among the omics sciences, metabolomics occupies a unique position as the molecular layer most proximal to phenotype, providing a dynamic readout of the cumulative effects of genetic, environmental, infectious, behavioral, and disease-related influences on human biology. As a result, metabolomics is increasingly serving as a bridge between molecular discovery and clinical application. This presentation will explore how integrative metabolomics, combined with other omics, and deep clinical phenotyping, advances precision medicine across a spectrum of biomedical challenges. Using examples from large population cohorts, biobanks, clinical trials, and national initiatives—including the Mass General Brigham Biobank and the NIH RECOVER Initiative—we illustrate how metabolomics can reveal biological mechanisms underlying poorly understood diseases such as Long COVID, identify molecular endotypes that enable precision therapeutic strategies in asthma, develop biomarkers that quantify biological aging, nutritional status, and metabolic resilience, and characterize how individual infectious exposure histories shape metabolic phenotypes and influence future health trajectories. Together, these examples highlight the evolving role of metabolomics as a foundational component of precision medicine, demonstrating how integrative multi-omic approaches can transform molecular measurements into biological understanding and biological understanding into clinical action.

PLENARY SESSION 2

From ^{13}C based metabolic flux analysis to genome scale metabolic modelling: identifying therapeutic vulnerabilities in cancer and multifactorial diseases

Monday, June 22
8:30 a.m. – 9:30 a.m.
Salon E

Marta Cascante, University of Barcelona – Spain

***Recipient of 2025 Metabolomics Society Honorary Fellowship**

Metabolic reprogramming is a hallmark of cancer and other multifactorial diseases, reflecting the ability of cells to dynamically redistribute metabolic fluxes in response to genetic, environmental, and therapeutic pressures. While metabolomics and other omic approaches provide valuable snapshots of metabolic states, translating these static measurements into functional understanding and therapeutically actionable vulnerabilities requires quantitative approaches capable of resolving intracellular fluxes and integrating them at the systems level. This presentation outlines how quantitative analysis of metabolic fluxes using ^{13}C based metabolic flux analysis (^{13}C MFA) has established fluxomics as a quantitative framework capable of inferring pathway usage and metabolic plasticity within central metabolic networks from isotopic labelling patterns, going beyond metabolite abundance alone. By resolving intracellular flux distributions, ^{13}C MFA has revealed functionally distinct modes of metabolic reprogramming that would remain hidden using static measurements alone, including, in the work discussed here, context dependent mitochondrial oxidation of non canonical carbon sources and adaptive glutamine dependence in response to targeted therapies.

An additional level of understanding of metabolic fluxes is achieved through genome scale metabolic models (GSMMs), which provide a systems level framework to explore metabolic phenotypes by integrating stoichiometric network structure with multi omics data, particularly transcriptomics. In this presentation, I will also describe the workflow we have developed to construct and analyse context specific GSMMs, in which experimental constraints, including metabolomics, uptake and secretion rates, and, when available, ^{13}C resolved flux information, are used to refine the space of feasible flux distributions. Application of this framework has enabled the identification of coordinated metabolic dependencies, stratification of disease states based on functional metabolic configurations, and differential responses to therapeutic or environmental perturbations. Using illustrative examples from our work, including ongoing and unpublished analyses, I will show how GSMM based approaches uncover context specific metabolic vulnerabilities and support translational applications, including patient derived organoids and clinically accessible metabolic readouts, across cancer and other multifactorial diseases.

PLENARY SESSION 3

Quantitative Metabolomics: Challenges and New Findings

Tuesday, June 23
8:30 a.m. – 9:30 a.m.
Salon E

Huiru Tang, Fudan University – China

CO-AUTHORS: Yulan Wang

Biological metabolomes are complex mixtures of diverse metabolite isomers with many of them having low levels but vital isomer-dependent functions, which are largely unknown. Quantification of metabolomes is therefore essential for efficiently revealing such diverse physiological functions of many metabolites. Given the presence of many chemical species in such complex mixtures with diverse structural and physiochemical properties as well as level differences in several orders of magnitude, however, quantitative metabolomics is nontrivial with demands to concurrently overcome numerous problems in sensitivity, isomeric resolution, availability of internal standards, batch effects or long-term stability for large cohort studies, de novo structure determination of unknown metabolites as well as effectiveness for inter-lab technology transfer. For reliable identification, this presentation will briefly report some results on de novo determination of absolute structures for completely unknown metabolites (Nature, 569:581, 2019; Cell Rep Phys Sci, 5:102041, 2024). I will then discuss some high-throughput quantitative strategies of the probe-propelled metabolomics instigated by sensitivity enhancement (PROMISE) for concurrently solving problems for sensitivity, resolution, internal standards, and batch effects (J Am Chem Soc, 145:25513, 2023; Anal Chem, 96:18141, 2024; Anal Chem, 97:1681, 2025; Anal Chem, 98:7356, 2026; J Pharm Anal, 15:101180, 2025). With numerous successful examples, I will also present some interesting applications of such methods for revealing new metabolomic phenotypic insights or molecular phenomes for important diseases (Nat Commun, 17:2461, 2026; J Hepatol, 82:781, 2025; Nat Aging, 4:1249, 2024; Immunity, 57:1087, 2024, Nat Microbiol, 7:707, 2022).

PLENARY SESSION 4

From African Biodiversity to Global Innovation: AI-Enabled Metabolomics for Plants, Natural Products, and Sustainable Futures

Wednesday, June 24
9:30 a.m. – 10:30 a.m.
Salon E

Fidele Tugizimana, University of Johannesburg – South Africa

***Recipient of 2025 Metabolomics Society Honorary Fellowship**

CO-AUTHORS: The Tugizimana Lab and collaborations

This plenary session presents an integrative scientific trajectory at the interface of computational metabolomics, plant biology, and translational innovation, illustrating how advanced analytical technologies and artificial intelligence (AI)-driven approaches are transforming our understanding of plant–microbe–environment interactions and natural product discovery. Central to this narrative is the application of untargeted LC–MS/MS metabolomics and computational workflows to decode the biochemical mechanisms underpinning plant adaptation, resilience, and productivity. Evidence from controlled and field-scale studies demonstrates that microbial- and seaweed-derived biostimulants induce coordinated metabolic reprogramming involving various biological pathways associated with enhanced growth, stress tolerance, and yield performance. These findings exemplify the evolution of metabolomics from a descriptive outlook to a predictive and mechanistic framework that informs the rational design of next-generation biostimulants and sustainable agricultural solutions. Complementing these agricultural applications, the session will explore the rich chemical diversity of African medicinal plants with a focus on genera such as Helichrysum, Plectranthus, and Momordica, as reservoirs of pharmacologically relevant natural products.

By integrating machine learning-assisted annotation, in silico structural prediction, and multi-omics approaches, these studies reveal previously inaccessible “dark metabolome” spaces, accelerating metabolite identification, chemotaxonomic characterization, and bioactive natural product discovery. Thus, this session highlights the convergence of AI, computational metabolomics, and cross-sector collaboration as drivers of sustainable and impactful science. Advances in eco-conscious workflows, and AI-enabled discovery are providing unprecedented insights into plant metabolism and plant–microbe–environment interactions. Simultaneously, partnerships between academia and industry are accelerating the translation of metabolomic knowledge into next-generation agricultural solutions and value-added natural products. As such, these developments reflect a shift toward predictive, systems-level biology, positioning metabolomics as a catalyst for innovation across agriculture, biotechnology, and natural product research. Africa’s biodiversity, scientific capacity, and global partnerships uniquely position the continent to transform molecular data into solutions for food security, environmental sustainability, and human well-being.

Metabolomics-driven cereal biotechnology: phenolic diversity, gastrointestinal transformation, and geographical indication

Wednesday, June 24
2:45 p.m. – 3:45 p.m.
Salon E

Mariana Simões Larraz Ferreira, Federal University of the State of Rio de Janeiro (UNIRIO) – Brazil

CO-AUTHORS: Carolina Thomaz dos Santos D'Almeida, Luciana Ribeiro da Silva Lima, Ana Bari Koifman, Esther de Mello Antonio, Cristina Yoshie Takeiti, Millena C. Barros Santos, Álvaro Fernández-Ochoa, Alexandre Guedes Torres

Rising demand for climate-resilient staple foods has renewed interest in cereals (e.g. sorghum, millets, and rice). Cereals combine genetic diversity and high levels of bioactive compounds with potential health benefits. Traditional varieties exhibit terroir-driven characteristics, and metabolomics has emerged as a powerful tool to assess authenticity. This study integrates untargeted/targeted metabolomics to characterize cereal species, germplasm genotypes, and milling fractions, aiming to discriminate rice terroir signatures and pollination effects, and to assess metabolite bioaccessibility through in vitro gastrointestinal digestion. Untargeted metabolomics was performed by UHPLC-HRMS or HPLC-QTOF-MS/MS with QC samples and MS/MS-based annotation (Progenesis QI, MSI guidelines). Targeted analysis used UHPLC-TQS (MRM) with external calibration and validation. MVA, metabolite annotation and networking were supported by MetaboAnalyst, SIRIUS and GNPS. HCA showed that rice phenolic composition was mainly driven by milling fraction, followed by crop year and pollination system. Polished rice exhibited high stability, while bran varied with climate and contained 15–25-fold higher phenolic levels, dominated by hydroxycinnamic acids. MVA separated aromatic landraces and germplasm panels, with aromatic rice displaying a distinct chemical profile. Sorghum genotypes were rich in apigenin and myricetin, along with glycosides, procyanidins, and hydroxycinnamic acids. HCA identified four subgroups differing in tannin content, grain color, and phenolic composition, all subjected to in vitro digestion. Targeted analysis confirmed high intergenotype variability, with apigenin and trans-ferulic acid as major compounds. FBMN revealed digestion phase-specific metabolite clusters: phenolics, saccharides, and lipids predominated in undigested/gastric samples, and amino acids and phenolic acids increasing in the intestinal phase. Specie-specific patterns were observed, and MVA showed sorghum bioaccessibility was least affected, while wheat and rye exhibited the greatest changes. This work revealed cereal phenolic diversity, supporting sorghum genotype selection, the geographical indication based on distinctive signatures of traditional rices, and the power of metabolomics to characterize digestion-driven changes in bioactive compound bioaccessibility.

VENDOR TALKS – SCIENTIFIC PARALLEL SESSIONS

PLATINUM SPONSORS

SESSION 5. STANDARDIZATION AND MULTI-SITE VALIDATION

Monday, June 22
1:50 p.m. – 2:05 p.m.
Salon C1

SCIEX
The power of precision

SCIEX

5.2: Quant-ready untargeted metabolomics through scanning-Q1 DIA: ZT Scan DIA

Abstract 486

Dr. Paul Baker, Senior Staff Scientist, Metabolomics and Lipidomics, United States

As untargeted metabolomics studies scale in sample number and chemical complexity, maintaining quantitative confidence becomes increasingly challenging. While data-dependent acquisition (DDA) provides selective MS/MS, its stochastic nature limits reproducibility and quantitative robustness. Conventional data-independent acquisition (DIA) improves coverage and consistency but introduces chimeric fragmentation that undermines precursor–fragment specificity and fragment-level quantitation.

ZT Scan DIA was developed to address this trade-off by introducing a scanning Q1 dimension that preserves precursor information and enables post-acquisition deconvolution. ZT Scan DIA 2.0 demonstrated that this approach substantially reduces chimeric MS/MS relative to conventional DIA, improving library matching and enabling MS/MS-level quantitative measurements across complex metabolomic datasets. However, increasing feature density in large-scale studies continued to challenge precursor selectivity and quantitative precision.

Here, we describe the quantitative advances introduced with ZT Scan DIA 3.0 on the ZenoTOF platform. By increasing acquisition speed and enhancing Q1 selectivity, ZT Scan DIA 3.0 narrows effective isolation widths while maintaining comprehensive MS/MS coverage. This further reduces spectral interference in crowded chemical space, producing cleaner fragment ion chromatograms that are inherently suitable for quantitation.

Using NIST SRM 1950 plasma as a benchmark matrix, ZT Scan DIA 3.0 shows improved identification quality compared with both DDA and ZT Scan DIA 2.0, driven by reduced chimericity rather than increased fragmentation. Importantly, these gains translate directly to quantitative performance, with MS/MS-based measurements exhibiting high signal-to-noise ratios, low coefficients of variation, and strong agreement with targeted high-resolution quantitative workflows. The ability to retrospectively extract quantitative data from untargeted datasets further supports cohort-scale and longitudinal study designs.

Together, these results demonstrate that scanning-Q1 DIA enables quant-ready untargeted metabolomics, integrating discovery, confident identification, and robust quantitation within a single acquisition strategy.

VENDOR TALKS – SCIENTIFIC PARALLEL SESSIONS

PLATINUM SPONSORS

SESSION 6. MICROORGANISMS

Monday, June 22
1:50 p.m. – 2:05 p.m.
Salon C2

SHIMADZU
Excellence in Science

Shimadzu do Brasil Comercio

6.2: How DIA with mass stability enhances your metabolomics workflow

Abstract 488

Laudicéia Alves de Oliveira, Ph.D, Product Specialist | LCMS | MALDISCIEX, Brazil

Mass accuracy is an important feature for LCMS metabolomics studies for it provides assurance for the identification and validation of biomarkers. However, metabolomics workflows usually involve long batches of samples within a few days of analysis. Also, DIA analysis although comprehensive, produces data that are not easy to process. In this work, we show a complete workflow for vegetal metabolomics using LCMS-9050, with mass accuracy stability of more than a week and easy DIA data processing capabilities.

SESSION 12. ION MOBILITY AND INTEGRATED WORKFLOWS

Tuesday, June 23
11:05 a.m. – 11:20 a.m.
Salon C2

BRUKER

Bruker

12.3: Transforming Metabolomics with Breakthrough NMR and MS Technologies

Abstract 484

Erica Forsberg, Vice President Metabolomics & Lipidomics North America, United States

Bruker continues to advance metabolomics through powerful innovations in both nuclear magnetic resonance spectroscopy and mass spectrometry. Our latest MS platforms, including timsMetabo, deliver unprecedented sensitivity, selectivity, and annotation confidence for small-molecule analysis, enabling deep 4D-Metabolomics and 4D-Lipidomics workflows that accelerate discovery across diverse sample types. These high-resolution capabilities are equally valuable for exposomics, where detection of low-abundance xenobiotics and environmental contaminants requires exceptional sensitivity and quantitative robustness. Complementary MALDI solutions provide label-free spatial mapping of metabolites, revealing molecular heterogeneity within complex tissues and supporting high-impact imaging studies.

Bruker's NMR portfolio further strengthens metabolomics and translational research through standardized, automated workflows for robust absolute quantification and untargeted analysis. The Avance IVDr platform enables fully automated, push-button metabolomics in biofluids, supporting large cohort studies, longitudinal monitoring, and clinically oriented research. Complementing this, Fourier 80 benchtop NMR brings accessible and automated NMR-based metabolomics to routine laboratory environments. To further enhance NMR workflows, the new ACP 2.0 software delivers scalable qNMR data analysis across diverse sample matrices, leveraging high-performance, model-based algorithms to achieve accurate quantification even in highly complex and overlapping spectra. Together, Bruker's integrated NMR, LC- MS and GC-MS technologies deliver comprehensive, scalable solutions that empower researchers to achieve deeper biological insight, improve annotation confidence, and accelerate applications ranging from precision medicine to plant, microbial, and environmental metabolomics.

VENDOR TALKS – SCIENTIFIC PARALLEL SESSIONS

PLATINUM SPONSORS

SESSION 13. IMMUNOMETABOLISM AND INFECTIOUS DISEASE

Tuesday, June 23
1:50 p.m. – 2:05 p.m.
Salon E

Agilent Technologies

13.2: Connecting Metabolomics to Function: A Unified Platform for Actionable Biology

Abstract 487

Fernando (Ralph) Tobias, LC/MS Application Scientist, United States

Advancing metabolomics research requires integrated, standardized workflows that reduce complexity and accelerate time to insight. Agilent Technologies delivers end-to-end omics solutions built on a unified platform spanning sample preparation, separation, detection, and data analysis, enabling reproducible, high-confidence results while lowering barriers to adoption. These harmonized workflows support metabolomics and broader omics applications with scalability and consistency across discovery and targeted studies. In addition, Agilent uniquely connects molecular profiling with functional biology through its cell analysis portfolio, including the Seahorse XF Analyzer, which enables real-time measurement of cellular metabolism to complement omics datasets and strengthen biological interpretation. Together, these capabilities provide a cohesive framework for generating robust, actionable insights with speed and confidence.

SESSION 14. REAL-TIME ANALYSIS

Tuesday, June 23
1:50 p.m. – 2:05 p.m.
Salon C1

Thermo Fisher Scientific

14.2: Software, hardware and intelligent acquisition strategies for guided unknown metabolite characterization

Abstract 485

Susan S. Bird, PhD, Sr Manager, Vertical Marketing - Metabolomics Lead, United States

The field of metabolomics has come a long way to effectively turn mass spectrometry signals into confident and reproducible chemical identities. It's well known that a single metabolite can generate multiple observable signals (isotopes, adducts, and multimers), while additional peaks arise from contaminants and unintentional fragmentation during ionization and ion transfer, requiring intelligent data reduction and filtering. As data processing and hardware advances continue to develop and address this complexity, the large chasm of uncharacterized small molecule compounds remains a top pain point for researchers applying global metabolomics strategies. Beyond spectral library matching, which is very effective at identifying known chemical identities, confident unknown annotations require intelligent sample acquisition as well as analysis.

Comprehensive MS/MS strategies such as the AcquireX Deep Scan workflow improve the efficiency of MS/MS collection by excluding background ions detected in blanks and iteratively targeting new relevant precursors in samples of interest across replicate injections. Combined with enhanced dynamic range methods, which attenuate the highly abundant compounds to reproducibly detect moderate-to-low abundant species often missed through ion-suppression, molecular networking can begin to piece together the substructure of common compound classes and provide orthogonal confidence. Real-Time Library Search (RTLS) offers a means to run ddMSn experiments with parameters adjusted based on matching compounds to a library. A similarity experiment allows the system to find the closest matching compound in the library in real time, then guide the instrument to collect ddMS3 data on matching fragments to confirm known substructures and non-matching fragments to characterize unknown structures.

In this presentation, we will showcase real-world examples utilizing the intelligently acquired consensus evidence as applied to a standard global experiment. The enhanced workflow is designed to reduce false positives and enable defensible reporting aligned with community based small-molecule annotation confidence frameworks.

VENDOR TALKS – SCIENTIFIC PARALLEL SESSIONS

GOLD SPONSORS

SESSION 5. STANDARDIZATION AND MULTI-SITE VALIDATION	Monday, June 22 2:25 p.m. – 2:40 p.m. Salon C1

Waters™

Waters Corporation

5.4: Advancing High-Resolution LC-MS Methods for High Sensitivity Metabolomics

Abstract 483

David Heywood, Principal Market Development Manager, UK

The power of measuring biology at the single cell level is within grasp. In our technology presentation we will discuss how new instrument developments combine with novel sample introduction and chromatographic approaches to push the boundaries of LC-MS methods for high sensitivity applications in Metabolomics and Lipidomics. We will discuss solutions and workflows that span high coverage characterisation, high-throughput and spatial mapping of metabolites and lipids at single-cell sample sizes.

PLATINUM SPONSOR POSTERS

PLATINUM SPONSORS

SCIEX

Redefining metabolomics performance: from sensitive, high-throughput quantitation to high-specificity DIA with ZT Scan DIA 3.0

Modern metabolomics demands analytical solutions that deliver speed, sensitivity, and confident molecular insight. Here, we highlight a suite of enabling technologies that collectively push the boundaries of throughput and specificity across diverse applications. Acoustic Ejection Mass Spectrometry (ECHO+ MS) with an open-port interface enables an ultra-fast, dilute-and-shoot workflow for glucose quantitation in fermentation media, achieving ~2.5-second analysis times per sample while maintaining excellent linearity and strong agreement with established HPLC-based assays. In parallel, high-resolution QTOF MS coupled with CID and electron-activated dissociation (EAD) enables comprehensive oxylipin analysis, combining pg/µL sensitivity with structurally diagnostic fragmentation to confidently resolve challenging isomeric species.

Building on these advances, next-generation data-independent acquisition (DIA) strategies are redefining how metabolomics data are collected and interpreted. ZT Scan DIA 3.0 on the ZenoTOF 8600 system introduces a continuously scanning Q1 dimension, fundamentally enhancing precursor specificity compared to traditional DDA and fixed-window DIA approaches. By encoding precursor information directly into fragment ion traces, ZT Scan DIA 3.0 simplifies spectral deconvolution and strengthens confidence in metabolite identification. The addition of narrow-window (as low as 1 Da) scanning further reduces co-isolation, enabling clearer differentiation of isobaric and isomeric metabolites without compromising sensitivity or acquisition speed.

Together, these innovations provide a powerful and scalable framework for metabolomics, enabling laboratories to move beyond traditional trade-offs between throughput, sensitivity, and specificity. ZT Scan DIA 3.0 represents a transformative step toward more confident, information-rich datasets, accelerating discovery in systems biology, bioprocessing, and precision health.

Thermo Fisher Scientific

Advancing Single-Cell Lipidomics with Increased Lipidome Depth and Confident Structural Annotation

Lipids play essential roles in cellular biology, and their heterogeneous distribution across individual cells has important functional implications. Bulk analyses mask this variability, making single-cell lipidomics critical for understanding true biological diversity. However, routine single-cell lipidomics is challenged by the chemical diversity of lipids, which requires both positive and negative ionization, by limited sensitivity, and by the need for robust, reproducible performance. Here, we present a nanoflow LC method with polarity switching on an Orbitrap Hybrid mass spectrometer equipped with an intelligent OptiSpray ion source interface, enabling sensitive, high-quality, and reproducible single-cell lipidomics.

Lipids were extracted from one million RK13 and Vero cells and diluted to single-cell equivalents (2 µL injection). Separation was performed using nanoflow LC on the nano UHPLC with an OptiSpray Reverse Phase Cartridge Column. Electrospray ionization was achieved using an OptiSpray Ion Source with automated spray optimization. Data were acquired in untargeted mode using polarity switching with top-4 data-dependent MS/MS and compared to single-polarity acquisition.

Given the limited material in single cells, maximizing information per injection is critical. Single-cell analysis also requires a large number of samples to be run. The OptiSpray automated emitter positioning ensured stable spray performance, reduced clogging, and improved robustness across repeated injections. Method performance was assessed using internal standards and QC evaluation tools.

Polarity switching significantly increased lipid coverage. Positive mode alone identified ~300 lipids with MS^2 spectra (>5 scans/peak), while polarity switching increased coverage to ~500 lipids per cell. Negative mode improved the detection of lipid classes such as PE and PI and enhanced structural annotation. For example, while positive-mode PC spectra are dominated by the 184.07 m/z head-group fragment, negative-mode fragmentation provides acyl chain information, increasing assignment confidence.

Novel Aspect

A robust, highly sensitive single-cell lipidomics method maximizing coverage and structural confidence from limited sample input.

SPONSOR LUNCH PRESENTATIONS

Monday, June 22	12:20 p.m. – 1:20 p.m.

SCIEX

SALON C1

Is your workflow giving you answers or just data?

Vinicius Verri Hernandes, Senior Scientist, University of Vienna, Austria

Integrating high-sensitivity exposomics and broad coverage metabolomics

Learn how exposomics driven metabolomics can link environmental and lifestyle exposures to measurable small molecule signatures, supporting exposure biology and biomarker discovery in population studies.

Nicola Zamboni, Adjunct Professor, Institute of Molecular Systems Biology, ETH Zurich

ZT Scan DIA 3.0 across scales

Explore how advances in metabolomics and lipidomics are enabling confident interpretation and data quality that holds up as studies scale.

Shimadzu do Brasil Comercio

SALON C2

WHEN YOU KNOW WHAT THE ANSWER IS .... OUR EXPERIENCE WITH THE LC-MS 8060RX

Jules Griffin, Professor, The Rowett Institute, University of Aberdeen, UK

In terms of study design in metabolomics, while open profiling methods are perfect for discovery science, many times in the clinic we need to test a hypothesis, and in such circumstances a targeted analysis makes more sense. Within the analytical core at the Rowett Institute we have developed a collection of assays that are designed to answer specific questions concerning metabolism. One of our most challenging assays developed to date has been for lipid mediators, which includes classic eicosanoids based on arachidonic acid but also oxidised phospholipids and other polyunsaturated fatty acids. We have been following this diverse range of signaling molecules in human blood to understand the metabolic consequences of heart failure. An assay under current development involves ceramides – both canonical ceramides based on serine and palmitate as the sphingoid base, and novel metabolites where serine is replaced with alanine or alternatively longer chain or more unsaturated fatty acids replace palmitate. As part of a multi-site collaborative project across the UK, we are tracking these ceramides in people at risk of metabolic associated inflammation. We are also profiling bioactive compounds in foods, including polyphenolics and heterocyclic amines and food additives such as parabens to understand their roles in human health. This talk will detail our experience of introducing the Shimadzu LC-MS 8060RX into a busy metabolomics and small molecule core facility where we apply these assays on a day-to-day basis.

SICRIT® – NEW DIMENSIONS IN MASS SPECTROMETRY

Jan Bucek, Head of Global Sales, Plasmion GmbH

Born in Germany in 2016, SICRIT® now stands as one of the most versatile LC-MS ionization sources on the market. From an operational standpoint, installation takes no longer than 60 seconds and is fully plug-and-play. Being compatible with the entire portfolio of Shimadzu LC-MS mass spectrometers, SICRIT® offers the coverage of EI, APCI, APPI, and ESI within one single, sensitive, and soft ionization technology.

Moreover, conventional hyphenation with LC is not the only option. SICRIT® can be deployed completely without any chromatography, resulting in rapid and sample prep-free analysis. Besides the already mentioned LC-SICRIT®-MS workflow, a GC can also be connected. In this case, however, to a conventional LC-MS instrument. This innovative approach tears down the typical LC-MS / GC-MS diversification and brings a previously unseen, highly practical combination of GC and any Shimadzu LC-MS mass spectrometer.

From highly non-polar to extremely polar compounds, while easily working with virtually any separation technique, SICRIT® brings completely new dimensions to modern mass spectrometry.

SPONSOR LUNCH PRESENTATIONS

Tuesday, June 23	12:20 p.m. – 1:20 p.m.

Agilent Technologies

SALON E

From Targeted analysis of VOCs to Lipidomics and Data Integration: The CEMBIO Experience with Agilent Platforms in Translational Metabolomics

Dr. Coral Barbas Arribas, Professor, Universidad San Pablo CEU

The effective application of metabolomics in both discovery-driven research and translational studies requires robust analytical platforms, reliable quantification strategies, and efficient data integration workflows. In this context, the Centro de Metabolómica y Bioanálisis (CEMBIO) has developed a comprehensive technological ecosystem based on Agilent platforms, enabling high-quality metabolomic analyses from targeted to largescale lipidomic studies, supported by advanced data management solutions.

In this lunch seminar, we will present an overview of CEMBIO's experience in implementing complementary metabolomics strategies using Agilent technologies, highlighting their application in biomedical research.

Bruker

SALON C1

What's for Dinner? timsMetabo™-based Metabolomics and Lipidomics of Omnivore and Vegetarian Human Fecal Reference Material NIST RM 8048

Dr. Michael Witting, Deputy Head Metabolomics and Proteomics Core, Helmholtz Munich, Germany

NIST RM 8048 is a newly released human fecal reference material designed to accelerate reproducible metabolomics and lipidomics. Built from pooled samples of vegetarian and omnivorous donors, it offers a biologically relevant, highly complex matrix for stress-testing real-world workflows. In this lunch seminar, we showcase how complementary reversed-phase and HILIC separations, combined with the power of timsMetabo, unlock deep, comprehensive coverage across polar, semi-polar, and lipid species. The result is a foundational, high-quality benchmark for this landmark reference material, including curated reference spectra and CCS values generated on timsMetabo™, providing the community with a robust starting point for harmonized, high-confidence metabolic profiling.

Phenomic Medicine: Application of NMR Molecular Data to Enhance Patient Outcomes

Prof Julien Wist, Professor of Computational Spectroscopy, Universidad del Valle, Colombia

Phenomic medicine requires accurate maps to estimate an individual's disease risk. Constructing such phenotypic risk maps necessitates the vertical integration of tens of thousands of molecular phenomes to capture a broad spectrum of medical conditions; this can be achieved by integrating multiple cross-sectional disease cohorts. The inherent robustness of NMR-based phenotypes enables the harmonization of data from diverse biobanks and epidemiological cohorts, even when measured at different facilities, into a single unified dataset. This scalability is evidenced by various contributions from the International Phenome Centre Network (IPCN). Furthermore, recent technological breakthroughs, including simplified sample collection, the transition to cost-effective platforms, and AI-enhanced data analytics, have made the clinical deployment of NMR-based phenotyping a realistic prospect.

SPONSOR LUNCH PRESENTATIONS

Tuesday, June 23	12:20 p.m. – 1:20 p.m.

Thermo Fisher Scientific

SALON C2

Debating the Dark Metabolome: Technologies to Illuminate the Unknown

Panel Discussion:

- **Rick Dunn, Professor of Analytical and Clinical Metabolomics, University of Liverpool**
- **Yasin El Abied, Postdoctoral Researcher, Institute of Analytical Chemistry, BOKU University**
- **Jennifer Kirwan, Professor of Veterinary Metabolomics, University of Veterinary Medicine Vienna**
- **Nicola Zamboni, Professor and Principal Investigator, Institute of Molecular Systems Biology, ETH Zurich**

Despite remarkable advances in analytical sensitivity and coverage, a substantial fraction of detected features in metabolomics experiments remain unidentified—the so-called “dark metabolome.” These unknowns represent both a grand challenge and a profound opportunity for discovery. This lunchtime seminar will explore a debate central to the field: Should we prioritize deeper annotation of existing datasets and computational approaches, or accelerate innovation in measurement technologies to expand biochemical knowledge?

We will examine the roots of the dark metabolome, including incomplete spectral libraries, limited chemical standards, biological context-dependence, and biases in extraction and ionization. Emerging solutions— multi-dimensional separations, data-independent acquisition, orthogonal fragmentation strategies, and AI-driven spectral prediction—are reshaping how we characterize unknown features.

By debating strategic priorities and highlighting enabling technologies, such as enhanced dynamic range and orbitrap tribrid mass spectrometers, this seminar aims to catalyze new approaches for illuminating the dark metabolome and pushing metabolomics toward a more comprehensive, and mechanistic science.

Thank you
to our SPONSORS!

PLATINUM

Agilent

BRUKER

SCIEX
The power of precision

SHIMADZU
Excellence in Science

ThermoFisher
SCIENTIFIC

Thank you to our SPONSORS!

GOLD

Waters™

SILVER

Cambridge Isotope Laboratories, Inc.
isotope.com

cmc Instruments GmbH

enveda®

exco

LECO
EMPOWERING RESULTS

Mass Analytica

Merck

Metabolon
SMALL MOLECULES, BIG INSIGHTS™

UNIVERSITY/NON-PROFIT

MSACL
Mass Spectrometry & Advances in the Clinical Lab

ORAL ABSTRACTS

SESSION 1. CARDIOMETABOLIC & ORGAN-SPECIFIC DISEASE	Monday, June 22	10:15 a.m. – 12:00 p.m.

1.1 **10:15 a.m. - 10:45 a.m.** **Abstract 351**

The Hidden Language of the Liver: Decoding Metabolic Secrets through Targeted and Untargeted Metabolomics and Lipidomics

PRESENTING AUTHOR: ***Antonia Garcia, CEMBIO, Universidad San Pablo-CEU, Spain***

CO-AUTHORS: *Constanza Fernández-Hernández, Sara Moreno-Talavera, Beatriz Linillos, María Bajo-Fernández, Hana Cermakova, Vanesa Alonso-Herranz, Jesús Fernández-Felipe, Ángeles López-Gonzálvez, Francisco J. Cueto, Mª Fernanda Rey-Stolle, Érica A. Souza-Silva, Francisco J. Ruperez, Coral Barbas, Ana Gradillas*

SUB-THEME: Biomarker validation and clinical translation

The liver is a high-flux, chemically diverse metabolic interface where central carbon and lipid metabolism, among other biotransformations, converge, making it an exceptional matrix for metabolomic and lipidomic studies.

Here, we present two complementary and methodologically novel analytical strategies and their applications to decode hepatic metabolism across distinct chemical domains and analytical requirements:

(i) Short-chain fatty acids (SCFAs). Within the intestinal tract, SCFAs are metabolized by colonocytes or absorbed into the portal vein, where they eventually reach the liver. Building on this liver–gut metabolic axis, we have optimized and validated a targeted workflow that enables absolute, robust, and sensitive quantification of 7 SCFAs: C2, C3, C4 (n-butyric and isobutyric acid), C5 (n-valeric, isovaleric, and 2-methylbutyric acid). SCFAs are metabolites seldom reported in the liver at reliable concentrations. We have thoroughly optimized and validated a method to quantify them based on HS-SPME–GC–MS after alkyl chloroformate derivatization, following a matrix-matched calibration with internal standard quantitation method.

(ii) In parallel, we implement an untargeted lipidomics pipeline based on LC–HRMS to achieve exhaustive molecular characterization of the liver lipidome, resolving lipid classes and a large diversity of molecular species through fragmentation, adducts-supported annotation, and RT elution order. This resulted in a highly curated lipidomic database that provides, for the first time, a structured lipidomic framework for liver tissue from mice.

Both approaches were applied in ongoing studies in mouse models. The integration of targeted metabolomics, providing quantitative accuracy and reproducibility, with discovery-driven lipidomics, enabling broad molecular coverage and structural characterization, offers a complementary framework to investigate metabolic alterations with potential biomarker relevance across metabolic and other pathological conditions. In addition, this combined strategy establishes a transferable workflow that improves both absolute metabolite quantification and comprehensive lipid species profiling in hepatic research.

1.2 **10:45 a.m. - 11:05 a.m.** **Abstract 240**

Metabolomics Identifies Environmental Chemical Signatures of Steatotic Liver Disease with PFAS as Key Contributors

PRESENTING AUTHOR: ***Tuulia Hyötyläinen, Orebro University, Sweden***

CO-AUTHORS: *Matej Orešič, Shashank Gupta, Andi Alijagic, Joao Barbosa, Jade Chaker, Maria Martin Grau, Daniel Duberg, Antonio J. Vidal-Puig, Stefania Carobbio, Ann Daly, Vlad Ratziu, Jorn Schattenberg, Elisabetta Bugianesi, Quentin M. Anstee*

SUB-THEME: Endocrine and metabolic diseases

Metabolic dysfunction–associated steatotic liver disease (MASLD) affects approximately 25% of the global population. While metabolic risk factors are central to disease development, environmental exposures are increasingly recognized as potential contributors. Endocrine-disrupting chemicals (EDCs) may influence MASLD development and progression, but the specific chemicals involved and the metabolic mechanisms underlying these effects remain poorly understood. This study aimed to identify environmental chemicals associated with MASLD severity and characterize metabolic pathways linking exposure to liver pathology.

★ AWARD WINNERS

SESSION 1. CARDIOMETABOLIC & ORGAN-SPECIFIC DISEASE	Monday, June 22	10:15 a.m. – 12:00 p.m.

We conducted an exposome analysis of plasma samples from 1,521 individuals in the European Steatotic Liver Disease registry. Targeted quantification of EDCs was integrated with metabolomics and lipidomics using liquid chromatography coupled to high-resolution mass spectrometry. Associations between exposures, MASLD stages, and metabolic traits were assessed with additional evaluation of sex-specific effects and findings were compared with results from in vitro and in vivo exposure models.

PFAS and parabens were associated with MASLD severity, correlating with histologically characterized steatosis, ballooning, and fibrosis, as well as with non-invasive fibrosis measures (FIB-4 and FibroScan) and markers of insulin resistance in a sex-specific manner. PFAS exposure was linked to disturbances in bile acid metabolism, lipid metabolism (phosphatidylcholines and sphingolipids), amino acids, and gut microbiota–derived metabolites, with stronger associations observed in males. Upregulation of several bile acids was associated with fibrosis, particularly in males (9 bile acids with adjusted p These findings identify PFAS as potential environmental contributors to MASLD severity and highlight mitochondrial dysfunction and the gut–liver axis as possible underlying mechanisms. Integrating exposomics with metabolomics may improve understanding of environmental drivers of liver diseases.

1.3	11:05 a.m. - 11:20 a.m.	Abstract 53

Metabolomic ageing clock monitors risks of cardiometabolic diseases

PRESENTING AUTHOR: ★ ***Manyi Jia, Imperial College London, United Kingdom***

CO-AUTHORS: *Manyi Jia, Kanta Chechi, Abbas Dehghan, Shamin Tahasildar, Declan O'Regan, Janet Lord, Parminder Raina, Mark Lathrop, Marc-Emmanuel Dumas*

SUB-THEME: Biological clocks

Biological age clocks capture aging-related epigenetic and phenotypic alterations at molecular resolution. In particular, metabolomic clocks built with circulating compounds tracking chronological age in large-scale cohorts are hypothesised to complement epigenetic clocks and capture latent dimensions of biological aging. Leveraging 1,458 metabolites detected by untargeted UHPLC-HRMS in the Canadian Longitudinal Study on Aging (CLSA, n=9,345), we built metabolomic aging clocks in 1,100 metabolically healthy individuals and scored the metabolomic age of 8,245 metabolically unhealthy individuals. We benchmarked penalised LASSO, Elastic Net and XGBoost models to evaluate prediction robustness and structural complexity. Linear and Cox regression were fitted to evaluate chronological age- and sex-adjusted association of metabolomic clocks with CMD prevalence and incidence. The metabolomic clocks developed with several algorithms typically involve 199-208 metabolites, explaining 40%-60% of age-related variance. We further confirmed the correlation between chronological, epigenetic and metabolomic age, and their derived age gaps. Particularly, metabolomic age was validated against gold standard epigenetics (Horvath clock, based on 353 CpG sites). This metabolomic clock suggested that participants with dysmetabolism were biologically older (+5.62 years) than metabolically health individuals. Such accelerated metabolomic aging was associated with baseline inflammation (CRP, TNF-a and IL-6), insulin resistance, dyslipidaemia, and hypertension. It also predicted follow-up events of CVD, T2D, COPD, highlighting the metabolomic clock's potential for predicting adverse metabolic and cardiorespiratory outcomes which are not captured by chronological age or epigenetics. Collectively, our work reveals that metabolomic aging associates with dysmetabolism and monitors longitudinal CMD risks, where microbiome-derived metabolites play a crucial role.

★ AWARD WINNERS

SESSION 1. CARDIOMETABOLIC & ORGAN-SPECIFIC DISEASE	Monday, June 22	10:15 a.m. – 12:00 p.m.

1.4 **11:20 a.m. - 11:40 a.m.** **Abstract 116**

The Metabolomic Signature of Subclinical Atherosclerosis

PRESENTING AUTHOR: ***Alessia Ferrarini, CNIC - Spanish National Center for Cardiovascular Reaserch, Spain***

CO-AUTHORS: *Alessia Ferrarini, Estefanía Núñez, Valentin Fuster, Rafael Barrero-Rodríguez, Annalaura Mastrangelo, María Gómez-Serrano, Rocio Tarifa, Riccardo Ponce Sanchez, Inés García-Lunar, Rocío Campo González, Antonio Fernández-Ortiz, Enrique Calvo, Ana Dopazo, Lucía Sánchez García, Enrique Lara-Pezzi, Borja Ibanez, Fátima Sánchez-Cabo, José Luis Martín-Ventura, Jesús Vázquez*

SUB-THEME: Cardiovascular diseases

Cardiovascular diseases (CVDs) remain the leading global cause of mortality, as highlighted in the American Heart Association's 2026 report. Atherosclerosis, the underlying driver of most CVDs, develops silently over decades and typically becomes clinically evident only at advanced stages or after acute cardiovascular events. Despite substantial advances in personalized medicine, the early detection and mechanistic understanding of subclinical atherosclerosis (SA) remain major challenges. Traditional cardiovascular risk factors (RFs) fail to identify many early-stage or new at-risk individuals, underscoring the need to uncover biological alterations that precede clinical manifestation.

In this study, we leverage untargeted metabolomics to characterize the plasma metabolomic and lipidomic signatures associated with SA in asymptomatic individuals, aiming to reveal functional pathways altered during the asymptomatic stages of disease. Comprehensive LC–MS/MS–based metabolomics and lipidomics were applied to 444 participants from the PESA study. Plasma metabolic profiles of individuals with or without SA, assessed through non-invasive imaging, were obtained using two complementary separation strategies (C18-RP and HILIC) with ESI± detection. Metabolic disturbances associated with SA indicators (peripheral plaque volume, maximal plaque thickness, coronary calcification, and spatial burden) were then evaluated in the same participants after three years of follow-up. SA-discriminating lipids were further validated in an independent subset from the AWHS cohort (n = 350) using shotgun MS-based lipidomics. Finally, transcriptomics data were integrated to elucidate functional pathways and assess alterations at multiple biological levels.

Robust associations emerged between metabolites, lipids, and SA markers independently of conventional RFs. Notably, consistent perturbations in glycerophospholipids (GPLs) and lysoglycerophospholipids (LysoGPLs) were observed across time and cohorts, while transcriptomics revealed specific dysregulation of GPLs metabolism. These findings demonstrate that systematic lipidomic alterations, beyond traditional cholesterol levels, are present in individuals without symptoms, highlighting new biological targets and supporting their potential as early, independent biomarkers for SA detection and risk assessment.

1.5 **11:40 a.m. - 12:00 p.m.** **Abstract 56**

When Heart Failure Mortality Presents as Metabolic: Metabolomic Signatures Predict 2-Year Mortalities Across Asia and Oceania

PRESENTING AUTHOR: ★ ***Lik Hang Wu, Kitasato University, Japan***

CO-AUTHORS: *Lik Hang Wu, Leroy Sivappiragasam Pakkiri, Jasper Tromp, Poh Leong Lim, XinYi Liu, Siew Pang Chan, Jielin Yang, Rishikesh Samant, Jiangwen Dong, Eugene Goh, Jieun Lee, Lieng Hsi Ling, Greg D. Gamble, David Sim, Kui Toh Gerard Leong, Poh Shuan Daniel Yeo, Hean Yee Ong, Fazlur Jaufeerally, Tze Pin Ng, Mayanna Lund, Gerry Devlin, Richard Troughton, Vicky Cameron, Carolyn Su Ping Lam, Robert Doughty, Arthur Mark Richards, Chester Lee Drum*

SUB-THEME: Cardiovascular diseases

Introduction. Heart failure (HF) is increasingly recognized as a systemic metabolic syndrome in which impaired blood perfusion contributes to metabolic decompensation in peripheral tissues. Distinct metabolic signatures have been reported for heart failure with preserved ejection fraction (HFpEF) and reduced ejection fraction (HFrEF), yet coordinated biochemical shifts are rarely quantified for prognostication, particularly in international cohorts that allow investigation of ethnic disparities.

SESSION 1. CARDIOMETABOLIC & ORGAN-SPECIFIC DISEASE	Monday, June 22	10:15 a.m. – 12:00 p.m.

Aim. Determine whether plasma metabolite network profiles differentially predict 2-year mortality in HFpEF and HFrEF, and whether these associations replicate across two ethnically diverse cohorts in Oceania and Asia.

Methodology. We quantified 288 plasma metabolites by LC-MS/MS in 1,520 participants from the PEOPLE (Oceania) and SHOP (Asia) cohorts to characterize baseline metabolic profiles in HF. Co-varying metabolite sets were identified using weighted co-expression network analysis (WGCNA). For each metabolite set, a score representing abundance was quantified using singular value decomposition and tested for association with 2-year all-cause mortality using multivariable Cox proportional hazards models.

Findings. Four metabolite sets common to HFpEF and HFrEF were annotated as nitric-oxide-related metabolic inflammation (netNO), purine metabolism (netPurine), acylcarnitine profile (netACa), and amino acid profile (netAA). Associations were subtype-specific but cohort-consistent: in HFpEF, lower netNO and higher netPurine predicted higher mortality in both PEOPLE-HFpEF and SHOP-HFpEF; in HFrEF, higher netACa and lower netAA predicted higher mortality in both PEOPLE-HFrEF and SHOP-HFrEF. Disparity analyses further showed an inverse association between netNO and body surface area among SHOP-HFpEF participants with concomitant hypertension and diabetes, a pattern not observed in PEOPLE-HFpEF.

Conclusion. Subtype-specific metabolite networks measured within 6 months following HF diagnosis predicted subsequent mortality and replicated across two international cohorts. While associations with comorbidities varied between the SHOP (Asia) and PEOPLE (Oceania) cohorts, our results highlight the potential of metabolomics-enabled risk stratification, motivating precision prognostication targeting metabolic inflammation in HFpEF and energy metabolism in HFrEF.

Agilent
Trusted Answers

Agilent Solutions for Metabolomics

Unlock the power of omics for research and drug discovery with complete and optimized workflows

Characterization, identification, and quantification of biological molecules can help researchers understand and decipher the complex cellular processes occurring at multiple levels. Integrating genomics, proteomics, lipidomics, and metabolomics often requires juggling multiple platforms, which can lead to data silos, inconsistent results, and wasted time, making it hard to identify biomarkers or validate targets with confidence. Agilent can help with a comprehensive portfolio of highly curated end-to-end workflows and analytical solutions including automated sample prep, sensitive detection, and advanced easy to use software for seamless data integration and interpretation. With Agilent, you can streamline analysis, accelerate discovery, improve reproducibility, and unlock deeper biological insights.

SESSION 2. METABOLITE ANNOTATION	Monday, June 22	10:15 a.m. - 12:00 p.m.

2.1 **10:15 a.m. - 10:45 a.m.** **Abstract 347**

Prospecting bioactive compounds in microorganisms through omics data mining and innovative validation platforms

PRESENTING AUTHOR: ***Ricardo da Silva, University of São Paulo, Brazil***

SUB-THEME: Multi-omics/Integrative omics

Studies of microbial communities associated with the most diverse environments have become increasingly important for the sustainable exploration of biodiversity. The numerous evolutionary adaptations of microbial communities to diverse environments make public data mining a source of potential biotechnological innovations for various applications. However, metagenomic data processing poses a series of challenges for mining, requiring sophisticated bioinformatics workflows to integrate multiple applications. Interactions between members of microbial communities and their environments are mediated by a wide range of metabolites. The main experimental approach used to study microbial metabolomes is liquid chromatography coupled to tandem mass spectrometry. With the expansion of public data repositories containing spectrometry experiments, several methods for correlating molecular and gene data have emerged. Despite advances in bioinformatics for functional annotation of protein sequences and associated metabolites, a significant portion of these sequences remains functionally uncharacterized. New predictive models for functional annotation have been using underlying structural and functional features that are shared and preserved in amino acid sequences. Although mining and predictive tools are an essential step in the prospecting of biosynthetic pathways, the expression of many genes has a complex regulation, requiring innovative strategies to promote gene expression. Once platforms for prospecting, prediction, and gene expression are established, the need to test the action of microorganisms and their genes under various experimental conditions becomes a major obstacle to bioactivity discoveries. The Computational Chemical Biology Laboratory (http://ccbl.fcfrp.usp.br/) integrates exploratory and predictive methods with validation methods that allow hypotheses from predictive methods to be tested, shortening the cycles for generating new biotechnological targets and refining computational methods. In this presentation, we will showcase studies that include genome mining, integration of metabolomics and genomics, and automation of microbial metabolomics monitoring.

2.2 **10:45 a.m. - 11:05 a.m.** **Abstract 272**

Benchmarking an ion mobility–resolved LC–MS/MS library against GNPS highlights the importance of instrument-specific spectral libraries

PRESENTING AUTHOR: ***Aninda Mazumdar, Mikrobiologicky ustav AV CR, Czech Republic***

CO-AUTHORS: *Tommaso Stefani, Michaela Plechatá, Mingxun Wang, Zdenek Kamenik*

SUB-THEME: Metabolite identification/annotation

Reliable metabolite annotation remains a major bottleneck in untargeted metabolomics. Although LC–MS/MS spectral libraries have expanded considerably, annotation rates remain limited, and most existing libraries lack collision cross section (CCS) information. Trapped ion mobility spectrometry coupled with LC–MS/MS (timsTOF HT) enables multidimensional molecular characterization by integrating accurate mass, retention time, fragmentation spectra, and CCS measurements. However, comprehensive ion mobility–resolved spectral resources for large collections of bioactive compounds are still lacking, and current libraries cover only a small fraction of chemical space, typically enabling annotation of only a small proportion of detected features in complex biological samples.

To address this limitation, we established a large-scale LC–IMS–MS/MS spectral library integrating MS/MS and CCS data for more than 10,000 bioactive compounds spanning diverse chemical classes. Compounds were analyzed using liquid chromatography coupled to a Bruker timsTOF HT system. The resulting data were processed using MZmine to extract features, generate consensus MS/MS spectra, and assign CCS values.

To evaluate the utility of this resource, we benchmarked the library against the GNPS2 public spectral database using multiple biological datasets, including gut microbiome fecal samples and microbial culture extracts, as well as global datasets.

SESSION 2. METABOLITE ANNOTATION	Monday, June 22	10:15 a.m. - 12:00 p.m.

Benchmarking included datasets acquired on the same timsTOF platform and Orbitrap-based datasets from public repositories.

Across datasets, the ion mobility–resolved library consistently improved metabolite annotation rates by 5–10% compared with GNPS2 spectral libraries, particularly for datasets acquired under similar chromatographic and instrumental conditions. Additionally, CCS values provided an orthogonal validation dimension that reduced false-positive identifications and increased structural confidence.

Together, this LC–IMS–MS/MS dataset expands current spectral resources by incorporating ion mobility information, increasing chemical coverage, enabling cross-platform validation, and supporting future AI-driven approaches in metabolite identification and natural product discovery.

2.3 **11:05 a.m. - 11:20 a.m.** **Abstract 128**

CASMI 2026: a real-world small molecule structure elucidation challenge, featuring hundreds of biologically-sourced challenge molecules (including many with novel structures) and the largest open-sourced library of novel small molecule LC-MS/MS spectra ever released

PRESENTING AUTHOR: ***David Healey, Enveda, United States***

CO-AUTHORS: *David Healey, Tobias Kind, Christoph Krettler, Erik DeBloois, James Taylor, Pelle Simpson, Marie Killian, Dejan Nikolic, Steffen Neumann, Emma Schymanski, August Allen*

SUB-THEME: Collaborative and open data science

The four years since the most recent Critical Assessment of Small Molecule Identification (CASMI) competition have seen an explosion of interest in methods for small molecule structure elucidation from LC-MS/MS spectra, particularly using deep learning models. To measure progress and promote further innovation, we announce a new CASMI challenge running for three months starting {June, July} 2026, designed to mimic the real-world task of metabolite identification from untargeted profiling of complex biological samples, where molecular structures range from well-characterized to completely novel.

The challenge set comprises spectra from approximately 400 molecules with a mix of known and novel structures, sourced entirely from complex biological samples from plants, mammals, fungi, and bacteria, with 2-D molecular structures confirmed by isolation and 2D-NMR or spectral comparison with synthetic standards. For each molecule, competitors will receive LC-MS/MS spectra from multiple adducts, collision energies, and ion modes, and must predict the 2-D molecular structure as SMILES strings. We provide top-k accuracy metrics and benchmark analyses of several commonly used elucidation methods.

In conjunction with the competition, we release Enveda-180, a fully open-source library of approximately 1.1 million MS/MS spectra across positive and negative ion modes, three collision energies, and multiple adduct forms, with accompanying LC retention times and collisional cross-sections. This dataset, gathered from approximately 184,000 structurally diverse synthetic small molecules, more than doubles the approximately 85,000 unique structures contained in all open-source MS/MS libraries combined.

The competition will be hosted on the machine learning community platform Kaggle to increase engagement and interest in this topic from the broader machine learning community. A $50,000 prize pool will be split among top-performing teams, and all winning methods will be fully open-sourced.

★ AWARD WINNERS

SESSION 2. METABOLITE ANNOTATION	Monday, June 22	10:15 a.m. - 12:00 p.m.

2.4 **11:20 a.m. - 11:40 a.m.** **Abstract 172**

Extending CEU Mass Mediator for Multi-Platform Metabolite Annotation in LC-MS, CE-MS and GC-MS

PRESENTING AUTHOR: ***Alberto Gil-de-la-Fuente, CEU-san Pablo University, Spain***

CO-AUTHORS: *Alberto Gil de-la-Fuente, Pablo Canadas-Miquel, Alejandra Oshea-Fernández, Pilar Bourg, Constantino A. Garcia, Sergio Saugar, Coral Barbas, Abraham Otero*

SUB-THEME: Metabolite and mass spectral databases

Metabolite annotation and identification remain major bottlenecks in untargeted mass spectrometry–based metabolomics and are critical for reliable biological interpretation of metabolomic experiments (Theodoridis et al., 2023; Hajnajafi and Iqbal, 2025). Computational approaches increasingly aim to improve annotation confidence by integrating orthogonal experimental information derived from separation techniques coupled to mass spectrometry.

CEU Mass Mediator (CMM) is an online platform designed to support metabolite annotation by integrating experimental and predicted physicochemical properties together with spectral information from multiple analytical platforms. Previous versions of CMM incorporated retention time prediction models and rule-based systems to support LC-MS data annotation.

Here we present the latest developments of the platform, which significantly expand its analytical coverage. A new set of services for the annotation of capillary electrophoresis–mass spectrometry (CE-MS) experiments has been implemented, supporting migration time (MT), relative migration time (RMT), and effective electrophoretic mobility (μeff) to improve robustness and reproducibility in CE-MS compound annotation (Drouin et al., 2020). Manual curation of CE-MS experimental data and literature resulted in a dataset of 1,193 compounds obtained under eight buffer conditions, representing, to our knowledge, the first general service for CE-MS metabolite annotation.

In addition, a new service has been developed for the annotation of gas chromatography–mass spectrometry (GC-MS) experiments using methyl chloroformate derivatization, integrating retention index (RI) information and electron ionization (EI) spectra to improve compound annotation (Bajo-Fernández et al.).

Finally, the platform has been fully refactored into a REST-native architecture, enabling programmatic access and facilitating integration with external metabolomics workflows and computational pipelines (Gil-de-la-Fuente et al., 2019). These developments expand the scope and interoperability of CMM, supporting metabolite annotation across multiple analytical platforms. CMM is freely available at http://ceumass.eps.uspceu.es/.

Acknowledgments: Research funded by project PID2024-162248NB-I00 from MICIU.

2.5 **11:40 a.m. - 12:00 p.m.** **Abstract 40**

FLARE: Fine-grained Learning for Alignment of spectra-molecule REpresentation Enhances Metabolite Annotation

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

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

SUB-THEME: Metabolite identification/annotation

Accurate metabolite annotation from tandem mass spectrometry remains a central bottleneck in untargeted metabolomics. Recent machine learning approaches have shown promising performance by aligning spectra and molecular structures in a shared embedding space using contrastive learning. However, these methods rely on global representations of spectra and molecules, despite the fact that spectrum–molecule correspondence is inherently local, arising from fragment-level relationships between spectral peaks and molecular substructures.

We present FLARE (Fine-grained Learning for Alignment of spectra–molecule REpresentations), a contrastive learning framework that explicitly models bidirectional peak–atom interactions. Instead of computing similarity solely from global embeddings, FLARE aggregates fine-grained peak-to-atom and atom-to-peak maximum similarities. This formulation enables

SESSION 2. METABOLITE ANNOTATION	Monday, June 22	10:15 a.m. - 12:00 p.m.

the model to capture chemically meaningful local correspondences that are otherwise obscured in global alignment approaches.

Importantly, FLARE is the first alignment-based framework to enable direct visual inspection of the model's decision mechanism. The learned peak–atom similarity scores expose which spectral fragments drive molecule matching, allowing predictions to be traced to specific molecular substructures and providing a new level of interpretability for spectra–molecule alignment models.

FLARE achieves state-of-the-art performance on the MassSpecGym retrieval benchmark, attaining 43.15% rank@1 accuracy and 7.84 MCES@1, improvements of over 63% and 36%, respectively, relative to prior methods. Through a systematic comparison between global and fine-grained-based contrastive learning, we show that fine-grained modeling is particularly beneficial for out-of-distribution molecules. In these settings, FLARE yields substantial rank improvements over global approaches, highlighting the generalizability of its formulation.

Beyond retrieval accuracy, FLARE learns structured embeddings that reflect molecular class organization and exhibit strong correlation with molecular fingerprint similarity, indicating that the model captures chemically meaningful relationships rather than dataset-specific heuristics.

SESSION 3. PLANTS	Monday, June 22	10:15 a.m. - 12:00 p.m.

3.1 **10:15 a.m. - 10:45 a.m.** **Abstract 354**

From UHPLC-MS Metabolomics to Metal Protection: Cinnamic Acid Derivatives as Green Corrosion Inhibitors

PRESENTING AUTHOR: ***Edwin Madala, University of Venda, South Africa***

SUB-THEME: Other

Several factors contribute to plant metabolome complexity, including glycosylation and isomerization, among others. Isomerization is primarily driven by biological processes, such as biosynthetic enzymes, as well as environmental factors like light and heat. Hydroxycinnamic acid (HCA)–containing compounds, such as chlorogenic acids, undergo trans–cis isomerization under UV light, a process that is expected to intensify with increasing sunlight exposure due to climate change. The formation of these photo-isomers presents a significant analytical challenge, as they further increase the complexity of already complex plant metabolomes. Therefore, advanced analytical and data visualization approaches are urgently needed to capture such dynamic transformations in nutraceutical compounds. LC–MS has proven to be a powerful technique for this purpose; however, it also presents challenges, as geometrical isomers often produce similar fragmentation patterns, making them difficult to annotate. Interestingly, our previous findings demonstrated that cis isomers of chlorogenic acids formed through light exposure exhibit enhanced binding to alkali metals compared to their trans counterparts, highlighting their potential for therapeutic and industrial applications, such as metal coatings. In this study, we demonstrate selected cases in which these light-induced geometrical isomers enhance metal protection against corrosion. A series of experimental optimizations of the LC–MS method were conducted to better elucidate the mechanistic processes underlying the formation of cis isomers; additionally, factors such as biological conjugation leading to positional isomers in organic acids (e.g., quinic, tartaric, citric and hexaric acids) contribute to structural diversity that is not easily resolved using conventional LC–MS, thereby necessitating well-optimized methods and advanced analytical techniques such as ion mobility spectrometry.

3.2 **10:45 a.m. - 11:05 a.m.** **Abstract 294**

Integrating metabolomics and MALDI-MS imaging reveals the efficacy of a novel elicitation strategy to induce spatially resolved chemical defenses in Eucalyptus.

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

CO-AUTHORS: *Javiera Nahuelcura, Areli Jara, Catalina Bravo, Ignacio Salinas*

SUB-THEME: Plant defense, pathogens and interactions

Metabolomics provides a comprehensive analytical framework for the global characterization of small-molecule profiles in biological systems using high-throughput mass spectrometry–based approaches. In untargeted metabolomics, data-driven analyses aim to capture the broad repertoire of downstream metabolites that reflect physiological or stress-induced biochemical states. However, conventional metabolomics workflows typically rely on tissue homogenization and solvent extraction, enabling robust identification and quantification of metabolites but inherently removing spatial information associated with their distribution within biological tissues. Consequently, linking metabolite profiles to their precise cellular or tissue-level localization remains a major limitation when interpreting metabolic functions. To address this challenge, we integrated untargeted metabolomics with MALDI-MS imaging, a technique that enables direct molecular mapping of intact cryo-preserved and microtome-sectioned tissues. MALDI-MS imaging preserves spatial context while simultaneously detecting multiple classes of metabolites, providing complementary information on molecular identity, relative abundance, and two-dimensional spatial distribution within biological samples. Using a biochemical elicitor derived from the specialist herbivore Gonipterus platensis, which feeds on young Eucalyptus foliage and causes severe crown defoliation, we investigated the systemic foliar metabolic response of Eucalyptus mother plants under controlled conditions. Following elicitation, leaf samples were collected at 0, 4, 8, 12, 24, 48, and 72 h for LC-MS–based untargeted metabolomics, while intact cryosectioned tissues were analyzed by MALDI-MS imaging. This integrative strategy captured early biochemical alterations after elicitation. Within 4 h we observed enzymatic cleavage of the glycosidic bond of Roseoside, releasing the volatile signaling molecule Vomifoliol from storage sites in secretory channels (SC). MALDI-Imaging revealed a localized decrease of Roseoside within SC at this stage, followed by restoration of metabolite homeostasis approximately 24 h after elicitation.

★ AWARD WINNERS

SESSION 3. PLANTS	Monday, June 22	10:15 a.m. - 12:00 p.m.

These findings provide new insight into early herbivory-induced defense responses in Eucalyptus and highlight the potential of combining untargeted metabolomics and MALDI-MS imaging to resolve the temporal and spatial dynamics of plant defense chemistry.

3.3 **11:05 a.m. - 11:20 a.m.** **Abstract 120**

The Metabolic Awakening of Barley Seedlings: A Time-Resolved View of how Germination Shapes Tissue-Specific Metabolic Networks

PRESENTING AUTHOR: ★ ***Claude Yasmine Hamany Djande, University Of Johannesburg, South Africa***

CO-AUTHORS: *Claude Y. Hamany Djande, Paul A. Steenkamp, Ian A. Dubery*

SUB-THEME: Plant metabolomics

Integration of internal metabolism with environmental cues is essential for the successful establishment and survival of seedlings, with light an important mediator of the transition from skotomorphogenesis to photomorphogenesis. However, detailed knowledge of the metabolic events associated with this critical transition in developing tissues of barley germlings/seedlings is currently lacking. Therefore, we systematically mapped the temporal and tissue-resolved metabolic reprogramming in seedlings grown under light/dark photoperiod and complete darkness, using untargeted LC-MS metabolomics to characterise seeds, shoots, and roots at 3 - 9 days post-imbibition. Dark-grown seedlings displayed substantial elongation but reduced biomass accumulation. Multivariate analyses revealed distinct, light-induced metabolic reprogramming in each tissue type. Among 192 annotated metabolites, seed metabolism was dominated by mobilisation and pre-emptive defence priming, with roots developing energy-centered networks. In darkness, shoots directed metabolism towards rapid elongation and synthesis of defensive hordatine isoforms A and B, alongside linolenic acid – and tryptophan derivatives. Light-triggered metabolic switches where shoots allocated resources towards photoprotective flavonoids and antifungal hordatine C. This work provides the first spatio-temporal metabolic atlas of metabolic awakening in germinating barley seeds and seedling establishment. It redefines skotomorphogenesis as an active escape-and-defence strategy and offers insight into photomorphogenesis in support of tissue-specific metabolomic differentiation.

3.4 **11:20 a.m. - 11:40 a.m.** **Abstract 77**

Metabolic crosstalk between foliar metabolism and BVOC emissions reveals seasonal plasticity in an urban Atlantic Forest tree

PRESENTING AUTHOR: ★ ***Fernanda Anselmo-Moreira, Environmental Research Institute (IPA-SP), Brazil***

CO-AUTHORS: *Patricia Menezes Ferreira Rodrigues, Bruno Ruiz Brandão da Costa, Cláudia Maria Furlan, Silvia Ribeiro de Souza*

SUB-THEME: Plant metabolomics

Urban trees in subtropical forests experience pronounced seasonal and anthropogenic stressors that reshape plant metabolism and plant–atmosphere interactions. However, the mechanisms driving seasonal variation in biogenic volatile organic compound (BVOC) emissions remain poorly understood, particularly whether these emissions arise from isolated biosynthetic processes or reflect broader metabolic crosstalk with central carbon pathways. We tested the hypothesis that seasonal BVOC responses reflect metabolic crosstalk linking BVOC release to carbon metabolism. Using Gymnanthes serrata as a model species, we investigated seasonal BVOC emissions and foliar metabolic profiles in an urban Atlantic Forest fragment in São Paulo, Brazil. Emissions were integrated with untargeted GC–MS metabolomics of polar and nonpolar phases and analyzed using multivariate statistics and low-level data fusion. BVOC emissions occurred predominantly during the dry season (151.37 ng g^{-1} DM h^{-1}) and were dominated by sesquiterpenes. The foliar metabolome showed clear seasonal differentiation, with dry-season enrichment of osmolytes and carbohydrates and rainy-season accumulation of fatty acids and phytosterols. Data fusion integrating BVOC emissions with polar and nonpolar metabolomes enhanced seasonal discrimination (R2=0.38, F=6.02, p=0.002) compared to data fusion with only polar and nonpolar metabolomes (R2=0.22,

SESSION 3. PLANTS	Monday, June 22	10:15 a.m. - 12:00 p.m.

F=2.78, p=0.002). Partial least squares discriminant analysis identified 32 metabolites with high Variable Importance in Projection (VIP > 1) and large effect sizes (|r| ≥ 0.5), including sesquiterpenes, sugars, polyols, proline, fatty acids, and phytosterols. Integrative analyses revealed coordinated metabolic shifts, providing evidence of crosstalk between BVOC biosynthesis and central carbon metabolism, with carbohydrate-related pathways emerging as the main axis of seasonal plasticity. These findings demonstrate that BVOC emissions in a subtropical urban tree are embedded within seasonal reprogramming of primary metabolism rather than directly coupled to lipid-derived processes, highlighting metabolic flexibility under environmental stress and its relevance for climate change research and urban management. (FAPESP grant numbers: 2020/07141-2, 2022/07326-8, 2022/13213-1).

3.5	11:40 a.m. - 12:00 p.m.	Abstract 148

Two generations, two carbon economies: the hornwort sporophyte enriches shikimate and the TCA cycle

PRESENTING AUTHOR: ***Ivan Hurtado Caceres, University of São Paulo, Brazil***

CO-AUTHORS: *Denilson F. Peralta, Claudia M. Furlan*

SUB-THEME: Plant metabolomics

Terrestrialization demanded biochemical solutions to withstand UV/desiccation and to build resistant walls; within this context, the shikimate-aromatic amino acid axis is viewed as an early evolutionary hub supplying aromatic and structural metabolites. Yet bryophyte metabolomics has largely centered on gametophytes (dominance and biomass), leaving a functional question underexplored: is carbon allocated differently across generations, particularly toward the reproductive sporophyte? Hornworts are a critical case because their sporophyte is photosynthetic and continuously growing, but nutritionally dependent on the gametophyte, and their chemistry remains fragmentary (volatiles or cuticular waxes) without a comparative pathway framework across life stages.

We compared the polar metabolomes of gametophytes (PG) and sporophytes (PS) of Phaeoceros carolinianus using untargeted GC-MS (n=10 per metagenesis phase) after biphasic extraction and MSTFA derivatization. Features were annotated via GNPS with curation against GMD/NIST/MassBank and confirmed with authentic standards when applicable (MSI levels 1–2). Analytical robustness included blank filtering (blank:sample >1:3) and TIC normalization. In MetaboAnalyst, OPLS-DA (VIP≥1) and a volcano framework (p PG was enriched in carbohydrates/osmolytes (xylulose, maltose, trehalose, ribose, glucose) and in starch–sucrose, galactose, and glycine/serine/threonine modules. PS was enriched in organic acids linked to respiration/anaplerosis (citrate, isocitrate, malate, 2-oxoglutarate, 2-hydroxyglutarate) and in TCA, glyoxylate-dicarboxylate, and phenylalanine/tyrosine/tryptophan biosynthesis modules, alongside glutathione metabolism. Shikimic acid emerged as an anchor feature in PS (log2FC PG–PS=−2.61), accompanied by phenylalanine.

We cautiously interpret that PS shows higher availability of aromatic precursors and an elevated energetic signature during development, without inferring flux or end-products. This framework provides reproducible markers and a comparative logic for Anthocerotophyta and other low-biomass bryophytes across contrasting habitats and historical life.

★ AWARD WINNERS

SESSION 4. MULTI-OMICS	Monday, June 22	1:30 p.m. - 3:00 p.m.

4.1 **1:30 p.m. - 1:50 p.m.** **Abstract 202**

MEANtools integrates multi-omics data to identify metabolites and predict biosynthetic pathways

PRESENTING AUTHOR: ***Kumar Saurabh Singh, Maastricht University, Netherlands***

CO-AUTHORS: *Kumar Saurabh Singh, Hernando Suarez Duran, Elena Del Pup, Olga Zafra Delgado, Saskia C. M. van Wees, Justin J. J. van der Hooft, Marnix H. Medema*

SUB-THEME: Multi-omics/Integrative omics

Plants produce a vast diversity of specialised metabolites that are essential for adaptation, yet many of their biosynthetic pathways remain unresolved because most discovery strategies rely on prior knowledge. We developed MEANtools, an unsupervised integrative multi-omics workflow that predicts candidate metabolic pathways de novo by linking transcript-metabolite associations with reaction rules and structural knowledge from public databases. MEANtools links correlated transcripts and mass features by testing whether enzyme-family-associated reaction rules can connect metabolite candidates into plausible pathway segments. In our approach, possible connections between metabolites and transcripts that show correlated abundance across samples are identified using reaction rules linked to the transcript-encoded enzyme families. MEANtools thus assesses whether these reactions can connect transcript-correlated mass features within a candidate metabolic pathway.

We validated MEANtools using a paired transcriptomic-metabolomic dataset for reconstruction of the falcarindiol biosynthetic pathway in tomato, where the tool correctly anticipated five of seven characterised pathway steps and proposed additional candidate pathways in specialised metabolism. We also applied MEANtools to a time-course transcriptomic and metabolomic study of Chrysanthemum seticuspe petals infected with Botrytis cinerea. Integration of both omics layers revealed infection-induced transcript-metabolite modules associated with flavonoid and anthocyanin accumulation, consistent with visible pigmentation at fungal penetration sites and supported by anti-fungal activity of selected compounds. Additionally, to better reflect biological dynamics, we further extended MEANtools with functions to capture lagged and nonlinear gene-metabolite relationships, enabling detection of pathway modules where metabolite accumulation follows delayed or non-linear transcriptional responses. Together, this work demonstrates how MEANtools, with recent developments including lag-aware, nonlinear integration, enables scalable hypothesis generation for specialised metabolism and defence pathways from paired multi-omics time series datasets.

4.2 **1:50 p.m. - 2:05 p.m.** **Abstract 164**

Integrated Blood Multi-Omics with 3D Probability Modelling Predicts Progression and Neuropathological Trajectory

PRESENTING AUTHOR: ★ ***Tereza Kacerova, University of Oxford, United Kingdom***

CO-AUTHORS: *Tereza Kacerova, Fay Probert, Daniel E. Radford-Smith, Jack J.J.J. Miller, Boris Shulgin, Stephen M. Smith, Elisabete Pires, Abi G. Yates, Matilda Kråkström, Eric Schiffer, Laia Montoliu-Gaya, Nicholas J. Ashton, Thomas Olsen, Henrik Zetterberg, Margaret M. Esiri, Helga Refsum, A. David Smith, James S. O. McCullagh, Alex M. Dickens, Daniel C. Anthony*

SUB-THEME: Neurological disorders

Prognostic blood-based tools, capable of predicting cognitive decline and underlying neuropathology at prodromal stages, remain limited. We tested whether integrated plasma multi-omics can anticipate both short-term structural progression and long-term clinical and pathological outcome. We implemented a multi-omics platform workflow combining targeted proteomics (pTau181, GFAP, NfL), NMR metabolomics including IVDr lipoprotein subclasses, and multi-LC-MS metabolomic and lipidomic profiling (AEC-MS and RPLC-MS). Following strong performance of targeted panels, pilot untargeted proteomics was introduced to explore pathway-level alterations associated with disease trajectory.

In a deeply phenotyped mild cognitive impairment (MCI) cohort (VITACOG; n=68) with two-year MRI follow-up, progression was defined by annualised cortical atrophy. Using repeated train-test splits with cross-validated logistic regression and independently trained random forest classifiers, integration of pTau181 with six metabolite features achieved AUC 0.91

SESSION 4. MULTI-OMICS	Monday, June 22	1:30 p.m. - 3:00 p.m.

(accuracy 80%), outperforming protein-only models (AUC ≤0.68), with key variables externally validated in the UK Biobank. These findings indicate that systemic metabolic state refines prognostication of short-term neurodegeneration in MCI.

We then examined longer-term outcome in a separate cohort (OPTIMA, n=143), in which baseline blood was obtained from individuals with Mini-Mental State Examination (MMSE) ≥24, approximately nine years prior to death and neuropathological assessment. A probability-based three-dimensional modelling framework projected cross-validated random forest probabilities into a biologically defined risk space (P_Alzheimer_disease, P_cerebrovascular_disease, P_cognitive_impairment), enabling geometric mapping of individuals along Alzheimer's, vascular, and neurodegenerative (cognitive decline in dementia) axes. This approach revealed a structured metabolic landscape in which Alzheimer and cerebrovascular pathologies diverged along distinct axes, mixed cases occupied intermediate space, and dementia status was resolved independently of the underlying histopathology.

Together, integrated NMR-LC-MS multi-omics coupled with probability-based modelling enabled scalable blood-based prognostication across temporal scales, linking early systemic metabolic state to both structural progression and eventual neuropathological trajectory.

4.3 **2:05 p.m. - 2:25 p.m.** **Abstract 174**

MetaboExpress: A Network-Based Framework for Discovering Gene–Function Relationships in Plants

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

CO-AUTHORS: *Gerd U. Balcke, Fiona Hui, Guillaume Patoine, René Meier, Henriette Uthe, Michael Wenk, Steffen Neumann, Jianguo Xia*

SUB-THEME: Metabolic networks

Analyzing MS/MS spectral similarity is central to linking structurally related metabolites with the genes encoding their biosynthesis and modification. Because similar fragmentation patterns often reflect shared substructures, grouping spectra enables the detection of biosynthetic origins, pathway diversification, and gene–metabolite associations—even when compounds remain incompletely annotated. Molecular networking extends this concept to pathway-level analyses, increasing statistical power in multi-omics integration.

Spectral similarity can be quantified using classical algebraic measures, such as modified cosine or Jaccard scores, as well as machine learning–based metrics that better approximate structural similarity. These scores support construction of molecular networks that can be partitioned into communities using diverse clustering algorithms.

However, to effectively integrate metabolomics with other omics layers, robust downstream interpretation depends on machine-readable, uniform annotations within each community based on standardized chemo-ontologies and inferred structural information.

To address both, we compiled high-resolution MS/MS spectral libraries from public repositories, covering major metabolite and lipid classes, and assigned ground-truth chemo-ontologies based on known structures. Spectra were processed with CANOPUS within the SIRIUS framework to predict chemical classes, and prediction performance was benchmarked against ground truth. We present the overall performance and specify the predictivity for individual compounds classes. We then generated similarity networks using modified cosine, modified Jaccard, MS2DeepScore, and DreaMS, clustered them with six algorithms, and systematically evaluated combinations based on annotation entropy and ontology dispersion across communities.

Using the optimal combination of similarity scoring and clustering, we annotate network communities with shared substructures and predicted biotransformations derived from mass differences. This structural information is then translated into related Gene Ontology terms, enabling direct integration with transcriptomic data.

With this setting, we reanalyzed a published metabolomics–transcriptomics dataset from Arabidopsis thaliana under high-light stress. Our results demonstrate that community-level molecular network analysis enhances the discovery of biologically meaningful gene–function relationships.

★ AWARD WINNERS

4. MULTI-OMICS	Monday, June 22	1:30 p.m. - 3:00 p.m.

4.4 **2:25 p.m. - 2:40 p.m.** **Abstract 98**

Annotation-independent construction of disease similarity networks from untargeted multi-cohort breath metabolomics

PRESENTING AUTHOR: ★ ***Britta Brugger, University Basel, Switzerland***

CO-AUTHORS: *Xue Li, Jianzhou Yao, Mélina Richard, Kapil Dev Singh, Xin Luo, Pablo Sinues*

SUB-THEME: Metabolic networks

Understanding relationships between diseases is central to systems medicine, yet metabolomics remains under-integrated in disease similarity frameworks due to pervasive annotation gaps in untargeted datasets. This limitation is particularly pronounced in breath metabolomics, where secondary electrospray ionization–high-resolution mass spectrometry (SESI–HRMS) generates thousands of reproducible but largely unidentified features. To address this challenge, we developed an annotation-independent framework that constructs disease similarity networks directly from feature-level metabolomics data.

We analyzed a multi-cohort dataset comprising 2,333 breath samples collected between May 2021 and January 2024 across seventeen disease conditions and healthy controls. Following preprocessing and ComBat batch correction, technical variance was reduced (R^2 from 53.2% to 3.0%) yielding 7,357 labelled metabolic features for downstream analysis. To extract biologically meaningful signal, we implemented a two-stage statistical pipeline. First, a global Kruskal–Wallis filter identified metabolites differing across at least one condition. Label permutation (scrambling while preserving group size) confirmed that structure exceeded random expectation. Second, Dunn's post-hoc testing combined with effect size filtering (fold change) and FDR control (q The resulting disease similarity network revealed two dominant metabolic axes. One cluster grouped respiratory and autonomic conditions, while a second axis linked urogenital, renal, and neurodegenerative diseases characterized by shared oxidative and inflammatory signatures. Subclinical kidney dysfunction occupies an intermediate position between health and overt disease, whereas advanced chronic kidney disease diverges markedly from both healthy controls and other disease states.

This work demonstrates that large-scale untargeted metabolomics data can support reproducible cross-disease network modelling without reliance on metabolite annotation. By leveraging high-dimensional feature space directly, our framework provides a scalable and annotation-independent strategy to advance systems-level disease biology and integrate metabolomics into disease similarity research.

4.5 **2:40 p.m. - 3:00 p.m.** **Abstract 254**

Inflammation Reprograms the Liver Lipidome and Metabolome: A Multi Omics Map of LPS Response

PRESENTING AUTHOR: ***Francisco Javier Rupérez, Universidad San Pablo CEU, Spain***

CO-AUTHORS: *Sara Moreno, Alejandra Delgado, Irene Blázquez, David Chamoso, Ángeles López-Gonzálvez, Francisco J. Rupérez*

SUB-THEME: Immunometabolism

Acute systemic inflammation profoundly disrupts liver metabolism, yet a comprehensive view of the associated metabolic reprogramming remains challenging due to the chemical diversity of hepatic metabolites. Here, we applied an integrated multi omics strategy with a strong emphasis on metabolomics to characterize liver remodeling in a murine model of lipopolysaccharide (LPS) induced inflammation.

Liver samples from LPS treated mice and controls were analyzed using data independent acquisition proteomics, RPLC MS based lipidomics, and an extensive multiplatform metabolomics workflow specifically designed to maximize coverage of polar metabolites. Untargeted metabolomics combined complementary analytical platforms, including HILIC MS, CE MS and GC MS, enabling broad interrogation of amino acids, organic acids, central carbon intermediates and other highly polar compounds that are poorly captured by single platform approaches.

SESSION 4. MULTI-OMICS	Monday, June 22	1:30 p.m. - 3:00 p.m.

Metabolomic analyses revealed robust separation between inflamed and control livers across all platforms and identified more than 60 significantly altered metabolites, indicating a profound reorganization of hepatic metabolism during inflammation. These changes were consistent with a coordinated metabolic shift affecting redox balance, energy metabolism and nitrogen handling. Lipidomic profiling further demonstrated marked remodeling of the hepatic lipidome, characterized by decreased lysophospholipids and fatty acids alongside increased carnitine species, suggesting enhanced lipid mobilization and adaptive energy metabolism. In parallel, proteomic data revealed extensive modulation of pathways related to innate immunity, including complement and coagulation cascades, phagosome formation, leukocyte migration and extracellular matrix interactions.

By integrating proteome, lipidome and polar metabolome, this study provides a comprehensive molecular map of LPS induced hepatic inflammation. Our results highlight the value of combining orthogonal metabolomics platforms to achieve a more complete representation of the inflamed liver metabolome and to better understand immune–metabolic coupling in inflammatory liver dysfunction.

Visit us at Booth P2

Connect with the Bruker team and learn...

...how Bruker's integrated metabolomics portfolio combines **TIMS-enabled mass spectrometry, biocrates standardized kits, and automated NMR solutions** to accelerate comprehensive, high confidence metabolomics and lipidomics research.

Join our lunch seminar

June 23, 12:20pm – 1:20pm

PD Dr. Michael Witting

Deputy Head Metabolomics and Proteomics Core and Executive Manager Metabolomics, Helmholtz Munich, Munich, Germany

'What's for dinner?'

Julien Wist

Professor of Computational Spectroscopy, Universidad del Valle, Valle del Cauca, Colombia

'Phenomic Medicine'

★ AWARD WINNERS

SESSION 5. STANDARDIZATION AND MULTI-SITE VALIDATION	Monday, June 22	1:30 p.m. - 3:00 p.m.

5.1 **1:30 p.m. - 1:50 p.m.** **Abstract 117**

Mapping the Blood Microsample Metabolome: A Multi-Platform Inter-Laboratory Study

PRESENTING AUTHOR: ★ ***Daniel Marques De Sa e Silva, Aristotle University of Thessaloniki, Greece***

CO-AUTHORS: *Daniel Marques de Sa e Silva, Marlene Thaitumu, Alexandra Tiganouria, Ana Sanchez Lorenzo, Dennisse Avella, Sara Londoño-Osorio, Pauline Couacault, Philippine Louail, Elisabeth Want, Kati Hanhineva, Coral Barbas, Michael Witting, Johannes Rainer, Georgios Theodoridis, Helen Gika*

SUB-THEME: At-home sample collection

Blood microsampling (BμS) has emerged as a promising approach for analyzing endogenous metabolites. Different BμS devices are currently available, and although they share similar concepts for sample collection, matrix variations may affect analytical outcomes. While some untargeted studies have been published, research comparing these devices to conventional matrices remains predominantly targeted; notably, there is a lack of studies specifically evaluating the qualitative annotations in untargeted workflows. This study aims to provide a comprehensive metabolic and lipidomic map of three BμS devices, namely Dried Blood Spots (Whatman), Volumetric Absorptive Microsampling (Mitra), and Capitainer cards, benchmarked against traditional plasma and whole blood.

For this purpose, venous blood, plasma, and BμS samples from ten fasting volunteers were analyzed across five laboratories. Using a multi-platform approach (RP-LC-MS/MS, HILIC-LC-MS/MS, GC-MS, and CE-MS), laboratories analyzed the samples using their own in-house workflow and databases. Manually curated level 1 and 2 annotations were harmonized using RefMet and ChEBI identifiers to access the metabolic and pathway coverage of the different matrices.

A total of 384 metabolites were collectively annotated. High comparability between matrices was observed, with 273 metabolites (71%) detected across all five compared sample types. 24 metabolites were unique to BμS and absent in blood and plasma. Lipidomic analysis identified 37 distinct lipid subclasses, with phosphatidylcholines and sphingomyelins being most abundant. While profiles were largely similar across matrices, some compounds were device-specific, such as unique lipid species captured only by the Capitainer device.

Overall, the results demonstrate that BμS devices provide a highly comparable metabolic and lipidomic coverage to traditional blood samples. Furthermore, this study contributes to advancing the field by providing an initial qualitative reference (indicating presence or absence of metabolites/lipids) for the application of BμS in large-scale metabolic studies.

5.2 **1:50 p.m. - 2:05 p.m.** **Abstract 486**

SCIEX: Quant-ready untargeted metabolomics through scanning-Q1 DIA: ZT Scan DIA

PRESENTING AUTHOR: ***Paul Baker, SCIEX, United States***

As untargeted metabolomics studies scale in sample number and chemical complexity, maintaining quantitative confidence becomes increasingly challenging. While data-dependent acquisition (DDA) provides selective MS/MS, its stochastic nature limits reproducibility and quantitative robustness. Conventional data-independent acquisition (DIA) improves coverage and consistency but introduces chimeric fragmentation that undermines precursor–fragment specificity and fragment-level quantitation.

ZT Scan DIA was developed to address this trade-off by introducing a scanning Q1 dimension that preserves precursor information and enables post-acquisition deconvolution. ZT Scan DIA 2.0 demonstrated that this approach substantially reduces chimeric MS/MS relative to conventional DIA, improving library matching and enabling MS/MS-level quantitative measurements across complex metabolomic datasets. However, increasing feature density in large-scale studies continued to challenge precursor selectivity and quantitative precision.

Here, we describe the quantitative advances introduced with ZT Scan DIA 3.0 on the ZenoTOF platform. By increasing acquisition speed and enhancing Q1 selectivity, ZT Scan DIA 3.0 narrows effective isolation widths while maintaining comprehensive MS/MS coverage. This further reduces spectral interference in crowded chemical space, producing cleaner fragment ion chromatograms that are inherently suitable for quantitation.

★ AWARD WINNERS

SESSION 5. STANDARDIZATION AND MULTI-SITE VALIDATION	Monday, June 22	1:30 p.m. - 3:00 p.m.

Using NIST SRM 1950 plasma as a benchmark matrix, ZT Scan DIA 3.0 shows improved identification quality compared with both DDA and ZT Scan DIA 2.0, driven by reduced chimericity rather than increased fragmentation. Importantly, these gains translate directly to quantitative performance, with MS/MS-based measurements exhibiting high signal-to-noise ratios, low coefficients of variation, and strong agreement with targeted high-resolution quantitative workflows. The ability to retrospectively extract quantitative data from untargeted datasets further supports cohort-scale and longitudinal study designs.

Together, these results demonstrate that scanning-Q1 DIA enables quant-ready untargeted metabolomics, integrating discovery, confident identification, and robust quantitation within a single acquisition strategy.

5.3	2:05 p.m. - 2:25 p.m.	Abstract 94

Hospital-derived pooled plasma reference material for clinical metabolomics

PRESENTING AUTHOR: ★ ***Barbora Pisklakova, Oslo University Hospital, Norway***

CO-AUTHORS: *Barbora Pisklakova, Aleš Kvasnička, Sander Johannes Thorbjørnsen Guttorm, Elise Sandås Sand, Hanne Bendiksen Skogvold, Helge Rootwelt, Katja Benedikte Prestø Elgstøen*

SUB-THEME: Standardization, Reproducibility, QA/QC and ring trials

The metabolomics community relies on commercially available reference materials, such as NIST SRM 1950 plasma (NIST-plasma) for harmonization, long-term quality control monitoring, and method validation. However, this material has several limitations: donors represent only healthy population of a narrow age range (40-50 years) and NIST-plasma is expected to expire in 2028. Since many clinically relevant metabolites are abundant only in specific pathological conditions, clinical metabolomics cannot rely solely on this source and must develop alternative strategies. Our objective was to create a new plasma reference material for long-term quality control monitoring that reflects the complexity of hospitalized patients and benchmark its metabolite/xenobiotic coverage to NIST-plasma.

The preparation of the new reference plasma material for long-term quality control (Oslo-LTQC) was done according to ISO/TR 33402:2025. The Oslo-LTQC was then characterized based on ISO 33405:2024 by certified laboratory analyses at Oslo University Hospital and by global metabolomics (PMID:34296888), and lipidomics (PMID:40825638). In addition, several batch-correction methods were tested. Acquired data from global metabolomics and lipidomics was processed using Compound Discoverer 3.4.

The Oslo-LTQC represents the biochemical complexity of samples from almost 4,000 individuals (aged 0–94 years) encompassing nearly all diagnoses and treatments from one of Europe's largest hospitals. Compared to NIST-plasma, we observed 36% and 15% increase in the number of detected compounds in positive and negative ionization mode, respectively. Moreover, many endogenous and exogenous compounds were detected only in Oslo-LTQC (e.g. 4-hydroxyphenylacetate, N-acetyltryptophan, xenobiotics). In addition, several clinically relevant endogenous metabolites showed significant increase compared to NIST-plasma (e.g. bile acids: log2FC=1.6, p-value=1.3x10-10 for glycochenodeoxycholic acid). Based on global metabolomics and lipidomics results, Oslo-LTQC showed more similar metabolic profiles with samples from clinical studies.

Without reference materials and harmonized quality control, clinical application of metabolomics assays is not feasible. Developing a sustainable, metabolically rich reference material significantly contributes to implementing metabolomics in clinical laboratories.

SESSION 5. STANDARDIZATION AND MULTI-SITE VALIDATION	Monday, June 22	1:30 p.m. - 3:00 p.m.

5.4 **2:25 p.m. - 2:40 p.m.** **Abstract 483**

WATERS: Advancing High-Resolution LC-MS Methods for High Sensitivity Metabolomics

PRESENTING AUTHOR: ***David Heywood, Waters Corporation, United Kingdom***

SUB-THEME: Single-cell Metabolomics

The power of measuring biology at the single cell level is within grasp. In our technology presentation we will discuss how new instrument developments combine with novel sample introduction and chromatographic approaches to push the boundaries of LC-MS methods for high sensitivity applications in Metabolomics and Lipidomics. We will discuss solutions and workflows that span high coverage characterisation, high-throughput and spatial mapping of metabolites and lipids at single-cell sample sizes.

5.5 **2:40 p.m. - 3:00 p.m.** **Abstract 142**

Preservation of Biological Variation Across Laboratories: A Fed–Fasted Multi-Site Ring Trial

PRESENTING AUTHOR: ***Anne Evans, Metabolon, United States***

CO-AUTHORS: *Janice C. Jones, Brad Cochran, Matthew Mitchell, Ray Moran, Richard Robinson*

SUB-THEME: Standardization, Reproducibility, QA/QC and ring trials

Metabolomics has long been constrained by limited methodological concordance across laboratories, limiting dataset comparability and slowing adoption in multi-site and large-cohort studies. We present a metabolomics profiling kit, as a decentralized method designed to establish a global standard for reproducible metabolomics. By embedding harmonized analytical methods, data processing and annotation methods optimized and refined over 25 years, and rigorous QA/QC into a scalable 96-well format, this kit enables consistent metabolite detection across studies and sites while preserving biological signal integrity. The workflow uses validated C18 positive- and negative-ionization methods with embedded system suitability testing (SST), plate level QC (PQC), and pooled reference materials on every plate. Raw data are processed through Metabolon's proprietary machine learning–driven annotation pipeline and returned via the Integrated Bioinformatics Platform (IBP), ensuring consistent feature alignment, identification confidence scoring, and statistical traceability. To evaluate inter-laboratory reproducibility, a global ring trial was conducted in which identical samples from a controlled fed–fasted study (40 plasma and 40 urine samples per site) were distributed to participating laboratories across North America, Europe, and Asia. All laboratories satisfied the predefined kit performance criteria for each matrix, detecting an average of approximately 730 metabolites in both plasma and urine, each with a median CV below 20%. The median correlation of metabolite abundances across biologically diverse samples was > 0.85 between labs. Fed–fasted differences were preserved across sites, with median Pearson correlations for fold-changes of 0.90 and consistent pathway-level enrichment concordance. Biologically expected shifts in amino acid, lipid, bile acid, and TCA cycle pathways were reproducibly observed. PCA showed that samples clustered by physiological state rather than laboratory origin, with clear separation of biological and technical variance. This kit enables reproducible, comparable metabolomics at a global scale, supporting collaborative consortia, strengthening confidence in cross-study data integration, and accelerating biological interpretation across diverse translational research.

SESSION 6. MICROORGANISMS	Monday, June 22	1:30 p.m. - 3:00 p.m.

6.1 **1:30 p.m. - 1:50 p.m.** **Abstract 280**

Complex metabolism in a simple system: cell-free metabolomics

PRESENTING AUTHOR: ***Mark Styczynski, Georgia Institute Of Technology, United States***

CO-AUTHORS: *Soor Vora, April Miguez, Yan Zhang, Monica McNerney*

SUB-THEME: Microbial metabolomics

One emerging biotechnology with significant promise is cell-free synthetic biology: the in vitro use of cell lysates to perform transcription and translation, with applications from biosensing to biomanufacturing. In principle, these lysates are simple and straightforward to control because their native programming (the genome) has been removed; in practice, large portions of the proteome come along with the desired expression machinery, causing poorly-defined side reactions that make cell-free systems less effective at protein expression and harder to engineer for impactful applications. In particular, residual metabolic activity likely has a major impact on the energy- and resource-intensive processes of transcription and translation.

We used GCxGC-MS and GC-MS for systems-scale time-course analysis of metabolic dynamics, with an emphasis on central carbon metabolism and amino acid metabolism. We measured protein expression via GFP fluorescence and used multivariate and univariate statistical analysis approaches to gain significant insights into the relationship between cell-free metabolism and expression.

We showed that in some cases, the impact of the residual metabolic activity on the metabolism was atually greater than the impact of protein expression. We showed that oxygenation has major impacts on both cell-free expression and cell-free metabolism, with those two processes fundamentally linked. We also showed that multiple metabolic pathways have critical impacts on the ability of cell-free systems to express protein, with amino acid metabolism causing unexpected metabolic dyanmics and protein epxression dynamics. Surprisingly, adding more reaction reagents throughout the reaction had a significant positive effect on protein expression, indicating some type of substrate inhibiition.

Taken together, these results provide a deeper understanding of the metabolism in cell-free systems that will enable us to better optimize and engineer them for critical biotechnological applications including biosensing and biomanufacturing.

6.2 **1:50 p.m. - 2:05 p.m.** **Abstract 488**

SHIMADZU: How DIA with mass stability enhances your metabolomics workflow

PRESENTING AUTHOR: ***Laudicéia Alves de Oliveira, Shimadzu, Brazil***

Mass accuracy is an important feature for LCMS metabolomics studies for it provides assurance for the identification and validation of biomarkers. However, metabolomics workflows usually envolve long batches of samples within a few days of analysis. Also, DIA analysis although comprehensive, produces data that are not easy to process. In this work, we show a complete workflow for vegetal metabolomics using LCMS-9050, with mass accuracy stability of more than a week and easy DIA data processing capabilities.

★ **AWARD WINNERS**

SESSION 6. MICROORGANISMS	Monday, June 22	1:30 p.m. - 3:00 p.m.

6.3 **2:05 p.m. - 2:25 p.m.** **Abstract 233**

Metabolic plasticity of an Andean lichen mycobiont (Stereocaulon sp.) revealed by untargeted LC-MS and GC-MS metabolomics

PRESENTING AUTHOR: ★ ***Christian Matías, Pontificia Universidad Católica del Perú, Peru***

CO-AUTHORS: *Erika Calla-Quispe*

SUB-THEME: Microbial metabolomics

Lichens represent complex symbiotic mutualistic associations where the mycobiont governs structural development and secondary metabolite production. However, the metabolomic diversity of Andean lichens remains largely unexplored, particularly under controlled in vitro conditions. This study aimed to evaluate how different nutritional environments influence the metabolic expression and chemical plasticity of an isolated mycobiont from the lichen Stereocaulon sp., and to assess which metabolites associated with the natural lichen thallus can be produced in axenic culture. The mycobiont was axenically isolated from spores obtained from apothecia of natural thalli, germinated, and maintained in three liquid media differing in carbon sources such as Lilly–Barnett with 10% glucose (LBMG), Lilly–Barnett with 10% mannitol (LBMM), and Malt Yeast Extract (MYE). Untargeted metabolomic profiling of cultured mycobiont extracts and lichen thalli was performed using gas chromatography–mass spectrometry (GC–MS/EI) and ultra-high-performance liquid chromatography–mass spectrometry (UHPLC–MS/HESI). These complementary analytical platforms enabled comprehensive coverage of volatile and semi-polar metabolites and facilitated comparative metabolome characterization across various growth conditions. Comparative metabolomic analysis revealed pronounced shifts in molecular feature profiles depending on culture medium composition. Several metabolites detected in the lichen thallus were absent in axenic cultures, suggesting that specific biosynthetic pathways may require symbiotic interactions or environmental triggers for full expression. In contrast, distinct metabolic signatures were observed in vitro, indicating that nutrient availability can reprogram mycobiont metabolic pathways. The results highlight the metabolic plasticity and biosynthetic potential of lichen-forming fungi and demonstrate how external nutritional cues can significantly modulate secondary metabolite production. This study provides one of the first untargeted metabolomic characterizations of an Andean lichen mycobiont, contributing to the understanding of lichen metabolic diversity and offering new perspectives for exploring their chemical potential under controlled cultivation conditions.

6.4 **2:25 p.m. - 2:40 p.m.** **Abstract 41**

Substrate-Driven Metabolomic Reprogramming and Bioactive Discovery in Lactic Acid Bacteria

PRESENTING AUTHOR: ★ ***Golnaz Heidari, Massey University, New Zealand***

CO-AUTHORS: *Pat Edwards, Paul Plieger, Gareth Rowlands, Andrew Sutherland-Smith, Wayne Young*

SUB-THEME: Microbial metabolomics

Microbial fermentation generates diverse health-relevant metabolites, yet systematic strategies linking fermentation inputs to bioactive output remain underdeveloped. Here, we applied untargeted metabolomics to map how carbon and nitrogen sources reshape the metabolome of a lactic acid bacterium, with the goal of discovering condition-dependent bioactives and connecting substrate selection to pathway-level mechanisms.

The bacterium was cultured on four carbon sources (glucose, sucrose, mannitol, xylose) combined with two nitrogen sources (yeast extract, pea peptone). Metabolite profiling of culture supernatants was performed using HILIC LC–MS/MS and Compound Discoverer. Peak annotations were manually curated to ensure high confidence. Compounds were annotated at level 1 (in-house standards) and level 2 (reference libraries). MetaboAnalyst was used for pathway analysis. Multivariate analysis (PCA), volcano plots, and pathway enrichment were used to characterize substrate-driven metabolomic shifts. High-priority metabolites were shortlisted by effect size across conditions and screened in silico for bioactivity using PASS.

A total of 550 metabolites were confidently identified. PCA revealed distinct metabolomic clustering driven by both carbon and nitrogen sources, while volcano plot analysis identified significant substrate-dependent up- and down-regulation patterns. Pathway analysis revealed that pyrimidine, purine, and tyrosine metabolism were consistently impacted across all substrates, while vitamin B6, tryptophan, and D-amino acid metabolism were selectively enriched under specific carbon

★ AWARD WINNERS

SESSION 6. MICROORGANISMS	Monday, June 22	1:30 p.m. - 3:00 p.m.

sources, demonstrating substrate-driven metabolic reprogramming. Notably, chemical diversity analysis revealed substrate-responsive accumulation of structurally diverse metabolite scaffolds across multiple compound families. In silico bioactivity screening of prioritized metabolites identified predicted activities spanning inflammation and immune modulation, oxidative stress, cytoprotection, neurological signaling, and anti-infective defense, among others.

This study establishes a metabolomics-guided framework for rationally designing fermentation conditions to maximize bioactive production. By linking substrate inputs to pathway-level metabolic reprogramming and functionally prioritized targets, this approach enables knowledge-driven selection of fermentation conditions for functional food and nutraceutical development.

6.5	2:40 p.m. - 3:00 p.m.	Abstract 257

Metabolomic evidence of fungal signatures associated with soybean seed rot in Brazil

PRESENTING AUTHOR: ★ ***Paula Derksen Macruz, State University of Maringá, Brazil***

CO-AUTHORS: *Paula Derksen Macruz, Esteban Charria-Girón, Mauricio Conrado Meyer, Francismar Correa Marcelino Guimaraes, Justin J. J. van der Hooft, Eduardo Jorge Pilau*

SUB-THEME: Agro-industrial crops and traditional foods

Soybean (Glycine max) is one of the most important crops worldwide, and Brazil is currently the largest global producer. Since 2019, a new disease characterized by soybean seed rot has been reported in Brazilian crop fields, with the causal pathogen still unclear. Understanding the biochemical processes associated with this disease is essential to support early diagnosis and disease management strategies. In this study, an untargeted metabolomics approach based on liquid chromatography–high-resolution tandem mass spectrometry (LC–HRMS/MS) was applied to investigate metabolic differences between healthy and rotten soybean seeds. Metabolomic data were processed using MZmine and statistically evaluated using MetaboAnalyst and FERMO prioritization. Metabolite annotation and structural relationships were explored through feature-based molecular networking using GNPS2, complemented by MS2Query and SIRIUS predictions. Multivariate analysis revealed distinct metabolic profiles between healthy and rotten soybean seeds. Several metabolites associated with fungal metabolism were detected, including fusaric acid and beauvericin, two well-known mycotoxins produced by Fusarium species. Importantly, all Fusarium-associated metabolites, including fusaric acid, beauvericin, and a cluster of fusaric acid analogues such as predicted dehydrofusaric acid and related derivatives, were detected exclusively in rotten soybean seeds. These findings suggest active fungal metabolism associated with disease development. In addition to fungal metabolites, several host-derived defense compounds were identified, including isoflavones (daidzein and genistein) and triterpenoid saponins (soyasaponins), suggesting activation of phenylpropanoid-derived defense pathways. Metabolites associated with plant hormonal regulation, such as indole-3-acetic acid, were also observed, indicating possible hormonal modulation during plant–pathogen interaction. These results reveal distinct metabolomic signatures associated with rotten soybean seeds and provide chemical evidence of active fungal metabolism during disease development. The exclusive detection of Fusarium-associated metabolites in diseased seeds, together with host defense compounds, highlights potential metabolomic biomarkers and provides new insights into plant–pathogen interactions involved in this emerging soybean disease.

SESSION 7. NEUROLOGICAL DISORDERS	Monday, June 22	3:30 p.m. - 5:15 p.m.

7.1 **3:30 p.m. - 3:50 p.m.** **Abstract 140**

Rapid Ambient Metabolomic Profiling of Sebum for Parkinson's Disease Biomarker Discovery Using Paper Spray Ionization-Ion Mobility Mass Spectrometry

PRESENTING AUTHOR: ***Breanna Dixon, University Of Manchester, United Kingdom***

CO-AUTHORS: *Depanjan Sarkar, Lea van Dissel, Minhui Zhu, Eleanor Sinclair, Monty Silverdale, Johannes PC Vissers, Perdita Barran*

SUB-THEME: Neurological disorders

Parkinson's disease (PD) is the fastest-growing neurological disorder worldwide, yet clinical diagnosis still relies on motor symptoms that emerge late in disease progression. There is a critical need for molecular biomarkers capable of detecting PD earlier. Sebum, the lipid-rich oily secretion of skin, is an underexplored biofluid that is non-invasively accessible and exhibits increased production in PD. Previous studies have shown that sebum contains compounds reflective of mitochondrial dysregulation in PD, highlighting its potential as a source of biomarkers obtainable through non-invasive sampling.

Here, we performed a direct metabolomic analysis of sebum in its native state. Data was obtained using paper spray ionization coupled with ion mobility mass spectrometry (PS-IM-MS) then analysed with the aim of identifying metabolic signatures which discriminate PD from controls. PS-IM-MS combines the rapid, minimal-preparation sampling of ambient ionization with high-resolution ion mobility separation, providing reproducible drift time measurements alongside m/z values and enhancing the analysis of complex species. A custom PS source was designed and 3D-printed, enabling efficient sample transfer from medical Q-tip swabs via a "touch and roll" approach onto paper triangles for analysis on a SELECT SERIES cyclic IMS instrument.

We analysed 275 clinical samples (PD n=147; controls n=128) and performed multivariate analysis using Elastic Net and Random Forest algorithms for feature selection and model training. A predictive panel of metabolite features was validated in test datasets, with structural identities assigned through accurate mass, collision cross-section values, and MS/MS confirmation. Pathway analysis was performed and revealed alterations in lipid-related pathways.

This study demonstrates that PS-IM-MS enables rapid, high-throughput metabolomic profiling of sebum with minimal sample preparation, capturing lipid signatures that discriminate PD from controls. The integration of ambient ionization and ion mobility offers a promising platform for non-invasive PD biomarker discovery, potentially supporting early diagnosis and monitoring of disease progression in clinical settings.

7.2 **3:50 p.m. - 4:10 p.m.** **Abstract 267**

Combining spatial metabolomics and transcriptomics to understand long term changes in the brain following a TBI

PRESENTING AUTHOR: ***Alex Dickens, University Of Turku, Finland***

CO-AUTHORS: *Matej Oresic, Daniel Anthony, Dominika Olesova, Ilia Evstafev*

SUB-THEME: Neurological disorders

Traumatic brain injury (TBI) is a leading cause of injury-related death and disability worldwide, affecting an estimated 64–74 million people each year. Although most individuals with mild TBI recover within weeks, approximately 15–30% experience persistent cognitive, behavioural, and physical symptoms that can last months or even years. Increasingly, TBI is therefore recognised as a chronic condition, with long-term neurological dysfunction thought to be partly driven by a post-injury metabolic "energy crisis." Understanding the cellular and metabolic changes that occur after injury is essential for identifying mechanisms that contribute to long-term pathology. In this study, we investigated spatially resolved molecular changes in the mouse brain following mild TBI.

A controlled cortical impact (CCI) model was used to induce mild TBI in the left cortex of mice. Injury was delivered using a linear impactor following craniotomy. Animals were sacrificed at 24 hours and three weeks post-injury. Spatial metabolomics

SESSION 7. NEUROLOGICAL DISORDERS	Monday, June 22	3:30 p.m. - 5:15 p.m.

were performed using a MALDI-2 timsTOFflex mass spectrometry platform. Spatial transcriptomics was conducted on sequential tissue sections using the 10x Genomics Visium platform.

Despite the injury being classified as mild, behavioural testing revealed anxiety-like behaviour persisting up to three weeks after injury, indicating lasting functional effects. Spatial transcriptomic analysis demonstrated continued immune activity within the brain at this time point. Much of this response was associated with GFAP-expressing astrocytes, alongside evidence of sustained microglial reactivity at the injury site. Spatial lipidomics revealed distinct accumulation patterns of ceramide lipids in the three-week samples, consistent with the presence of fibrotic astrocytes. In contrast, macrophage-associated regions showed increased levels of diacylglycerols and triacylglycerols.

By integrating multiple spatial omics layers, we can resolve cell-type-specific metabolic changes within injured brain tissue. This approach provides mechanistic insight into the metabolic responses following TBI and highlights potential pathways that may represent targets for future therapeutic development.

7.3	4:10 p.m. - 4:30 p.m.	Abstract 121

Does lipid metabolism play a central role in Late Onset Alzheimer's disease? Evidence from human cohorts and murine models

PRESENTING AUTHOR: ***Julian Griffin, University of Aberdeen, United Kingdom***

CO-AUTHORS: *Qi Zhong, Brenan Durainayagam, Rui Pinto, Nirmal Verma, Elaine Holmes, Paul Elliot, Florin Despa, Abbas Dehghan, Julian L. Griffin*

SUB-THEME: Neurological disorders

Introduction: Many of the genes associated with late onset Alzheimer's disease (AD), including ABCA1, ABCA7, Trem2 and ApoE, are also associated with lipid metabolism. Previously we applied metabolome wide association studies (MWAS) with single nucleotide polymorphs (SNPs) known to be associated with AD in two European cohorts of ~2600 people with metabolomic data. We identified strong associations between SNPs in ABCA7 and lactosyl-ceramides, and SNPs in ApoE and arachidonic acid (ARA) containing lipids.

Aim: To determine the mechanistic basis of the associations between lipid metabolism and SNPs in ABCA7 and ApoE.

Methods: Open profiling ultra high-performance liquid chromatography ion mobility mass spectrometry was applied to mouse and cell culture models of late onset AD.

Results: Pursuing the associations identified for ABCA7, we demonstrated using Mendelian randomisation that ceramides were on the causative pathway for AD-risk, while in brain tissue of ABCA7 mice, sphingolipid metabolism was altered particularly for sphingomyelins, ceramides, and hexosyl-ceramides. To identify which cells were most affected we studied microglia, demonstrating that hexosyl-ceramides increase in LPS-activated microglia, implicating sphingolipid metabolism in the pro-inflammatory state of the AD-brain.

To examine associations between ApoEe4 (associated with increased AD-risk) and ARA containing phospholipids, we performed untargeted lipidomic profiling of brain, plasma, and liver from humanised APOE knock-in mice overexpressing human islet amyloid polypeptide (hIAPP) which promotes amyloid plaque formation in the brain. In the 6-month APOE4/4 hIAPP mice, docosahexaenoic acid and ARA-containing glycerophospholipids, including plasmalogens, were decreased in the brain compared with control strains. The lipids impacted are similar to those described previously for lysosomal storage disorders, suggesting that ApoEe4 lipid alterations may be caused by impaired lysosome/endosome processing of lipids.

Conclusion: Lipidomics of human and mouse models of late onset AD highlight profound changes in lipid metabolism that contribute to disease progression, although via fundamentally different mechanisms.

SESSION 7. NEUROLOGICAL DISORDERS	Monday, June 22	3:30 p.m. - 5:15 p.m.

7.4 **4:30 p.m. - 4:50 p.m.** **Abstract 88**

Unravelling metabolic drivers of neurodegeneration: Cross-species convergence from a Leigh syndrome model

PRESENTING AUTHOR: ***Roan Louw, North-West University, South Africa***

CO-AUTHORS: *Karin Terburgh, Janine Scholefield*

SUB-THEME: Neurological disorders

Selective neuronal vulnerability is a hallmark of neurodegenerative disease, yet the metabolic determinants that dictate why specific brain regions degenerate remain poorly understood. Here, we leverage Leigh syndrome (LS), a genetically defined mitochondrial disorder caused frequently by complex I deficiency, as a tractable human model to dissect core metabolic mechanisms of neurodegeneration.

Using semi-targeted and untargeted metabolomics, we profiled discrete brain regions, skeletal muscle, and biofluids from the Ndufs4 knockout mouse of LS. Lesion-prone regions exhibited pronounced NADH/NAD^+ disequilibrium, disrupted branched-chain amino acid catabolism, tricarboxylic acid (TCA) cycle remodelling, and altered one-carbon metabolism. In contrast, lesion-resistant regions maintained relative metabolic stability, revealing region-specific metabolic changes associated with vulnerability versus resilience.

To bridge these findings to human biology, we generated a CRISPR/Cas9-engineered NDUFS4 knockout induced pluripotent stem cell (iPSC) model—the first of its kind derived from an African genetic background, addressing a critical gap in disease modelling diversity. Metabolomic profiling of these iPSCs recapitulated key redox and TCA-linked metabolic disruptions observed in vivo. Importantly, independent metabolomic data from mitochondrial disease patients demonstrated strong concordance with the signatures identified in both mouse and iPSC systems, validating the translational relevance of these models.

Collectively, this work positions metabolomics as a systems-level framework for linking experimental models to human neurodegenerative pathology. By integrating animal models, genetically defined human iPSCs, and patient metabolomes, we demonstrate conserved metabolic reprogramming underlying neuronal vulnerability. Beyond LS, this approach establishes a scalable blueprint for interrogating metabolic drivers of neurodegeneration and highlights the power of diverse stem cell models to accelerate biomarker discovery and therapeutic targeting.

7.5 **4:50 p.m. - 5:10 p.m.** **Abstract 169**

UPLC-MS/MS Method to Evaluate a Neurotransmitter Panel in Women Using Cannabis During Pregnancy

PRESENTING AUTHOR: ***Corentin Fracapane, Université de Sherbrooke, Canada***

CO-AUTHORS: *Virginie Gillet, Annie Ouellet, Christiane Auray-Blais*

SUB-THEME: Pregnancy, maternal and neonatal health

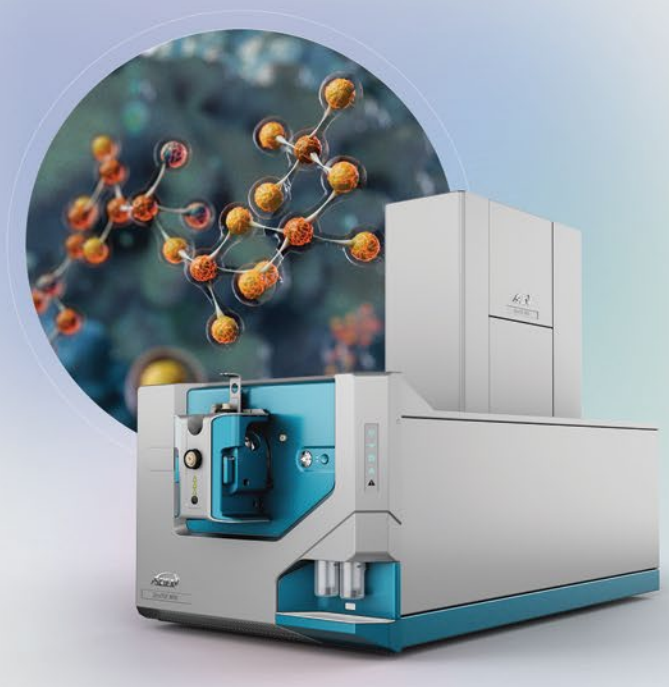
Cannabis use, especially during pregnancy, has increased during recent years. Δ9-tetrahydrocannabinol (THC), the main psychoactive compound of cannabis, acts through cannabinoid type-1 (CB1) receptors widely expressed in the central nervous system and modulates several neurotransmitter systems. These interactions may influence cognitive function and mental health. However, current analytical approaches generally quantify neurotransmitters individually, limiting the assessment of coordinated neurochemical changes. Moreover, no studies have evaluated systemic neurotransmitter alterations during pregnancy in relation to cannabis exposure. To address these limitations, we have developed and validated an innovative multiplex robust ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) method for the simultaneous quantification of six neurotransmitters, mainly γ-aminobutyric acid, L-glutamate, serotonin, epinephrine, dopamine, and acetylcholine, in plasma. The method was validated according to Food and Drug Administration (FDA) bioanalytical guidelines and applied to plasma samples collected at 35 weeks of gestation from 48 pregnant participants, including 19 cannabis-exposed and 29 control women. Sample preparation involved protein precipitation followed by chromatographic separation on an Atlantis Premier BEH Z-HILIC Vanguard FIT column (2.5 µm, 2.1

SESSION 7. NEUROLOGICAL DISORDERS	Monday, June 22	3:30 p.m. - 5:15 p.m.

mm x 100 mm, Waters Corp.) coupled to an Acquity I-Class Xevo TQ-XS (Waters) triple quadrupole mass spectrometer operating in positive electrospray ionization mode with an 11-min run time. Deuterated internal standards were used for quantification. Calibration curves showed excellent linearity (R2 > 0.995), with limits of quantification ranging from 0.025 to 5 ng/mL and a linear range up to 100 ng/mL. Neurotransmitter reference control values were established. The method demonstrated satisfactory sensitivity, precision, accuracy, and stability for all analytes. Comparative analyses of neurotransmitter profiles between cannabis-exposed and control pregnancies are ongoing and will be presented. In conclusion, this validated multiplex approach provides a novel analytical platform to investigate systemic neurotransmitter profiles associated with cannabis exposure.

SCIEX

The power of precision



Join the SCIEX lunch seminar
Monday, June 22 | 12:20 p.m.
Visit us at Booth #P3

Is your workflow giving you answers or just data?

Kick off Metabolomics 2026 with a proof-driven lunch seminar featuring Nicola Zamboni and Vinicius Verri Hernandes

Confidence you can see.

From routine runs to deeper insight, with more answers per sample.

Speaker highlights:

Vinicius Verri Hernandes
Senior Scientist ,
University of Vienna, Austria

Learn how exposomics-driven metabolomics can **link environmental and lifestyle exposures to measurable small-molecule signatures**, supporting exposure biology and biomarker discovery in population studies.

Nicola Zamboni
Adjunct Professor,
Institute of Molecular Systems Biology, ETH Zurich

Explore how advances in metabolomics and lipidomics are enabling **confident interpretation** and **data quality that holds up as studies scale.**

See how proof-led, workflow-ready performance can strengthen small-molecule analysis as your science evolves.

Skeptics welcome.

danaher

SESSION 8. ANALYTICAL METHODS AND WORKFLOW INNOVATIONS	Monday, June 22	3:30 p.m. - 5:15 p.m.

8.1 **3:30 p.m. - 3:50 p.m.** **Abstract 250**

A Validated Single-Tube Dual-Extraction Platform for Combined Placental Metabolomics and Lipidomics to Investigate In Utero Exposures

PRESENTING AUTHOR: ***Ellen De Paepe, Ghent University, Belgium***

CO-AUTHORS: *Adam Csereszny e, Lieselot Y. Hemeryck, Tiffany De Troyer, Matthijs Vynck, Roger Pero-Gascon, Sarah De Saeger, Tim Nawrot, Adrian Covaci, Lynn Vanhaecke*

SUB-THEME: Novel samples/matrices

From conception onwards, humans are exposed to various internal and external factors, collectively called the exposome, which can trigger metabolic adaptations during the vulnerable in utero period. Despite the placenta's central role in fetal development, few studies comprehensively characterize its metabolome and lipidome.

In this context, we optimized and validated a single-tube dual-extraction protocol using methanol, ultrapure water, methyl-tert-butyl ether and acetonitrile, for combined (semi-)polar metabolomics and lipidomics of placental tissue via UHPLC-Orbitrap-HRMS. A structured Design of Experiments approach was implemented to systematically optimize homogenization and extraction steps to maximize molecular coverage and reproducibility. This approach was based on 55 targeted compounds (log P=-3.24-21.9) and untargeted feature count. All metabolites and most lipids demonstrated excellent linearity (R^2≥0.99), intra-assay (n=10) and inter-day precision (n=20) (CV≤20%). Robust untargeted coverage was achieved, consistently detecting 2,811 metabolite and 5,412 lipid features (CV≤30%).

The platform's applicability was demonstrated using placental samples from the ENVIRONAGE cohort (n=373 mother-child pairs, recruited between 2011-2018). Within the FLEXiGUT project, cord and maternal blood biochemistry, placental telomere length, organic pollutants, mycotoxins, DNA-adductome, lifestyle and dietary intake data were collected. Metabolomics and lipidomics data normalization, and batch correction to account for year of sample collection, were performed using Metanorm and ComBat, followed by covariate adjusted and Benjamini-Hochberg corrected partial Spearman correlation, and linear mixed model analyses.

Foetal sex was associated with creatinine and cortisone, while gestational duration negatively correlated with amino acids (|ρ|>0.15). Maternal lifestyle was also reflected, with trigonelline correlating with fries consumption (ρ=0.43), and maternal smoking with the nicotine metabolite cotinine (ρ=0.33) and tobacco-derived quinic acid (ρ=0.22).

Overall, our method proved suitable for studying different biological factors and exposures in utero. Ongoing pathway and integrative analyses using mummichog, MOFA and DIABLO will further connect prenatal exposures with placental metabolism and early-life outcomes, for which results will be presented.

8.2 **3:50 p.m. - 4:10 p.m.** **Abstract 266**

A tailored HILIC–TIMS–MS strategy to unlock Bis(monoacylglycero)phosphates profiling in lysosomal disorders

PRESENTING AUTHOR: ***Fabrizio Merciai, University of Salerno, Italy***

CO-AUTHORS: *Eduardo Maria Sommella, Pietro Campiglia*

SUB-THEME: Non-targeted and semi-targeted methods

Bis(monoacylglycero)phosphates (BMPs) are negatively charged glycerophospholipids that play a key role in lysosomal membrane organization and trafficking. Their dysregulation is widely recognized as a hallmark of lysosomal storage diseases and increasing evidence links altered BMP homeostasis to rare neurodegenerative conditions, such as Batten disease. Despite their biological relevance, BMPs remain poorly characterized in MS-based lipidomics studies. Their low abundance, unusual physicochemical properties, structural similarity to phosphatidylglycerols complicate both extraction and confident identification. Consequently, a lipidomics workflow specifically optimized to improve BMPs recovery and structural characterization is still lacking. Here, we evaluated lipid extraction strategies together with an LC–TIMS–MS approach to improve BMPs detection and characterization. Human plasma and mouse brain samples were spiked with deuterated BMPs

SESSION 8. ANALYTICAL METHODS AND WORKFLOW INNOVATIONS	Monday, June 22	3:30 p.m. - 5:15 p.m.

and phosphatidylglycerol and subjected to different extraction protocols, including biphasic Folch and Matyash methods as well as single-phase butanol–methanol (Asherley) and isopropanol extractions. Lipid extracts were analyzed using a high-throughput negative-ion HILIC-DDA-PASEF method, while PRM-PASEF was investigated as a pseudo-targeted strategy to enhance MS/MS spectral quality and structural confidence. For deeper structural characterization, offline Paternò–Büchi derivatization was explored using acetone and 6-azauracil. Single-phase extractions consistently outperformed biphasic protocols in terms of BMP recovery and analytical coverage. In particular, the Asherley method provided the most balanced performance, achieving high recovery of labeled BMPs and improved coverage of endogenous species with low intra-assay variability, further enhanced by ammonium acetate supplementation. The optimized HILIC–TIMS–MS workflow enabled sensitive BMP detection, while PRM-PASEF improved the quality of diagnostic fragment ions. Exploratory Paternò–Büchi derivatization showed higher reactivity for 6-azauracil and enabled tentative localization of double-bond positions in BMP species, such as BMP 20:4/22:6 in brain samples. This approach provides a robust framework for improved BMP profiling in biological matrices and may support studies of lysosomal dysfunction in neurodegenerative and lysosomal storage disorders.

8.3	4:10 p.m. - 4:30 p.m.	Abstract 134

Helios2D: A Cell-Preserving Method for Mass Spectrometric Analysis of Living Cells under Near-Native Conditions

PRESENTING AUTHOR: ***Timm Schaefle, University of Tübingen, Germany***

CO-AUTHORS: *Timm Schaefle, Benedikt Loeffler*

SUB-THEME: New developments in instrumentation

In this project, we present a novel plasma-based ion source enabling the mass spectrometric analysis of living cells, without compromising cell viability. This approach allows microorganisms and cells to be cultivated under near-native conditions on agar plates and analyzed directly. The cell-preserving properties enable time-resolved investigation of the metabolic profiles of a sample over several days, permitting molecular changes to be monitored throughout different growth phases. The method can be extended to co-cultures, where competitive interactions may activate otherwise silent gene clusters, facilitating the identification of potentially novel antimicrobial agents or other bioactive metabolites. These close-to-nature analytical conditions permit direct investigation of samples without liquid cultivation, extraction, or other preparative steps prior to analysis.

The method utilizes a closed ion source design with integrated sensors for precise atmospheric control. By establishing an argon atmosphere, the formation of reactive oxygen and nitrogen species, typically generated by cold plasma, is inhibited, thereby eliminating any sterilizing effects. A vertical argon inlet tube, accommodating a small solvent vial, establishes and maintains the argon atmosphere, supports the ion transport, and enables in-situ calibration and in-situ derivatization of analytes. This enhances signal intensities and reveals analytes that would otherwise remain undetected. Furthermore, operating the source at reduced pressure significantly increases sensitivity and expands the range of detectable molecular species.

Using the ion source, monocultures and co-cultures of bacteria and fungi were analyzed repeatedly to investigate their metabolic profiles, revealing distinct metabolic alterations over several days, particularly in the presence of interacting microorganisms.

Additionally, a “Culture Compound Analysis” (CCA) approach was introduced to monitor the cellular metabolic response of microorganisms to bioactive compounds over several hours.

Future experiments include the investigation of human cells, particularly tumor cells, to explore near real-time differentiation between healthy and cancerous cells and to study their metabolic responses to promising anti-tumor agents.

SESSION 8. ANALYTICAL METHODS AND WORKFLOW INNOVATIONS	Monday, June 22	3:30 p.m. - 5:15 p.m.

8.4 **4:30 p.m. - 4:50 p.m.** **Abstract 35**

Workflow optimization for integrated mass spectrometry-based metabolomics and lipidomics from dried blood microsamples

PRESENTING AUTHOR: ***Eleonora Bossi, University Of Milano-bicocca, Italy***

CO-AUTHORS: *Marta Nobile, Simone Serrao, Pierluigi Reveglia, Antonietta Ferrara, Gaetano Corso, Lia Crotti, Gabriella Malfatto, Cornelia Prehn, Michael Witting, Giuseppe Paglia*

SUB-THEME: At-home sample collection

Blood microsampling has attracted interest as a viable alternative to conventional venipuncture for a wide range of applications including metabolomics. It offers several advantages such as minimal invasiveness, ease of collection, suitability for multiple sampling and longitudinal designs1. This study aims to optimize a liquid chromatography-mass spectrometry-based workflow enabling both untargeted metabolomics and lipidomics analyses from a single dried blood spot (DBS). Workflow optimization was also performed for targeted metabolomics on DBS using the TMIC MEGA assay. For the untargeted workflow the optimization was performed on three commercially available microsampling devices: Capitainer, Whatman (whole blood) and Telimmune (plasma). Among the five extraction solutions tested, pure methanol provided the best compromise for simultaneous extraction of polar metabolites and lipids from the same spot. A two-step consecutive extraction protocol was developed: methanol followed by water improved the recovery of more polar metabolite classes and was successfully applied in a clinical study on cardiac rehabilitation2. Short-term stability of polar metabolites and lipids was assessed at room temperature (RT) for up to five days. Stability of all evaluated compound classes was preserved for up to five days at RT in Capitainer, delivering the best performance. Regarding targeted metabolomics workflow optimization, the manufacturer's protocol was modified for both Panel A and Panel B. Compared to the initial tests, an increased metabolite coverage was observed in DBS with 323 detected metabolites. Longitudinal comparison with paired serum samples showed that relative temporal changes were preserved in DBS. Overall, this study suggests that methanol extraction enables integrated metabolomics and lipidomics analysis from a single spot and a two-step approach can further enhance polar metabolite coverage. Targeted protocol optimization on the TMIC MEGA assay also improved metabolome coverage in DBS, highlighting the importance of tailored device selection and extraction protocol optimization based on study aims, matrices, and analytical scope.

8.5 **4:50 p.m. - 5:10 p.m.** **Abstract 166**

A Multiomics Approach to Understanding and Targeting Glioblastoma

PRESENTING AUTHOR: ***Elizabeth Want, Imperial College London, United Kingdom***

CO-AUTHORS: *Nelofer Syed, Harry Whitwell, Brittannie Willis*

SUB-THEME: Cancer

Background: Glioblastoma Multiforme (GBM) is the most common and lethal form of primary brain tumour in adults. Despite radical surgery, radiation and chemotherapy, recurrence is common and life expectancy is months, indicating a clear need to identify novel therapies to improve patient outcomes.

The GBM microenvironment is complex and heterogeneous, with interactions between tumour cells, normal brain cells, immune and stem cells. Reprogramming of metabolic pathways by cancer cells contributes to tumour growth and progression by enabling an immunosuppressive environment. Modulating metabolism - and immune system regulation - may improve therapy opportunities for GBM patients. Animal models indicate that combining cancer drugs, radiation, and metabolic therapies may improve survival. We are investigating arginine deprivation as a treatment for GBM and the metabolic response of GBM cells.

Methods: We employed a comprehensive systems biology approach using state-of-the-art multiomics technologies, e.g. mass spectrometry imaging, imaging mass cytometry and spatial transcriptomics. We generated metabolomic, proteomic and transcriptomics data from 2D and 3D in vitro models and mouse models of GBM, both treated with ADI-PEG20 and untreated. We developed a novel pipeline enabling the spatial mapping of metabolites and lipids using DESI mass spectrometry imaging

SESSION 8. ANALYTICAL METHODS AND WORKFLOW INNOVATIONS	Monday, June 22	3:30 p.m. - 5:15 p.m.

of brain tissue slices. We then used laser capture microdissection (LCM) to capture and profile regions of interest (ROI) from tumour and healthy tissue.

Results: Together with DESI-metabolomics, proteomics analysis of these ROI revealed significant molecular changes in response to ADI-PEG20 treatment, both in small molecules and lipids. We showed that arginine deprivation potentiates the effects of radiation or chemotherapy in in vivo models, reducing tumour size and extending lifespan.

Conclusion: We have shown clear anti-GBM efficacy of ADI-PEG20 in an intracranial disease model - a novel approach to treating GBM. These findings aid our understanding of GBM metabolism and arginine deprivation, paving the way for clinical trials.

★ AWARD WINNERS

SESSION 9. FOOD	Monday, June 22	3:30 p.m. - 5:15 p.m.

9.1 **3:30 p.m. - 3:50 p.m.** **Abstract 83**

Value chain-oriented Metabolomics: Integrating agrobiodiversity bioprospecting with process optimization for functional food design-A Green Onion Case Study

PRESENTING AUTHOR: ★ ***Mateo Mejia, Universidad EAFIT, Colombia***

CO-AUTHORS: *Juan C. Henao, Catalina Giraldo*

SUB-THEME: Agro-industrial crops and traditional foods

Green onion ranks among the most widely consumed vegetables globally and plays a relevant role in food security in Latin America. Beyond its nutritional value, it represents a source of structurally diverse bioactive metabolites whose functional potential can be altered by postharvest processing. A persistent gap in agroindustry lies between chemical characterization of agrobiodiversity and transformation processes aimed at preserving and enhancing functional metabolites. From a regional collection of 152 materials in Antioquia and the Andean region of Colombia, comprising Allium fistulosum L. and a hybrid (A. galantum × A. pskemense), multivariate analysis of morphoagronomic, physicochemical, and genotypic variables defined a representative subset. This study applied a value chain–oriented metabolomics framework linking bioprospecting, material prioritization and process optimization, using chemical richness as a criterion for functional food design. Thirty accessions were characterized by untargeted and targeted LC–MS metabolomics (QTOF, QQQ). Chemometric analyses (PCA, PLS-DA, hierarchical clustering) and molecular networking supported material prioritization. Drying and cooking were optimized using response surface methodology, with antioxidant activities and phenolic metabolites as response variables. Untargeted metabolomics showed robust genotype discrimination (R2 = 0.99; Q2 = 0.75), while targeted analysis based on 18 phenolics confirmed separation (R2 = 0.75; Q2 = 0.69). 20% of the 30 accessions were prioritized based on their superior chemical profiles. Process optimization yielded one-to-two-order of magnitude increases in specific metabolites and up to 20-24-fold increases in total phenolics in dehydrated and cooked products. Antioxidant responses were assay and process-dependent, indicating that chemical preservation does not ensure functional proportionality. Systematic metabolomic monitoring across value addition stages enabled the development of chemically differentiated products with experimentally verified functional potential. The approach provides a transferable framework supported by robust LC–MS platforms for both exploratory and targeted validation of bioactive metabolites within regional agrifood value chains.

9.2 **3:50 p.m. - 4:10 p.m.** **Abstract 20**

Transition Dairy Cow Health: A Multiomics Approach

PRESENTING AUTHOR: ***Simone Rochfort, Agriculture Victoria Research, Australia***

CO-AUTHORS: *A.J. Chamberlain, M.S. Tahir, Z. Liu, C.M. Reich, J.E. Hemsworth, P. Thakur, B.A. Mason, T.V. Nguyen, I.M. MacLeod, C. Bath, V. Ezernieks, L.C. Marett, M.J. Berkhart, M.E. Goddard*

SUB-THEME: Other

Introduction and Aim: Dairy cows typically enter negative energy balance (NEB) after calving, when energy demands of lactation exceed feed intake. NEB increases the risk of metabolic disease (e.g., ketosis) and can impair reproductive performance. This study aimed to characterise the biological processes associated with NEB using multi-omic profiling and identify genomic variants associated with NEB-related molecular phenotypes to support genomic selection for improved transition cow health.

Methodology: 374 spring-calving Holstein cows were sampled over two years. Blood and milk were collected at 5–14 days in milk (DIM), representing peak NEB, and at 65–80 DIM, when cows had returned to positive energy balance (PEB). Cows ranged from 2–11 years of age (parity 1–9) and produced an average of 33 L/day. Molecular phenotypes included serum metabolomics and lipidomics (LC-MS), serum biochemistry, transcriptomics (RNA-Seq), and milk lipidomics (LC-MS). All animals were genotyped and imputed to whole-genome sequence (Beagle v5.2), and genome-wide association studies (GWAS) were performed for each molecular trait.

Findings and Significance: Pathway analysis of lipidomics and metabolomics data indicated marked mobilisation of body fat during NEB, accompanied by shifts in amino acid biosynthesis and catabolism consistent with protein remodelling. Linear

★ AWARD WINNERS

SESSION 9. FOOD	Monday, June 22	3:30 p.m. - 5:15 p.m.

modelling of NEB vs PEB enabled identification of metabolites associated with energy balance status, and principal component analysis generated an individualised "metabolic shift index" from the change in PC1 between states. One of the most significant variant association with this index mapped to ApoB, a gene central to lipid and cholesterol transport. Additional results will be discussed.

Contribution to Discipline: This study introduces the concept of a metabolic shift index derived from paired serum metabolomics to quantify physiological transitions in early lactation. It also demonstrates the utility of metabolite-coupled GWAS (mGWAS) in identifying candidate variants relevant for genomic selection targeting transition cow health.

9.3 **4:10 p.m. - 4:30 p.m.** **Abstract 167**

Untargeted UHPLC–Ion Mobility–MS Metabolomics for Scottish Honey Authentication: Geographic, Seasonal, and Blend Signatures

PRESENTING AUTHOR: ★ ***Renata Garbellini Duft, University of Aberdeen, United Kingdom***

CO-AUTHORS: *Julian L. Griffin*

SUB-THEME: Foodomics and food security

Introduction: Honey is highly vulnerable to authenticity issues: mislabelled origin and undisclosed blending erode consumer trust and harm local producers. Phenolic and related semi-polar compounds are informative authenticity markers, yet linking these chemical fingerprints to provenance remains challenging because of isomerism and matrix effects. Aim: To develop an untargeted UHPLC–IM–MS-based metabolomics workflow integrating collision cross section (CCS) to improve confidence in the annotation of phenolic and non-phenolic discriminant markers relevant to honey authenticity. Methods: We analysed 39 honey samples: Scottish seasonal honeys (spring, summer, and heather), European honeys, supermarket blends, Chinese-labelled and infused honeys. A low-solvent liquid–liquid extraction was followed by UHPLC–IM–MS acquisition on an Agilent 6560 platform. Analytical robustness was demonstrated using pooled QCs and a QC dilution series (mean $|\Delta RT| \approx 0.005$ min; mass accuracy within ±4 ppm; ΔCCS within ±1.5%). Results: Discriminant features showed diagnostic MS/MS and strong dilution linearity ($R^2 = 0.93–0.99$), and integration of accurate mass, retention time, MS/MS fragmentation, and CCS strengthened confidence in multiple phenolic and non-phenolic discriminant markers. A cross-validated OPLS-DA model separated Scottish from European honeys, with separation associated with markers including 3-phenyllactic acid, isokaempferide, and gallic acid. It also separated paired spring and summer honeys collected from the same apiaries, indicating a seasonal signal independent of location (suberic acid, luteolin-7-glucuronide, quercetin). The workflow captured broader composition shifts: supermarket blends and Chinese-labelled honeys showed elevated sugar features (fructose, arabinose) alongside reduced flavonoid signals (naringenin, isorhamnetin), while infused honeys were characterised by ingredient-derived phytochemicals (trans-cinnamic acid, chlorogenic acid, curcumin) yet retained a base-honey fingerprint. Conclusion: Integrating a simple, low-solvent extraction with CCS into untargeted UHPLC–IM–MS strengthens honey authentication by improving confidence in discriminant markers across geography, seasonality, and commercial modification, and provides, to our knowledge, the first ion mobility-supported metabolomic characterisation of Scottish honey.

9.4 **4:30 p.m. - 4:50 p.m.** **Abstract 262**

Untargeted Metabolomics Applied to Study the Chemical Composition of Propolis from Vale do Jequitinhonha (Brazil)

PRESENTING AUTHOR: ***Adriana Nori De Macedo, Federal University of Minas Gerais, Brazil***

CO-AUTHORS: *Adriana Nori de Macedo, Brícia Marques Parreiras, Mayra Conceição Meira Silva, André Rodrigo Rech*

SUB-THEME: Agro-industrial crops and traditional foods

Propolis is a bee product with a complex chemical composition, influenced by botanical origin and environmental conditions. Vale do Jequitinhonha, a mesoregion within Minas Gerais State (Brazil), is marked by a high plant diversity, where apiculture stands out as an important occupation and income source for the local population. Although honey production in Vale do

SESSION 9. FOOD	Monday, June 22	3:30 p.m. - 5:15 p.m.

Jequitinhonha has become a well-established activity, information is still lacking about the characteristics of propolis originating from this mesoregion. Therefore, the present study aimed to evaluate the chemical composition of propolis produced in different microregions within Vale do Jequitinhonha, using an untargeted metabolomics approach to generate initial information about this apicultural product. A total of 54 propolis samples from different microregions in Vale do Jequitinhonha were gently provided by beekeepers. Ethanolic extracts were prepared and analyzed by liquid chromatography-mass spectrometry. In addition, propolis extracts were spectrophotometrically screened in the visible-light range (400-800 nm) to objectively evaluate their colours, an important characteristic often used to classify propolis types. Metabolomics and spectrophotometric measures were combined using correlation-based statistical analyses. A pronounced chemical variability was revealed using multivariate analysis, with partial differentiation for propolis from Diamantina microregion and substantial overlap for the other microregions. Visible spectrophotometry allowed objective classification of propolis extracts into green (n=19), red (n=5) and others (n=30). Compounds already reported in Brazilian green and red propolis (e.g., artepillin C, caffeoylquinic acid derivatives, liquiritigenin and formononetin) were detected, although the samples did not originate from classical regions of occurrence of these types of propolis. A total of 77 molecular features were tentatively annotated, predominantly corresponding to phenolic acids, flavonoids, and other related phenolic subclasses. Correlation-based analysis of metabolomics and spectroscopic data indicated that propolis colour arises from the combined contributions of multiple phenolic compounds rather than from a single pigment molecule.

9.5	4:50 p.m. - 5:10 p.m.	Abstract 38

Discovery-driven untargeted metabolomics reveals compound transformations during static in vitro gastrointestinal digestion of cereal matrices

PRESENTING AUTHOR: ***Luciana Lima, UNIRIO, Brazil***

CO-AUTHORS: *Millena C. Barros Santos, Álvaro Fernández-Ochoa, Mariana Simões Larraz Ferreira*

SUB-THEME: Foodomics and food security

Cereal grains are staple foods and important sources of energy and bioactive phytochemicals (phenolic compounds, vitamins, and fatty acids). However, their bioaccessibility may undergo significant changes during digestion, mainly due to the enzymatic activity and pH variations. This work aimed to assess the cereal metabolite modifications during a static in vitro gastrointestinal digestion model combined with an untargeted metabolomics approach and bioinformatic tools. Wholemeal and bran fractions (n=15) from barley, pearl millet, oat, rye, triticale, wheat, and sorghum (with tannins, tannin-free) were cooked before being subjected to the in vitro digestion analysis. Extracts from cooked samples (undigested) and aliquots from the gastric and intestinal phases, including controls, were analyzed using an RP-C18 column in an HPLC-ESI(-)-Q-TOF-MS/MS platform. Data preprocessing was performed using MZmine and Notame R-package, multivariate analysis in MetaboAnalyst, metabolite annotation with GNPS and SIRIUS, and Feature-Based Molecular Networking (FBMN) analysis in GNPS. The FBMN revealed clusters exclusive to undigested samples or specific to each digestion phase. Phenolic compounds (chlorogenic acids, flavonoid aglycones), saccharides (e.g., maltotriose, sucrose), and lipids (e.g., glycerophospholipids) were predominantly detected in the undigested and gastric-phase samples; while amino acids (e.g., phenylalanine), phenolic acids (e.g., ferulic acid, 4-methoxycinnamic acid), and other saccharides (e.g., maltose, raffinose) were mainly observed in the intestinal phase. Cereal-specific shifts were also observed, with unique compounds detected in rye, oat, sorghum, and millets, highlighting the influence of grain-specific composition. Metabolic changes were likely driven by enzymatic activity and pH effects during digestion. Multivariate analysis indicated that sorghum was the least affected, whereas wheat and rye exhibited the greatest alterations. Importantly, the untargeted metabolomics approach enabled comprehensive and unbiased detection of digestion-driven biochemical transformations, demonstrating its strong potential as an innovative strategy to elucidate complex metabolic dynamics and bioaccessibility changes in food digestion studies.

SESSION 10. MICROBIOTA & INFECTIOUS DISEASE	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

10.1 **10:15 a.m. - 10:45 a.m.** **Abstract 365**

Dietary Determinants of Colorectal Cancer Risk: From Association to Causation

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

CO-AUTHORS: *Kaoutar Abaakil, Maria Glymenaki, Yang Bi, Maria Valdivia*

SUB-THEME: Precision health

Colorectal cancer (CRC) is the third most common cancer globally. Although population-wide screening and earlier diagnosis have reduced incidence overall, a striking rise is occurring in younger adults. This shift strongly implicates modifiable environmental factors, with diet emerging as a central driver. The global transition towards Westernised dietary patterns - high in animal fats and processed foods and low in fiber - has profound consequences not only for metabolic regulation but also for the structure and function of the gut microbiota.

The gut microbiome, defined by both the composition and functional activity of the gut microbiota, is now recognized as a central intermediary linking diet to colorectal carcinogenesis; however, disentangling causality from association remains a major challenge. In this talk, I will examine how dietary exposures influence CRC risk. Drawing on integrative studies combining metabolic phenotyping with molecular biology, I will focus on the biological activity of microbial metabolites, such as tyramine, and host–microbial co-metabolites including trimethylamine *N*-oxide (TMAO). By illuminating how these metabolic signals influence CRC risk factors, this work helps to redefine our understanding of the diet–microbiome–host axis and points towards new opportunities for precision prevention in CRC.

10.2 **10:45 a.m. - 11:05 a.m.** **Abstract 93**

Imidazole Propionate: From Untargeted Metabolomics Discovery to a Mechanistic Driver of Atherosclerosis

PRESENTING AUTHOR: ***Annalaura Mastrangelo, Spanish National Center for Cardiovascular Research, Spain***

CO-AUTHORS: *Iñaki Robles-Vera, Diego Mañanes, Miguel Galán, Marcos Femenía-Muiña, Ana Redondo-Urzainqui, Rafael Barrero-Rodríguez, Eleftheria Papaioannou, Joaquín Amores-Iniesta, Ana Devesa, Manuel Lobo-González, Alba Carreras, Katharina R. Beck, Sophie Ivarsson, Anders Gummesson, Georgios Georgiopoulos, Manuel Rodrigo-Tapias, Sarai Martínez-Cano, Ivan Fernández-López, Vanessa Nuñez, Alessia Ferrarini, Naohiro Inohara, Kimon Stamatelopoulos, Alberto Benguría, Danay Cibrian, Francisco Sánchez-Madrid, Vanesa Alonso-Herranz, Ana Dopazo, Coral Barbas, Jesús Vázquez, Juan Antonio López, Alicia González-Martín, Gabriel Nuñez, Konstantinos Stellos, Göran Bergström, Fredrik Bäckhed, Valentín Fuster, Borja Ibañez, David Sancho*

SUB-THEME: Cardiovascular diseases

Atherosclerosis remains the leading cause of cardiovascular morbidity and mortality worldwide, with many individuals at risk for early vascular disease that remain undetected. Identifying metabolic biomarkers and pathways that enable early detection while revealing causal disease mechanisms is therefore a major priority. While metabolomics studies have uncovered numerous disease-associated metabolites, relatively few have been mechanistically linked to cardiovascular disease progression.

Using untargeted LC–MS metabolomics, we profiled thousands of circulating metabolic features and identified the microbiota-derived metabolite imidazole propionate (ImP), a product of microbial histidine metabolism by the gut microbiota, as strongly associated with atherosclerosis in both atheroprone mice and humans. In the PESA cohort, circulating ImP levels were associated with the presence and burden of subclinical atherosclerosis and were elevated in individuals with metabolically active plaques characterized by vascular inflammation. These findings were independently validated in the IGT cohort, supporting the translational relevance of ImP across human populations.

To determine whether ImP directly contributes to disease pathogenesis, we performed mechanistic studies in vivo. Chronic administration of ImP induced atherosclerosis in atherosclerosis-prone mice even under chow diet conditions and independently of systemic lipid levels. Instead, ImP promoted a pro-inflammatory state involving activation of innate and adaptive immune responses at systemic and vascular levels.

★ AWARD WINNERS

SESSION 10. MICROBIOTA & INFECTIOUS DISEASE	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

Mechanistically, ImP signals through the imidazoline-1 receptor (I1R/nischarin) in myeloid cells, triggering vascular inflammation and promoting plaque development. Genetic or pharmacological disruption of the ImP/I1R signaling axis protected mice from atherosclerosis induced either by exogenous ImP or by high-cholesterol diet, underscoring novel therapeutic targets in atherosclerosis.

Together, these findings establish imidazole propionate as a microbiota-derived metabolic driver of atherosclerosis, illustrating how untargeted metabolomics can uncover causal pathways linking microbial metabolism, immune activation, and cardiovascular disease while revealing new opportunities for alternative and potentially synergic therapies for cardiovascular diseases.

10.3	11:05 a.m. - 11:20 a.m.	Abstract 231

Intramammary milk transplantation induces microbial and metabolic remodeling in experimental caprine mastitis

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

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

SUB-THEME: Infectious diseases

Goat milk represents a vital socioeconomic activity while gaining attention for its nutraceutical properties. Mastitis, a mammary dysbiosis, compromises milk production and quality. This study investigates intramammary milk transplantation as a therapeutic approach to restore mammary homeostasis. We aimed to characterize microbiota and metabolomic alterations during experimental infection and recovery following milk transplantation. Six primiparous Chamois-coloured goats (*Capra hircus*) underwent intramammary infection with *Staphylococcus warneri* (1.2×10^8 CFU/mL) in the right udder half (CEUA-UFV 62/2018). Subclinical infection was monitored for 10 days, followed by nine-day transplantation with 120 mL/day milk from a healthy donor. Milk samples were collected during Healthy (D-1, D0), Infected (D+1–D+10), Transplantation (D+12–D+18) and Resilience (D+20–D+22) periods. Microbiota was analyzed using on-farm chromogenic culture system for mastitis, while UPLC-MS/MS metabolomic data were annotated and analyzed using MetaboAnalyst (v6.0). Multivariate modelling VIP scores >2.0 and ROC-based AUC>0.7 with $p<0.05$ filtering identified significant metabolites. Following Transplantation, one animal achieved clinical cure. Microbiota analysis revealed shifts throughout the experiment. Healthy period was characterized by Gram-negative predominance alongside *Lactococcus* and *Streptococcus agalactiae*. Infection introduced coagulase-negative *Staphylococcus* (likely *S. warneri*), while still maintaining Gram-negative prevalence. Transplantation increased bacterial richness, reducing Gram-negative prevalence and eliminating coagulase-negative *Staphylococcus*. A total of 145 metabolites were annotated. Comparison between Healthy and Resilience phases revealed six metabolites showing significant fold-change alterations (VIP 2.02–3.61; AUC 0.76–0.92; $p<0.05$). There was increased host-peptide Lys-Met-His ($\log_2$FC=1.15), L-Monomenthyl glutarate ($\log_2$FC=2.13), and triglyceride 8:0_10:0_14:1 ($\log_2$FC=1.30), together with decreased bacterial peptide H-D-Cha-Pro-N(Me)Arg-Al ($\log_2$FC=−2.24), sphingolipid 58:5;3O ($\log_2$FC=−3.70), and monoacylglycerol 0:0-22:2(13Z;16Z)-0:0 ($\log_2$FC=−1.31). This study shows that microbiota restructuring after transplantation coincided with changes in peptide and lipid metabolites, compatible with the observed clinical improvement, reflecting reduced bacterial peptide abundance, decreased sphingolipid levels associated with inflammatory signaling, and restoration of lipid metabolism during the resilience phase. This approach offers a promising non-antibiotic strategy for mastitis management. Acknowledgments: CAPES, FAPEMIG and CNPq

SESSION 10. MICROBIOTA & INFECTIOUS DISEASE	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

10.4 **11:20 a.m. - 11:40 a.m.** **Abstract 185**

Integrated Multiplatform Metabolomics and Microbiota Profiling of HPV Infection in an Underdiagnosed Rural Peruvian Cohort

PRESENTING AUTHOR: ***Victor González-Ruiz, CEU-San Pablo University, Spain***

CO-AUTHORS: *Isabel Fernández-Prades, Miguel Ángel Aguilar, Juana del Valle, Miguel Fernández-García, Coral Barbas*

SUB-THEME: Infectious diseases

Introduction and Aim: Cervical cancer, driven primarily by Human papillomavirus (HPV), constitutes a global health crisis [1], particularly in regions like rural Peru where screening access is limited and sexually transmitted infections persist as a taboo. Despite its prevalence, the metabolic interactions between HPV, the host, and the vaginal microbiota remain poorly defined. This study aims to identify early diagnostic metabolic signatures of HPV infection within this vulnerable population using an integrated, multiplatform metabolomics strategy.

Methodology: We applied an untargeted multiplatform strategy to vaginal swabs from a cohort of 75 women (HPV+ n=26; HPV- n=49). To ensure comprehensive metabolome coverage, capillary electrophoresis-mass spectrometry (CE-MS) was utilized for polar metabolites, while liquid chromatography-mass spectrometry (LC-MS) targeted lipidomic profiling. Simultaneously, 16S rRNA sequencing served to characterized the cervicovaginal microbiota. To address experimental noise (mixed host-microbiome metabolome, hormonal cycles) and ion suppression (swab contaminants), nonlinear modeling based on random forest was used to enhance group discrimination.

Findings and Significance: While linear models showed limited performance due to high intrinsic biological noise, nonlinear approaches successfully discriminated HPV(+/-) individuals. We identified a discriminatory subset of metabolites such as glycerophospholipids, sphingolipids, 3-methylhistidine and choline, indicating significant alterations in epithelial barrier integrity and cellular stress in the epithelium. ROC analyses confirmed the high diagnostic performance of these profiles, even in early, asymptomatic stages. Correlation analyses further revealed specific links with microbial genus, suggesting a tight interplay between bacteria and epithelium's metabolic rewiring upon HPV infection.

Contribution to Discipline: Our work advances infectious disease metabolomics by providing a robust framework for early, non-invasive diagnosis of HPV infection. It demonstrates that integration of multiplatform metabolomics and microbial metagenomics enable access to a better mechanistic insight into how the host's response is modulated by bacteria, and opens a path toward accessible, metabolite-based screening tools for high-risk populations.

SESSION 10. MICROBIOTA & INFECTIOUS DISEASE	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

10.5	11:40 a.m. - 12:00 p.m.	Abstract 306

Untargeted Metabolomics Reveals HIV and cART-Associated Metabolic Changes Linked to Cardiovascular Risk

PRESENTING AUTHOR: ***Jacques-gernore Frans, North-West Unversity, South Africa***

CO-AUTHORS: *Aurelia Williams, Emile Janse Van Rensburg, Du Toit Loots, Nicolene Odendaal*

SUB-THEME: Cardiovascular diseases

Since the introduction of combination antiretroviral therapy (cART), mortality rates among people living with HIV (PLWH) have declined while the development of comorbidities such as cardiovascular disease (CVD) has increased. Although metabolic changes associated with HIV and CVD are independently cited, these studies have often relied on conventional biochemical techniques and rarely assessed relationships of the metabolic features to CVD risk markers. This study employed untargeted GC×GC-TOFMS to compare serum metabolic profiles of HIV-uninfected individuals (HIV−, n = 147), PLWH naïve to cART (HIV+, n = 70), and PLWH receiving cART (cART, n = 60). The aim was to identify metabolic markers associated with HIV pathogenesis and cART exposure and assess the relationship of these markers with established CVD risk markers. One-way ANOVA identified metabolite features significantly altered across the three groups (adjusted $p \leq 0.05$). HIV-related alterations were linked to immune–metabolic remodelling, dyslipidaemia, and increased gut permeability, whereas cART increased amino acid utilization, enhanced lipolysis, altered inflammatory eicosanoid pathways, oxidative stress, and upregulated antioxidant defence. Spearman's rank correlations between the ANOVA-identified metabolites and 26 established CVD risk markers revealed nine moderately significant associations ($\rho \geq 0.3$, $p \leq 0.005$). α-Linolenic acid (a polyunsaturated fatty acid) for example correlated with total cholesterol and triglycerides (typical markers of CVD). Butanoic acid (a short-chain fatty acid related to gut integrity) correlated with the cardiovascular and chronic inflammatory marker soluble urokinase plasminogen activator receptor (suPAR). These correlations highlight key dysfunctional pathways associated with CVD, including altered polyunsaturated fatty acid metabolism, inflammation, and gut intestinal integrity, and may represent potential targets for clinical management to reduce cardiovascular risk in PLWH.

SHIMADZU

Excellence in Science

Unlock the Power of Your Analysis with Shimadzu Solutions

Visit us!

www.shimadzu.com.br

SESSION 11. MACHINE LEARNING AND AI SYSTEMS	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

11.1 **10:15 a.m. - 10:45 a.m.** **Abstract 363**

From Rigid to Self-Evolving Multi-Agent AI System for Metabolomics

PRESENTING AUTHOR: ***Louis-Félix Nothias, CNRS & Université Côte d'Azur, France***

CO-AUTHORS: *Martin Legrand, Tao Jiang, Madina Bekbergenova, Lucas Pradi, Matthieu Feraud, Benjamin Navet, Yousouf Taghzouti, Fabien Gandon, Elise Dumont, Louis-Félix Nothias*

SUB-THEME: Machine Learning and AI

The growing scale and complexity of metabolomics data demand new paradigms for computational analysis. At the HolobiomicsLab, we are developing multi-agent AI frameworks that trace an arc from structured, tool-specific architectures to self-evolving systems for autonomous scientific reasoning.

MetaboT, our first-generation system, enables interactive mass spectrometry data analysis through agents that translate natural-language questions into SPARQL queries across metabolomics knowledge graphs, reaching 83.67% accuracy on curated benchmarks (doi:10.21203/rs.3.rs-6591884/v1). Yet its predefined agent roles and fixed workflows limit adaptability to novel research questions.

Mimosa (arXiv:2603.28986) addresses these limitations as an evolving multi-agent framework that automatically synthesizes task-specific workflows and iteratively refines them through experimental feedback. It combines dynamic tool discovery via the Model Context Protocol, a meta-orchestrator that generates and mutates workflow topologies, code-generating agents that invoke established scientific libraries, and an LLM-based judge that drives workflow refinement through local search. On ScienceAgentBench, Mimosa reaches a 43.1% success rate, outperforming both single-agent and static multi-agent baselines — while revealing that models respond heterogeneously to iterative learning.

Alongside Mimosa, we developed Perspicacité, an agentic literature assistant that searches, filters, and synthesizes publications across PubMed, arXiv, and OpenAlex, grounding analyses in the existing scientific record. Mimosa v2, currently in development, integrates Perspicacité to couple experiment-aware workflow evolution with literature-driven hypothesis generation — moving toward AI systems that not only execute analyses but situate them within the broader scientific literature.

Both Mimosa and Perspicacité are released as modular, open-source platforms (github.com/HolobiomicsLab), providing a foundation for community-driven AI-assisted research. We discuss implications for metabolomics and beyond.

11.2 **10:45 a.m. - 11:05 a.m.** **Abstract 195**

Adduct-Specific Machine Learning for Accurate Collision Cross Section Prediction in Untargeted Metabolomics

PRESENTING AUTHOR: ***Facundo Fernandez, Georgia Institute of Technology, United States***

CO-AUTHORS: *Rithika Gorrepati, Olatomiwa Bifarin, Facundo M. Fernandez, Erin S. Baker*

SUB-THEME: Machine Learning and AI

Collision cross section (CCS) values obtained through ion mobility–mass spectrometry are frequently used to aid metabolite annotation in untargeted LC–MS workflows. However, experimentally measured CCS values are only available for a small subset of detected metabolites, limiting their use in large metabolomics datasets. Computational CCS prediction offers a scalable alternative, but most existing models are trained on very small datasets and treat different ion adducts as structurally identical in the gas phase.

We developed adduct-specific machine learning models for CCS prediction using structures derived from the METLIN-CCS small molecule database. After standardizing structures with RDKit, the dataset included 58,034 ion entries: 24,827 $[M+H]^+$, 18,160 $[M–H]^-$, and 15,047 $[M+Na]^+$. For each molecule, about 1,800 molecular descriptors were calculated using RDKit and Mordred. To avoid structural overlap between splits, molecules were grouped by InChIKey and split into training (60%), validation (20%), and test (20%) sets. Descriptors with low variance or high correlation were removed before model training.

SESSION 11. MACHINE LEARNING AND AI SYSTEMS	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

Linear Support Vector Regression (SVR) models, implemented in scikit-learn (v1.3.1), were trained separately for $[M–H]^-$, $[M+H]^+$, and $[M+Na]^+$ adducts on the PACE Phoenix HPC cluster. Descriptor generation, feature selection, and five-fold cross-validation for model training were parallelized across 24 CPU cores, typically taking about 7–8 hours per adduct-specific model.

The models showed strong predictive performance for protonated and deprotonated ions. Independent test sets produced R^2 values of 0.913 for $[M+H]^+$ and 0.865 for $[M–H]^-$, with mean relative errors of ~1.9% and ~2.5%, respectively. Predictions for $[M+Na]^+$ ions were less accurate (R^2 = 0.819, mean relative error ~2.2%), likely reflecting greater structural variability associated with gas-phase sodium binding.

We are now extending this framework to neural network models, including graph neural networks and conformer-based molecular features, to evaluate whether incorporating 3D structural information improves CCS prediction across different ion adducts.

11.3	11:05 a.m. - 11:20 a.m.	Abstract 232

Aligning Metabolomic Networks via Graph Learning for Common Subnetwork Discovery

PRESENTING AUTHOR: ***Edwin Alvarez Mamani, Pontificia Universidad Católica del Perú, Peru***

CO-AUTHORS: *Deybis Paucar Pinto, Alfredo J. Ibañez, Madina Mansurova*

SUB-THEME: Machine Learning and AI

Metabolomic data analysis is challenging due to its high complexity and the noise inherent to mass spectrometry data, where only about 5% of detected signals correspond to biologically relevant metabolites. In this context, identifying such metabolites within metabolomic networks remains an important problem in bioinformatics. We propose a graph neural network (GNN)-based approach for aligning metabolomic networks to discover common subnetworks.

The proposed method uses GC–MS and LC–MS data as input, provided in CSV format with metabolite identifiers (Id), retention time (Rt), mass-to-charge ratio (m/z), and signal intensities across point-of-origin and fresh/dried presentations with their biological and analytical replicates. Our workflow includes: (i) computing partial correlation matrices; (ii) constructing metabolomic networks where edges exist if correlation coefficient ≥ |0.5|, with node features derived from Rt, m/z, and signal intensities; (iii) learning node embeddings using the transferable graph autoencoder framework (T-GAE) architecture with graph isomorphism network (GIN) and graph isomorphism network with edge features (GINE) operators; and (iv) aligning networks by computing node embeddings similarities using the K-NN algorithm to identify correspondences between metabolites.

It was tested with different datasets. For cocoa samples, it was possible to determine both the origin of the cocoa beans and whether the samples were fresh or had undergone the postharvest processing. To evaluate the quality of the results, the silhouette metric was used, expecting metabolites associated with the same phenotype to form well-defined clusters. These results demonstrate the usefulness of the proposed method for identifying and filtering representative metabolites within metabolomic networks.

We believe that our graph neural networks approach can improve the identification of representative metabolites through metabolomic network alignment and be applied for complex data processing/analysis.

★ AWARD WINNERS

SESSION 11. MACHINE LEARNING AND AI SYSTEMS	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

11.4 **11:20 a.m. - 11:40 a.m.** **Abstract 239**

Deriving Insights from Mass Spectrometry Imaging and Single Cell Datasets with Biologically Informed Neural Networks

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

CO-AUTHORS: *Elena Chekmeneva, Elizabeth J Want, Timothy Ebbels*

SUB-THEME: Machine Learning and AI

Mass spectrometry imaging (MSI) enables the spatial mapping of hundreds of metabolites and has provided insight into metabolic variation across cell types, tissues, and disease states. However, the functional interpretation of MSI data remains challenging, typically focussing on the spatial distribution of specific ions, rather than higher level sets such pathways or chemical classes. Further, detected ions are typically annotated to molecular formulas rather than specific metabolites, and a single formula can correspond to multiple candidate structures.

Biologically informed neural networks (BINNs) have gained prominence as interpretable deep learning models that integrate biological knowledge directly into network architecture. BINNs link molecular measurements to nodes representing pathways or chemical classes, enabling predictions to be interpreted in terms of biological processes. Here, we adapt BINNs for metabolomic MSI datasets. To accommodate structural ambiguity, the mapping between molecular formulas and candidate structures is treated as learnable parameters rather than fixed assignments.

We applied this framework to publicly available single-cell metabolomics datasets generated from the HT-SPACEM workflow. A BINN classifier achieved strong predictive performance while providing interpretable biological insights. The fatty acid chemical class was among the most influential features distinguishing co-cultured NCI-H460 and NIH3T3 cells, consistent with previously reported findings. In a glycolysis inhibition experiment, monosaccharide and carbohydrate classes were identified as top-ranking nodes, validating the expected perturbation. To extend the approach to unsupervised settings, we also developed a variational autoencoder BINN to generate chemical-class activity maps. In bronchopulmonary dysplasia imaging datasets, preliminary analyses show spatially coherent chemical-class signals corresponding to tissue morphology and higher spatial correlation than null models.

Together, these results highlight the potential utility of BINNs as an interpretable framework for pathway and chemical-class analysis of MSI data, particularly emphasising their ability to account for annotation uncertainty, a widely appreciated and fundamental challenge in metabolomics.

11.5 **11:40 a.m. - 12:00 p.m.** **Abstract 256**

Multimodal Mass Spectrometry Imaging Data Integration using Machine Learning and Network Analyses

PRESENTING AUTHOR: ★ ***Lukas Kopecky, Imperial College London, United Kingdom***

CO-AUTHORS: *Georgia Lorentzen, Elmeri Latvanen, James McKenzie, Zoe Hall, Lauren Ford, Duncan Roberts, Zoltan Takts, Elizabeth Want, Timothy Ebbels*

SUB-THEME: Metabolic modeling

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. However, due to the different physicochemical properties of metabolites, multiple analytical assays must be applied to adjacent tissue sections, resulting in partially overlapping data blocks from potentially non-aligned tissue locations.

Conventional approaches analysing each data block separately do not capture inter-block relationships, complicating interpretation of multi-assay data. Here, we propose an integrative workflow for multimodal MSI analysis.

Method: We co-registered adjacent tissue section MS data via affine transformation. Next, we employed a multi-block – partial least squares model coupled with permutation testing (MAMSI) workflow to model different tissue types and estimate the

SESSION 11. MACHINE LEARNING AND AI SYSTEMS	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

importance of predictors. Network analyses allowed us to investigate the structural and spatial distribution of metabolites differentiating tissue types.

We validated the workflow using three datasets: a colorectal cancer tissue section acquired using DESI MSI in +/- ionisation modes, a cirrhotic liver tissue dataset acquired using multiple MSI instruments (MALDI, LD-REIMS), and a fibrotic liver tissue dataset in +/- ionisation modes both acquired from the same tissue section.

Results: Our integrative model resulted in >95% cross-validation accuracy across all datasets and yielded up to 20% additional statistically significant features compared to single-modal methods. The network analyses revealed metabolite clusters that, after annotation, could be linked to the disease of interest, for example, phosphatidylserine (36:1) in liver fibrosis.

Further, our method enabled more precise classification of “unknown” tissue pixels than a unimodal approach, especially in situations when one of the modalities has a low signal-to-noise ratio.

Conclusion: Our workflow provides a streamlined modelling framework for the analysis of multimodal MS images. It has the potential to benefit the metabolomics and MSI communities by offering interpretable integrative analysis of multimodal MSI data.

★ AWARD WINNERS

SESSION 12. ION MOBILITY AND INTEGRATED WORKFLOWS	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

12.1 **10:15 a.m. - 10:45 a.m.** **Abstract 375**

The role of chromatography and ion mobility in metabolite identification

PRESENTING AUTHOR: ★ ***Michael Witting, Helmholtz Zentrum Muenchen, Germany***

CO-AUTHORS: *Fleming Kretschmer, Eva-Maria Harrieder, Klidel Fae Rellin, Aiko Barsch, Sebastian Böcker*

SUB-THEME: Metabolite identification/annotation

Metabolite identification in LC-MS-based non-targeted metabolomics relied heavily on the development and growth of tandem mass spectral databases, as well as on multiple analytical and machine learning approaches leveraging MS information. While this development is important and is continually improving, several other readily available parameters are used only in later stages of the metabolite identification workflow: chromatographic retention times (RT) and ion mobility-derived collisional cross sections (CCS). Both are often only used when comparing against chemical reference standards.

In recent years, their use has been increasing; e.g., the CCS compendium and multiple databases and prediction tools have been developed for CCS. However, a comprehensive RT database was missing. In many instances, RT is believed to be of little use for cross-laboratory metabolite identification, in contrast to MS and CCS information. RepoRT has been developed as a comprehensive data resource for RTs, including important chromatographic metadata.

Here, the RepoRT database and a model for transferable retention time prediction are presented, along with the use of CCS in trapped-ion mobility metabolomics and lipidomics. Their value in metabolite identification workflows is highlighted, underscoring their role similar to that of MS data.

12.2 **10:45 a.m. - 11:05 a.m.** **Abstract 39**

Cyclic Ion Mobility Separations of Oxidized Free Fatty Acids

PRESENTING AUTHOR: ***Eric Gier, Georgia Institute of Technology, United States***

CO-AUTHORS: *Facundo M. Fernández*

SUB-THEME: Ion mobility spectrometry

Oxylipins are oxygenated polyunsaturated fatty acids (PUFAs) that regulate inflammatory and vascular functions and are involved in various diseases, including cancers and heart conditions. Oxidized free fatty acids (oxFFAs) arise from enzymatic and nonenzymatic oxidation of PUFA precursors, yielding numerous regio-, stereo-, and structural isomers. This isomeric diversity is difficult to fully resolve using chromatography alone; however, combining it with ion mobility spectrometry (IMS) improves peak capacity. Cyclic IMS–mass spectrometry (cIMS–MS) provides high resolution through multipass separations and is compatible with workflows lacking front-end separations such as MS imaging. Here, we evaluate the resolving power and collision cross section (CCS) accuracy for multipass cIMS–MS separations of 23 oxFFAs.

OxFFA standards were analyzed as isomeric groups by direct infusion cIMS–MS under individually optimized traveling-wave (TW) conditions. In negative ion mode, deprotonated adducts produced broad, overlapping ATDs with multiple conformations observed after extended path lengths. Sodium and other metal adducts displayed narrower ATDs and larger %ΔCCS values, enabling baseline resolution ($R_{p-p} > 1.5$) for most isomer pairs in fewer than ten passes. Groups of three and four metal-adducted isomers were often baseline resolved prior to the onset of wrap-around, a phenomenon in which faster-moving ion populations complete an additional lap of the mobility cell. When wrap-around limited resolution, cIMS–IMS experiments isolated regions of the ATD to extend effective path length and increase resolution.

CCS values of metal-adducted species were calculated from perturbation-corrected periodic drift times, including charge and reduced-mass corrections, across four TW conditions using an updated version of our group's freely available Multipass CCS Refiner. Multipass CCS values were consistent across TW conditions and in agreement with literature values within 1.5%. These results highlight the ability of cIMS–MS to differentiate complex oxFFA isomer populations, provide reliable CCS values, and support applications in MS imaging.

SESSION 12. ION MOBILITY AND INTEGRATED WORKFLOWS	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

12.3 **11:05 a.m. - 11:20 a.m.** **Abstract 484**

BRUKER: Transforming Metabolomics with Breakthrough NMR and MS Technologies

PRESENTING AUTHOR: ***Erica Forsberg, Bruker, United States***

SUB-THEME: Applications in Metabolomics and Lipidomics

Bruker continues to advance metabolomics through powerful innovations in both nuclear magnetic resonance spectroscopy and mass spectrometry. Our latest MS platforms, including timsMetabo, deliver unprecedented sensitivity, selectivity, and annotation confidence for small-molecule analysis, enabling deep 4D-Metabolomics and 4D-Lipidomics workflows that accelerate discovery across diverse sample types. These high-resolution capabilities are equally valuable for exposomics, where detection of low-abundance xenobiotics and environmental contaminants requires exceptional sensitivity and quantitative robustness. Complementary MALDI solutions provide label-free spatial mapping of metabolites, revealing molecular heterogeneity within complex tissues and supporting high-impact imaging studies. Bruker's NMR portfolio further strengthens metabolomics and translational research through standardized, automated workflows for robust absolute quantification and untargeted analysis. The Avance IVDr platform enables fully automated, push-button metabolomics in biofluids, supporting large cohort studies, longitudinal monitoring, and clinically oriented research. Complementing this, Fourier 80 benchtop NMR brings accessible and automated NMR-based metabolomics to routine laboratory environments. To further enhance NMR workflows, the new ACP 2.0 software delivers scalable qNMR data analysis across diverse sample matrices, leveraging high-performance, model-based algorithms to achieve accurate quantification even in highly complex and overlapping spectra. Together, Bruker's integrated NMR, LC- MS and GC-MS technologies deliver comprehensive, scalable solutions that empower researchers to achieve deeper biological insight, improve annotation confidence, and accelerate applications ranging from precision medicine to plant, microbial, and environmental metabolomics.

12.4 **11:20 a.m. - 11:40 a.m.** **Abstract 73**

Toward integration of enantiomeric resolution into mass spectrometry-based metabolomics workflows through fragmentation of non-covalent complexes

PRESENTING AUTHOR: ***Annelaure Damont, CEA, France***

CO-AUTHORS: *Chenqin Cao, Jean-Claude Tabet, François Fenaille, Christophe Junot, Annelaure Damont*

SUB-THEME: Quantitation in metabolomics

D-amino acids and D-hydroxy acids are increasingly recognized as clinically relevant metabolites, with altered concentrations reported in cancers, neurological disorders and kidney dysfunction. However, conventional metabolomics workflows based on LC-HRMS do not resolve enantiomers, and chiral analysis still relies largely on dedicated HPLC separations. Here, we introduce a mass spectrometry–based chiral strategy that differentiates L- and D- enantiomers without chromatographic resolution that can be directly interfaced with existing LC-HRMS metabolomics methods.

Our approach relies on the gas-phase formation of non-covalent ternary complexes between a chiral reference (Ref), the enantiomer of interest (E), and a metal cation (M). The resulting ionic diastereomeric complexes are expected to display distinct fragmentation patterns, enabling enantiomer discrimination by tandem high-resolution mass spectrometry. Using an Orbitrap Fusion™ instrument, seven enantiomeric amino acid pairs were studied in combination with two different chiral references and Mg^{2+} as metal cation. Mixtures of Ref and E (5 µg/mL) with Mg^{2+} (1 µg/mL) in water were introduced into the ESI source by flow injection analysis to produce the complexes that were further fragmented in both resonant and non-resonant conditions.

Chiral recognition was quantified using the intensity ratio (R) of two diagnostic fragment ions. For example, using NMDA as Ref to differentiate cis-4-L/D-hydroxy-proline enantiomers, significantly different abundances were observed for two product ions at m/z 270.0457 and 255.0824, giving intensity ratios of 0.61 (R(L)) and 0.29 (R(D)), corresponding to a chiral selectivity factor Rchiral of 2.1 (Rchiral = R(L)/R(D)). Overall, 5 out of 9 enantiomeric systems showed robust chiral discrimination, with Rchiral values between 1.5 to 2.1. Enantioselective calibration curves enabled accurate determination of enantiomeric excess with <

SESSION 12. ION MOBILITY AND INTEGRATED WORKFLOWS	Tuesday, June 23	10:15 a.m. - 12:00 p.m.

5 % error in %L. Importantly, preliminary coupling to LC-HRMS via post-column infusion of Mg^{2+} and Ref has demonstrated its compatibility with the analysis of complex mixtures, thus opening up new perspectives for chiral metabolomics.

12.5 **11:40 a.m. - 12:00 p.m.** **Abstract 206**

Uniting targeted quantification and untargeted ion mobility in one unified workflow for small molecule analysis

PRESENTING AUTHOR: ***Alice Limonciel, biocrates life sciences ag, Austria***

CO-AUTHORS: *Aiko Barsch, Cristian De Gobba, Tuan Hai Pham, Guido Dallman, Alice Limonciel, Matthew R. Lewis*

SUB-THEME: Ion mobility spectrometry

A hybrid LCMS workflow was developed to combine the quantitative robustness of a standardized targeted platform with the discovery power of untargeted ion mobility-enabled metabolomics. Targeted quantification leverages reproducible retention times, validated calibration models, and stable isotope–labeled internal standards to deliver absolute concentrations across diverse metabolic pathways. In parallel, full-scan high-resolution MS with ion mobility separation and broadband collision-induced dissociation (bbCID) generates rich ion mobility-resolved MS/MS data that support non-targeted feature discovery and structural annotation.

Eleven human plasma samples and the NIST SRM 1950 reference plasma material were prepared using a dual derivatization strategy (PITC and 3NPH) and analyzed in triplicate. Data were processed with dedicated software for absolute quantification and for untargeted feature extraction, annotation, and insilico derivatization. Quantitative precision was high, with median coefficient of variation (CV) of 6.5% in spiked plasma and 3.4% in NIST SRM 1950. Accuracy assessment for representative metabolites with certified reference values showed generally Untargeted analysis enabled confident annotation of 474 derivatized targets across PITC and 3NPH chemistries, complemented by additional features identified exclusively through predicted derivatization patterns. In a proof-of-concept donor comparison, principal component analysis revealed a distinct separation of one lipemic sample. Rule-based lipid annotation indicated that lysophosphatidylcholines (absent from the targeted panel but present in the untargeted ion-mobility dataset) drove this differentiation. Additional endogenous and exogenous small molecules were annotated through in-silico prediction, expanding the accessible chemical space. All spectra were saved in a digital metabolome archive available for future discovery work.

Integrating these complementary approaches closes the gap between quantitative accuracy and metabolome depth, enabling more comprehensive interpretation of biological responses with particular relevance for exposomics and molecular epidemiology.

SESSION 13. IMMUNOMETABOLISM AND INFECTIOUS DISEASE	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

13.1 **1:30 p.m. - 1:50 p.m.** **Abstract 48**

Serum NMR Metabolomics Directed Multi-omics Investigation of Acute Respiratory Distress Syndrome (ARDS) Severities and Their System Biology Pathways

PRESENTING AUTHOR: ***Leo Cheng, Corewell Health Research Institute, United States***

CO-AUTHORS: *Rajshree Ghosh Biswas, Ella Zhang, Clara Benatzky, Aaron Ziegler, Mulong Du, Li Su, David C. Christiani*

SUB-THEME: Respiratory diseases

Acute Respiratory Distress Syndrome (ARDS) is a life-threatening condition marked by rapid respiratory failure, diffuse lung injury, pulmonary edema, and severe hypoxemia, often requiring mechanical ventilation and prolonged ICU care. It can result from pneumonia, sepsis, trauma, and other inflammatory insults that trigger widespread epithelial and endothelial damage. Despite advances in supportive care, ARDS remains associated with high mortality and long-term morbidity, underscoring the need for improved mechanistic understanding and better diagnostic and therapeutic strategies. However, its underlying molecular and metabolic drivers are not fully defined.

To address this gap, we applied high-resolution magic angle spinning (HRMAS) NMR to profile serum metabolomics in ICU patients with and without ARDS. Metabolomic data were integrated with clinical outcomes and serum proteomics measured using the Olink platform, which combines proximity extension assay with next-generation sequencing for sensitive, high-throughput protein quantification.

This retrospective study analyzed 174 pre–COVID-19 serum samples from the Molecular Epidemiology of ARDS Study repository, including 74 ARDS patients (64 with matched proteomics) and 100 non-ARDS ICU controls. HRMAS NMR was performed on 10–20 µL serum at 4 °C and 5000 Hz using a 600 MHz spectrometer. Thirty-seven spectral regions of interest (ROIs) were identified. Seven ROIs significantly differed between ARDS and controls. Among ARDS patients, five ROIs distinguished survivors from non-survivors at 28 days, and one region (2.14–2.12 ppm), representing potential metabolites, was significant across all comparisons.

Several ROIs correlated with ARDS clinical severity scores. NMR-guided multi-omics integration revealed associations between 13 ROIs and 218 proteins after FDR correction, representing epithelial injury, inflammation, mitochondrial dysfunction, and oxidative stress, that were undiscernible with proteomics alone. These findings demonstrate that integrated metabolomic and proteomic axes can uncover new and novel system biology signatures in ARDS, potentially improving diagnosis, prognostication, and therapeutic targeting beyond clinical measures alone.

13.2 **1:50 p.m. - 2:05 p.m.** **Abstract 487**

AGILENT TECHNOLOGIES: Connecting Metabolomics to Function: A Unified Platform for Actionable Biology

PRESENTING AUTHOR: ***Fernando (Ralph) Tobias, Agilent Technologies, United States***

SUB-THEME: Connecting Metabolomics to Function

Advancing metabolomics research requires integrated, standardized workflows that reduce complexity and accelerate time to insight. Agilent Technologies delivers end-to-end omics solutions built on a unified platform spanning sample preparation, separation, detection, and data analysis, enabling reproducible, high-confidence results while lowering barriers to adoption. These harmonized workflows support metabolomics and broader omics applications with scalability and consistency across discovery and targeted studies. In addition, Agilent uniquely connects molecular profiling with functional biology through its cell analysis portfolio, including the Seahorse XF Analyzer, which enables real-time measurement of cellular metabolism to complement omics datasets and strengthen biological interpretation. Together, these capabilities provide a cohesive framework for generating robust, actionable insights with speed and confidence.

SESSION 13. IMMUNOMETABOLISM AND INFECTIOUS DISEASE	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

13.3 **2:05 p.m. - 2:25 p.m.** **Abstract 288**

Unraveling new pathways in sepsis pathogenesis using LC-MS/MS-based metabolic profiling

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

CO-AUTHORS: *Ricarda Jürgens, Nadine Bieß, Christian Strauß, Matthias Behrens, Julien Park, Angelika Rambold, Melanie Mersch-Dini, Michael Schäfers, Hans-Ulrich Humpf, Judith Austermann, Johannes Roth*

SUB-THEME: Immunometabolism

The human immune response is a complex defense system against various pathogens or foreign substances. Dysregulation of this immune reaction can lead to sepsis which is characterized by impaired responses to secondary infections and high mortality rates. Key modulators of this severe disease state are innate immune cells such as monocytes and macrophages, which show a reduced inflammatory response during sepsis-associated immune tolerance. Recent research highlights the metabolism of immune cells as an important factor contributing to the development of this tolerance.

Here, we investigated the metabolic mechanisms underlying immune tolerization using an established murine in vitro model. Immune tolerance was induced by repeated stimulation of monocytes with lipopolysaccharide (LPS), which is a bacterial marker molecule presented on the membrane of gram-negative bacteria. Immune tolerization was confirmed by reduced tumor necrosis factor alpha (TNF-α) secretion upon secondary stimulation with LPS compared to primary stimulation.

We were able to confirm the commonly described effects on the tricarboxylic acid cycle characterized by high levels of itaconate accompanied with enrichment of succinate as a result of succinate dehydrogenase inhibition. Using specific treatment and knock-out experiments we demonstrated that itaconate is not functionally relevant in immune tolerance, which is contrary to common knowledge. Instead, we identified glutamine metabolism as key driver of this phenotype, which is closely related to redox homeostasis and energy metabolism. This finding was supported by elevated cellular glutamine levels in monocytes isolated from sepsis patients exhibiting impaired glutaminolysis.

These results refine the current understanding of sepsis pathogenesis and underline the critical role of immune cell metabolism in developing immune tolerance.

13.4 **2:25 p.m. - 2:40 p.m.** **Abstract 268**

Simultaneous quantitative and qualitative analysis of oxylipins using high resolution mass spectrometry with CID and EAD based fragmentation

PRESENTING AUTHOR: ***Paul Baker, SCIEX, United States***

CO-AUTHORS: *Paul Norris, Thomas Horvath*

SUB-THEME: Immunometabolism

Oxylipins are bioactive lipid mediators that regulate inflammation, immune responses, and other physiological processes. Their structural diversity, low endogenous abundance, and extensive isomerism require analytical methods that provide both high quantitative sensitivity and confident structural confirmation. Traditionally, triple quadrupole mass spectrometers have been favored for quantitative oxylipin analysis.

This study demonstrates that a high-resolution quadrupole time-of-flight (QTOF) mass spectrometer equipped with electron-activated dissociation (EAD) can deliver triple quadrupole–level quantitative performance while enabling isomer-specific structural characterization within a single analytical workflow. Targeted oxylipin analysis was performed on a hybrid QTOF mass spectrometer. External calibration curves were generated for 70 oxylipins spanning concentrations from 0.00864 to 494.4 pg/µL. Quantitative data were acquired while collecting full product ion spectra without compromising duty cycle. Data processing followed published oxylipin community guidelines, including replicate injections (n ≥ 5 at low concentrations), evaluation of %CV (Quantitative analysis demonstrated excellent sensitivity across the oxylipin panel. For 20-OH-LTB4, the lower limit of quantitation (LLOQ) was 0.0864 pg/µL (0.43 pg on-column; S/N = 10.5), while 14,15-dihydroxyeicosatrienoic acid (14,15-DiHETrE) showed an LLOQ of 0.0329 pg/µL (1.645 pg on-column; S/N = 8.32). Qualitative analysis using EAD enabled clear differentiation of isomeric oxylipins. For example, 11-HETE produced unique fragment ions at m/z 151.0350 and

★ AWARD WINNERS

SESSION 13. IMMUNOMETABOLISM AND INFECTIOUS DISEASE	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

245.1152, whereas 11,12-EET generated a diagnostic fragment at m/z 231.0984. Extracted ion chromatograms of EAD-derived fragments showed clean, isomer-specific peaks that were not achievable using CID alone.

High-resolution QTOF mass spectrometry combined with EAD enables simultaneous quantitative and qualitative oxylipin analysis with triple quadrupole–level sensitivity and enhanced isomer specificity. This integrated workflow supports confident quantitation and structural confirmation in a single experiment, addressing a key analytical challenge in oxylipin-focused metabolomics studies.

13.5	2:40 p.m. - 3:00 p.m.	Abstract 283

COVID Dogolomics: Integrating Canine Olfaction and GC–MS Volatilomics to Identify Urinary VOC Signatures of Post COVID Syndrome

PRESENTING AUTHOR: ★ ***Lea Woyciechowski, TU Braunschweig, Germany***

CO-AUTHORS: *T. More, S. Meller, K. Zacharias, F. Twele, A. Dopfer-Jablonka, T. Welte, T. Illig, G. Behrens, H. A. Volk, and K. Hiller*

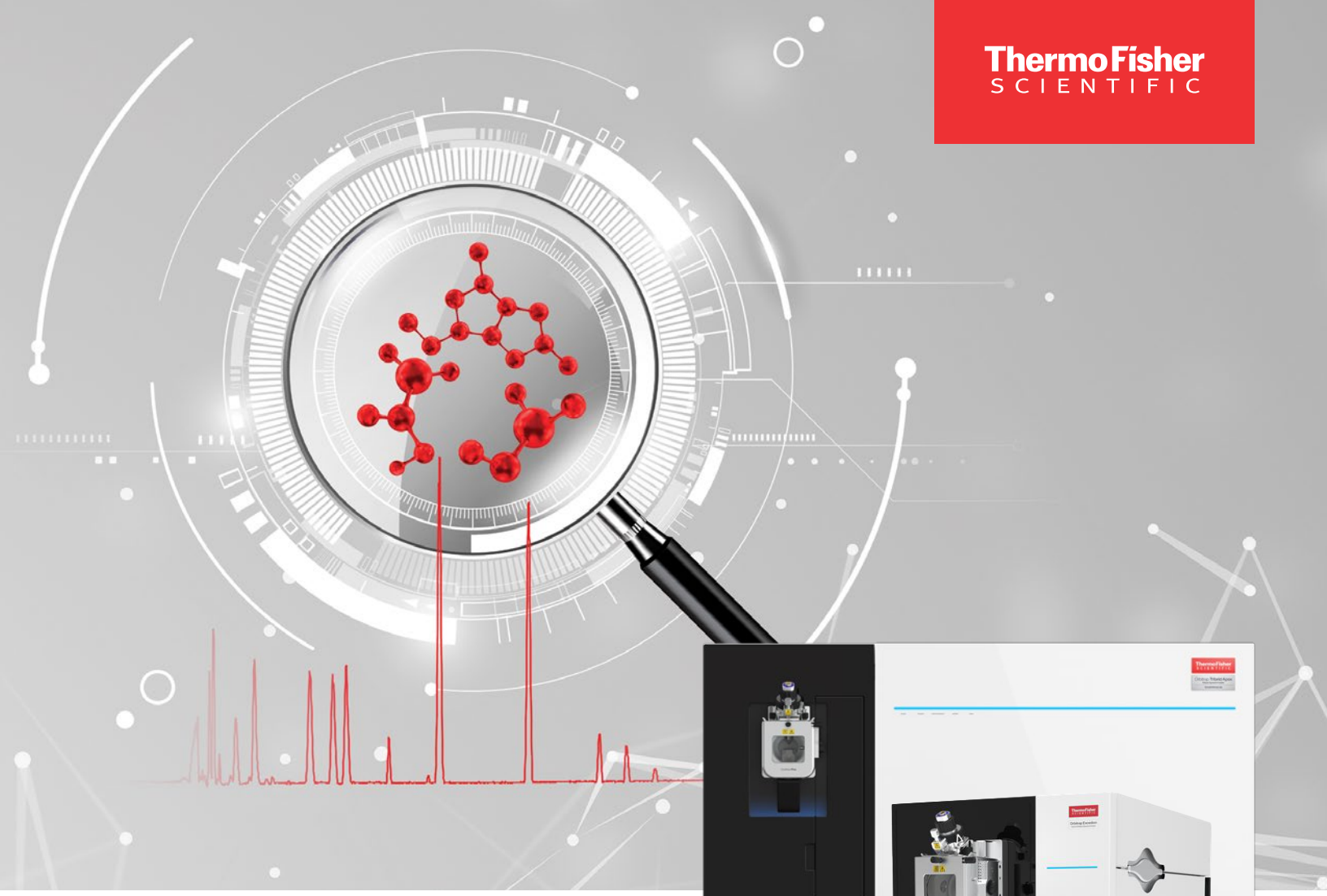
SUB-THEME: Immunometabolism

Volatile organic compounds (VOCs) are small, highly volatile molecules (

We established and analytically validated a headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME GC–MS) workflow optimized for limited urine volumes. Quality control included pooled reference samples, internal normalization strategies, and technical reproducibility assessment. Urinary VOC profiles from PCS patients and controls were analyzed using supervised machine learning. Random forest and regularized regression models identified discriminatory VOC patterns capable of separating PCS from controls. Feature selection revealed a subset of metabolites contributing most strongly to classification performance, supporting the presence of a disease-associated volatilomic signature.

Importantly, model outputs show concordance with canine detection performance, suggesting that dogs and mass-spectrometry-based profiling capture overlapping biochemical information. This systems-level Dogolomics framework links biological odor signatures with analytical chemistry and explainable artificial intelligence. The approach provides a foundation for objective, non-invasive diagnostics in PCS and may be extended to related immune-mediated and post-infectious syndromes.

ThermoFisher SCIENTIFIC



SQUAD analysis

Quantify what you know. Discover what you don't.

Accelerate your metabolomics research with integrated workflows powered by Thermo Scientific™ Orbitrap™ technology. From sample to insight, our solutions combine high-resolution mass spectrometers, intelligent data acquisition, and advanced software to enable simultaneous quantitation and discovery in a single analysis. Visit our booth to explore two new instruments designed to expand metabolome coverage, simplify complex data, and deliver confident quantitative results—faster.

Visit us at Metabolomics 2026 Booth #P5

Learn more at **thermofisher.com/metabolomics**

thermo scientific

SESSION 14. REAL-TIME ANALYSIS	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

14.1 **1:30 p.m. - 1:50 p.m.** **Abstract 201**

Integrated real-time breath analysis for therapeutic drug monitoring and outcome prediction in epilepsy

PRESENTING AUTHOR: ***Pablo Sinues, University of Basel, Switzerland***

CO-AUTHORS: *Kai Fricke, Diego Manuel Baur, Kapil Dev Singh, Felix Schmidt, Kathrin Müsch, Noriane Andrina Sievi, Marco Di Donato, Anton Schmick, Jonas Herth, Silvia Ulrich, Pablo Sinues, Marian Galovic, Malcolm Kohler*

SUB-THEME: Breath analysis/Volatolomics

Optimizing antiseizure medication (ASM) therapy requires both accurate assessment of drug exposure and early identification of treatment failure or adverse effects, yet conventional therapeutic drug monitoring is invasive and provides limited insight into pharmacodynamic response. Secondary electrospray ionization high-resolution mass spectrometry (SESI-HRMS) offers a noninvasive way to capture both drug-related and endogenous metabolic information from exhaled breath.

In a single-center observational study, 117 adults with epilepsy contributed 127 valid paired breath and serum measurements during stable ASM treatment. Breath spectra were analyzed to estimate serum concentrations of valproic acid (total and free), lamotrigine, levetiracetam, and lacosamide, and to classify treatment response and clinically significant side effects using machine-learning models with nested cross-validation.

Breath-based prediction showed good agreement with serum concentrations across ASMs, with Lin's concordance correlation coefficients of 0.777 for total valproic acid, 0.848 for free valproic acid, 0.781 for lamotrigine, 0.733 for levetiracetam, and 0.895 for lacosamide; in-range agreement reached 83.8%, 91.7%, 88.2%, 71.2%, and 100.0%, respectively. In the same cohort, breath metabolomic profiles predicted treatment response with an AUC of 0.843 and ASM-related side effects with an AUC of 0.880, whereas serum ASM concentrations showed no meaningful association with either outcome. Pathway analysis linked non-response mainly to altered tyrosine/phenylalanine and intermediary energy metabolism, and side effects to broader amino acid metabolic disturbances.

These findings support a unified breath-based precision monitoring approach in epilepsy, in which a single noninvasive sample may provide both pharmacokinetic information and clinically relevant metabolic signatures of efficacy and tolerability.

14.2 **1:50 p.m. - 2:05 p.m.** **Abstract 485**

THERMO FISHER SCIENTIFIC: Software, hardware and intelligent acquisition strategies for guided unknown metabolite characterization

PRESENTING AUTHOR: ***Susan S. Bird, Thermo Fisher Scientific, United States***

The field of metabolomics has come a long way to effectively turn mass spectrometry signals into confident and reproducible chemical identities. It's well known that a single metabolite can generate multiple observable signals (isotopes, adducts, and multimers), while additional peaks arise from contaminants and unintentional fragmentation during ionization and ion transfer, requiring intelligent data reduction and filtering. As data processing and hardware advances continue to develop and address this complexity, the large chasm of uncharacterized small molecule compounds remains a top pain point for researchers applying global metabolomics strategies. Beyond spectral library matching, which is very effective at identifying known chemical identities, confident unknown annotations require intelligent sample acquisition as well as analysis.

Comprehensive MS/MS strategies such as the AcquireX Deep Scan workflow improve the efficiency of MS/MS collection by excluding background ions detected in blanks and iteratively targeting new relevant precursors in samples of interest across replicate injections. Combined with enhanced dynamic range methods, which attenuate the highly abundant compounds to reproducibly detect moderate-to-low abundant species often missed through ion-suppression, molecular networking can begin to piece together the substructure of common compound classes and provide orthogonal confidence. Real-Time Library Search (RTLS) offers a means to run ddMSn experiments with parameters adjusted based on matching compounds to a library. A similarity experiment allows the system to find the closest matching compound in the library in real time, then guide the instrument to collect ddMS3 data on matching fragments to confirm known substructures and non-matching fragments to characterize unknown structures.

SESSION 14. REAL-TIME ANALYSIS	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

In this presentation, we will showcase real-world examples utilizing the intelligently acquired consensus evidence as applied to a standard global experiment. The enhanced workflow is designed to reduce false positives and enable defensible reporting aligned with community based small-molecule annotation confidence frameworks.

14.3 **2:05 p.m. - 2:25 p.m.** **Abstract 92**

Real-time metabolomic approach for the monitoring of Chinese hamster ovary cell metabolism

PRESENTING AUTHOR: ***Yuandi Zhao, University of Edinburgh, United Kingdom***

CO-AUTHORS: *Bart Pander, Luke Johnston, Catalina Guerra Cornejo, Karl Burgess*

SUB-THEME: Non-targeted and semi-targeted methods

Metabolomics is a powerful tool for identifying metabolites in a biological system, which provides important insights during bioprocesses, such as the monitoring of cellular metabolism, nutrient consumption, and by-product formation. However, most existing metabolomic approaches rely on offline sampling and analysis with only a handful of time points can be collected, limiting bioprocess monitoring and control. In the meanwhile, sample instability has introduced significant challenges to the effectiveness of metabolomics. The development of real-time metabolomics (RTMet) provides the resolution to address these challenges, enabling more direct and accurate monitoring into dynamic changes during the bioprocesses. In this study, we presented a direct infusion mass spectrometry (MS) based real-time metabolomic approach. It was applied to monitor the metabolism of Chinese hamster ovary (CHO) cells, which are the preferred cell factory for monoclonal antibody (mAb) production in biopharmaceutical manufacturing. Two different CHO cell lines were used for this study, including a non-producer CHO K1 cell line and a novel engineered trastuzumab (Herceptin) producing CHO K1-HERC cell line. The metabolites of these two cell lines were continuously monitored in real-time for 7 days, during which samples were collected to assess the cell viability and for subsequent metabolite characterisation by nuclear magnetic resonance (NMR) spectroscopy. The successful application of the RTMet in CHO cells confirmed previous metabolomic observations at an unprecedented temporal resolution, offering deeper insights into cellular dynamics and cultivation. We showed that culture dynamics and interventions could be measured in greater detail both in terms of temporal resolution and the number of metabolites, highlighting its potential as a promising tool for bioprocess analytical testing.

14.4 **2:25 p.m. - 2:40 p.m.** **Abstract 28**

Urinary Metabolome dynamics in 13C-labeled mice

PRESENTING AUTHOR: ***Eric Ezan, CEA, France***

CO-AUTHORS: *Annelaure Damont, Anaïs Legrand, Kathleen Rousseau, Laurent Bellanger, Séverine Boiry, Frédéric Gibiat, Jean-Jacques Leguay, Christophe Junot, François Fenaille, Eric Ezan*

SUB-THEME: Flux analysis

Specific pathway analyses of metabolic fluxes traditionally rely on administering stable isotope precursors and quantifying their incorporation into selected metabolites by mass spectrometry-based approaches.

In this study, we introduce a comprehensive strategy to interrogate whole-metabolome in vivo dynamics by feeding mice a fully 13C-enriched diet (>97%). The diet, composed of 13C-labeled spirulina and in-house produced 13C-labeled wheat flour, was administered to three mice for six weeks, while controls received an unenriched diet.

All animals exhibited normal growth and their urine was collected daily for 39 days. LC-HRMS-based metabolomics was carried out with a C18 column coupled to an Orbitrap mass spectrometer equipped with an electrospray ionization source operating in both positive and negative modes. Following data processing with W4M and MetExtract II workflows, metabolites were confidently annotated by integrating 12C/13C MS and MS/MS data. Isotopic data were extracted for each metabolite to study their individual 13C-enrichment trajectories.

Among the hundreds of detected features, 238 metabolites were confidently annotated, including organic and amino acids, nucleic acids and derivatives, dipeptides, and acyl-conjugated species of carnitine, glycine and glucuronide, all of which

SESSION 14. REAL-TIME ANALYSIS	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

covering multiple biochemical pathways. Global urinary labelling was rapid, exceeding 90% 13C enrichment within 22 days. Strikingly, enrichment kinetics were highly metabolite-specific, reflecting differences in metabolic function, origin, and biosynthesis in vivo. Rapid labelling characterized central biochemical pathways, whereas slower incorporation suggested release from deeper storage compartments.

This innovative model provides a powerful framework to resolve systemic metabolite dynamics and can be extended to investigate metabolic remodelling in response to nutritional, pharmacological, or pathological perturbations.

14.5 **2:40 p.m. - 3:00 p.m.** **Abstract 118**

Real Time Metabolomics and Bayesian Optimization for Surfactant Production in Bacillus subtilis

PRESENTING AUTHOR: ★ ***Catalina Guerra, University of Edinburgh, United Kingdom***

CO-AUTHORS: *Karl Burgess, Diego Oyarzun, Bart Pander*

SUB-THEME: Bioprocessing

While many bioprocesses can be prototyped at 'lab scale' increasing process volume to commercial levels is challenging and prone to failure. Process analytical testing is key to assure a correct fermentation development. This project aims to optimize non-ribosomal lipopeptide synthesis of sustainable antimicrobial surfactants in Bacillus subtilis.

Two techniques will be considered: Real Time Metabolomics (RTMet) and gsMOBO. RTMet is a technique that connects a bioreactor to a mass spectrometer allowing real-time analysis of the biochemistry underpinning bioprocesses. gsMOBO is a computational approach for media design that integrates genome-scale metabolic models with multiobjective optimization. Both techniques aim to link the measured exctracellular compounds produced in fermentation with the predicted behaviour of the metabolic network and its necessity for different nutrients.

As a proof of concept, RTMet was implemented in a 20 ml bioreactor, injecting cell-free broth samples every 10 minutes into the mass spectrometer. Meanwhile, gsMOBO was applied to iBsu1103, a B. subtilis model and different components of M9 medium were varied to optimize growth and surfactin production. Results show a trade off between both objectives, some media components were widely distributed, showing how exploration is balanced with exploitation.

RTMet is a powerful tool that allows to monitor in real time fermentation compounds in the liquid phase to identify key metabolites. On the other hand, gsMOBO provides a broadly applicable tool for the design of cost-effective and productive culture media. Both techniques can be combined: one to improve media composition towards maximising production, and the other one to monitor closely different compounds that reflects the state of the bioprocess. As future work, some gsMOBO optimal media compositions will be analysed using RTMet to compare predicted and measured surfactin titres.

SESSION 15. PLANTS AND FOOD	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

15.1 **1:30 p.m. - 1:50 p.m.** **Abstract 104**

Integrated metabolomics reveals genotype-driven phenolic diversity in sorghum germplasm

PRESENTING AUTHOR: ***Carolina T. S. D'Almeida, Federal University of the State of Rio de Janeiro, Brasil***

CO-AUTHORS: *Natalia Gomes Xavier, Carolina Thomaz dos Santos D'Almeida, Maria Eduarda Magalhães da Silva, Hygor Marcos Ribeiro de Souza, Alexandre Guedes Torres, Mariana Simões Larraz Ferreira, Jurandir Vieira de Magalhaes, Maria Marta Pastina*

SUB-THEME: Foodomics and food security

Sorghum (Sorghum bicolor L.) is a climate-resilient cereal notable for its high phenolic content and functional potential. This study characterized and quantified the phenolic profile of 29 grain sorghum genotypes using integrated untargeted and targeted metabolomics to assess phenotypic variability among diverse materials associated with bioactive loci. Grains were cryogenically milled, and phenolics compounds were obtained by sequential hydroalcoholic extraction followed by alkaline and acid hydrolysis. Untargeted analysis was performed by UHPLC-HRMS (Orbitrap Q-Exactive Plus™, ESI-), with data acquired in data-dependent MS/MS mode (dd-MS2 top 3, m/z 100–1000; 35,000 FWHM), enabling fragmentation-based metabolite annotation. Analytical standards and pooled QC samples were injected every six runs. Data processing and annotation were conducted in Progenesis QI following MSI guidelines, based on accurate mass (≤2 ppm) and fragmentation matching to a curated phenolic database. Targeted quantification was performed by UPLC–TQ-S in MRM using external calibration, with TargetLynx™ XS processing and manual validation of peak integration; all analytes showed good linearity (R^2>0.995) over the evaluated concentration ranges. Untargeted analysis detected 25,246 molecular features, which were reduced to 253 high-confidence phenolic features after database filtering and QC criteria. Major compounds comprised flavonoids (mainly apigenin and myricetin), glycosylated derivatives, B-type procyanidins, and hydroxycinnamic acids. PCA explained 57% of total variance and hierarchical clustering revealed two major sample groups (G1 and G2). G1 comprised light-pericarp, low-tannin genotypes enriched in phenolic acids; whereas G2 grouped brown/red, tannin-rich genotypes enriched in flavonoid and proanthocyanidin-related metabolites. Targeted analysis confirmed high inter-genotype variability. Apigenin ranged from non-detectable to 35.01 ng/100 mg. trans-Ferulic acid was predominant among hydroxycinnamic acids, followed by p-coumaric and caffeic acids. Chlorogenic acid was detected at trace levels (< 1 ng/100 mg), highlighting the sensitivity of UPLC–TQ-S platform. This integrative metabolomics approach revealed structured phenolic diversity in sorghum germplasm and supports genotype selection for bioactive enrichment.

15.2 **1:50 p.m. - 2:05 p.m.** **Abstract 109**

Anaerobic processing enhances γ-aminobutyric acid (GABA) production and α-glucosidase inhibitory activity in tempe

PRESENTING AUTHOR: ***Chiaki Tsushi, Shimadzu Corporation, Japan***

CO-AUTHORS: *Eiichiro Fukusaki, Sastia P. Putri*

SUB-THEME: Fermented foods

Tempe is a traditional Indonesian fermented soybean food.Tempe is rich in protein, essential amino acids, and dietary fiber, as well as bioactive components such as isoflavones and GABA. GABA is well known as an inhibitory neurotransmitter and has been reported to affect relaxation and sleep. It has also been reported to lower blood pressure and blood glucose levels. However, tempe has a relatively low GABA content compared to other GABA-rich foods such as tomato, potato, and pumpkin. Therefore, this study focused on increasing GABA in tempe to improve its functionality. We applied three different treatments （anaerobic treatment, high-pressure processing, and freeze-thaw treatment) to increase GABA in tempe and analyzed the results. We found that anaerobic treatment, was the most effective method to increase GABA in tempe by approximately 16 times, from 37.6 mg/100 g to 596.9 mg/100 g. Furthermore, a comprehensive analysis of the components using metabolomics revealed that branched-chain amino acids (BCAAs) and tryptophan, which have been implicated in pathways associated with postprandial glucose homeostasis, as well as GABA, were significantly increased. Multiple mechanisms are involved in lowering blood glucose levels, one of which is intestinal glucose absorption suppression by α-glucosidase inhibition. Therefore, we measured α-glucosidase inhibitory activity as the functional evaluation, and anaerobic tempe showed higher

★ AWARD WINNERS

SESSION 15. PLANTS AND FOOD	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

inhibitory activity than normal tempe. Furthermore, this study demonstrates for the first time that high concentrations of GABA and tryptophan exhibit α-glucosidase inhibitory activity, suggesting that the increase in GABA and tryptophan by anaerobic treatment may have associate with the increase in α-glucosidase inhibitory activity in tempe.

15.3 **2:05 p.m. - 2:25 p.m.** **Abstract 90**

The MetaBó-Bainne Study – Characterisation of the milk metabolome from a seasonal pasture-based dairy system using 1H-NMR spectroscopy

PRESENTING AUTHOR: ★ ***Paula Rojas Gómez, University College Cork, Ireland***

CO-AUTHORS: *Paula Rojas-Gómez, Raghunath Pariyani, Michael Dineen, Denis Lynch, Lorraine Bateman, Eoghan Roche, Anita R. Maguire, Noel A. McCarthy, Thomas Brendan Murphy, James A. O'Mahony, and Tom F. O'Callaghan*

SUB-THEME: Foodomics and food security

Milk is a widely consumed highly nutritious food whose macro- and micronutrient composition has been extensively characterised. However, the milk metabolome remains comparatively underexplored in dairy science, despite its potential to identify biomarkers linking animal physiology, diet, and milk quality. This is particularly relevant in seasonal calving pasture-based systems, where fluctuations in milk composition directly influence techno-functional properties and processing efficiency as a result. This study aimed to characterise the milk metabolome of a seasonal pasture-based spring-calving dairy system using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Over 41 weeks, ten dairy farms were visited weekly for the collection of raw bulk tank milk samples (n = 410) and three commercial pasteurised skimmed milks were also purchased weekly (n = 123), representing one of the most comprehensive longitudinal datasets of the milk metabolome. A total of 38 milk metabolites were quantified, of which 30 exhibited significant seasonal variation. Multivariate analysis identified key metabolites associated with seasonal metabolic shifts. Winter (early lactation) milk was enriched in ketone bodies, O-phosphocholine, creatinine, and glucose-1-phosphate, reflecting increased metabolic stress and negative energy balance. In contrast, autumn (late lactation) milk contained higher concentrations of choline and urea, indicative of improved energy status but reduced nitrogen use efficiency. Furthermore, this publication goes beyond the metabolite analysis by including pathway analysis, showing how seasonality affects different metabolic routes, providing a link between the animal's physiology and milk composition. These findings highlight the potential of milk metabolomics as a valuable tool for monitoring physiological status and guiding interventions to enhance sustainability, animal health and processing efficiency in dairy systems. This publication represents a central component of the MetaBó-Bainne project and underpins the development of the "Pasture-based milk metabolome database" (www.metabobainne.ie), translating the objective of this project into a practical reference tool for future dairy metabolomics research and industry applications.

15.4 **2:25 p.m. - 2:40 p.m.** **Abstract 150**

Untargeted Metabolomics Integration of MS and NMR for the Taxonomic Investigation of Psychotria and Palicourea

PRESENTING AUTHOR: ***Daniele De Oliveira Silva, Federal University of Alfenas, Brazil***

CO-AUTHORS: *Paula Pio de Oliveira Salem, Lara Priscila D' Martin Costa, Paulo Roberto Santos da Silva, Danielle Ferreira Dias, Daniela Aparecida Chagas-Paula, RuAngelie Edrada-Ebel, Marisi Gomes Soares*

SUB-THEME: Plant metabolomics

The tribe Psychotrieae (Rubiaceae) is recognized for its taxonomic complexity, with the genus Psychotria historically regarded as polyphyletic[1]. Although recent phylogenetic studies have resulted in the segregation of new genera, such as Palicourea, their phytochemical differentiation remains insufficiently explored[2]. While Psychotria is generally associated with polyphenols, Palicourea is linked to complex alkaloids; however, these associations lack robust diagnostic markers that could support molecular phylogenetic relationships[2]. To address this gap, LC-MS and NMR analyses were conducted to identify chemotaxonomic markers distinguishing Psychotria and Palicourea. Twenty-nine herbarium specimens were analyzed using

★ AWARD WINNERS

SESSION 15. PLANTS AND FOOD	Tuesday, June 23	1:30 p.m. - 3:00 p.m.

UPLC-HRMS/MS and [1]H-NMR. Data integration used block scaling, followed by multivariate analyses (PCA, PLS-DA, OPLS-DA), which were validated through CV-ANOVA and permutation testing. Variable selection employed stringent criteria (VIP, p-value, and Mann-Whitney/FDR) to ensure robustness and minimize overfitting. The PLS-DA model effectively discriminated between the genera (R2 = 0.86; Q2 = 0.41; pCV-ANOVA = 0.02). Psychotria exhibited a diverse chemical profile, including flavonoids, terpenoids, alkaloids (Level 3, MSI), other nitrogenous molecular formulas (Level 4, MSI), and aromatic region signals. In contrast, Palicourea displayed greater internal variability, with a distinct subgroup identified by OPLS-DA (R2 = 0.99, Q2 = 0.87, pCV-ANOVA providing new insights into the chemical differentiation between the studied genera.

[1]Santos AF, et al. Aust Syst Bot 2021;34:527–40.

[2]Berger A, et al. Phytochem Rev 2022;21:941–86.

15.5 **2:40 p.m. - 3:00 p.m.** **Abstract 177**

Untargeted UHPLC-HD-QTOF/MS Metabolomics Reveals Pathway-Level Modes of Action and Defence Priming by Laminarin and Paenibacillus alvei T22 in Triticum aestivum

PRESENTING AUTHOR: ★ ***Manamele Dannies Mashabela, University Of Johannesburg, South Africa***

CO-AUTHORS: *Lizelle A. Piater, Tarekegn Terefe, Pavel Kerchev, Neerakkal Sujeeth, Msizi I. Mhlongo*

SUB-THEME: Plant metabolomics

Laminarin (β-glucan seaweed extract) and Paenibacillus alvei T22 (PGPR) individually enhance wheat growth and stress tolerance, yet their combined metabolic effects and integration under biotic stress remain uncharacterised at the systems level. We applied untargeted metabolomics to dissect the pathway-level modes of action of each agent, alone and in combination, on Triticum aestivum under normal and Pst stress conditions. Seedlings (cv. Gariep) received laminarin (1% v/v foliar), PGPR (seed biopriming, OD_{600} = 0.5), their combination, or control (Study I); Study II added Pst inoculation across five groups. Leaf metabolomes (n = 3; W1–W3) were profiled by SYNAPT XS HD-QTOF/MS (HSS T3 C18; ESI±; m/z 100–1500 Da), processed in MzMine 4.7.8 (Level 2 MSI), and modelled in MetaboAnalyst 6.0 (PLS-DA, sPLS-DA, VIP, KEGG MetPA). Profiling of annotated metabolites yielded significant chemometric separation (Accuracy = 1.0, R^2 = 0.98, Q^2 = 0.897) and three distinct pathway-level mechanisms. Laminarin drove a maximal TCA flux (enrichment p = 4.03×10^{-7}, ratio ≈50), peak Phe/Tyr/Trp, and full phenylpropanoid cascade activation (cinnamic → ferulic acid; C-glycosyl flavones). PGPR induced an ISR-targeted phenylpropanoid flux (feruloyl agmatine, coumaroyl putrescine) with controlled TCA upregulation, preserving the growth-defence balance. The combination achieved regulatory integration, preventing extremes while sustaining targeted defence priming, an emergent non-additive mechanism sustained over three weeks. Under Pst challenge, priming drove stepwise metabolic divergence (sPLS-DA Component 1: 15.9% Pst-only → 40.9% combined). Pst alone depleted constitutive benzoxazinoid (BX) pools via ZmAuxRP1-mediated suppression, while combined priming restored and amplified BXs above controls, maximised hydroxycinnamic acid-polyamine conjugates (feruloyl putrescine, sinapoyl hydroxyagmatine) and revealed emergent MAMP-ISR crosstalk absent in either agent alone (orientin uniquely elevated). These results provide the first systems-level mechanistic framework for microbial-non-microbial biostimulant interaction and the rational basis for next-generation consortia targeting durable stripe rust resistance.

SESSION 16. CHEMICAL EXPOSURES AND BIOACTIVE METABOLITES	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

16.1 **3:30 p.m. - 3:50 p.m.** **Abstract 261**

Plasma-Based Biomonitoring of Environmental PAH Exposure Using a Targeted GC-MS/MS Exposomics Platform

PRESENTING AUTHOR: ***Jutarop Phetcharaburanin, Khon Kaen University, Thailand***

CO-AUTHORS: *Jakkapong Kluebsoongnoen, Kriangsak Jenwitheesuk, Ronnarit Boonyarat, Wassana Jamnongkan, Theerayut Bubpamala, Peeraya Suksuratin*

SUB-THEME: Exposome research

Polycyclic aromatic hydrocarbons (PAHs) are persistent environmental pollutants generated from incomplete combustion processes and are widely present in air pollution, industrial emissions, and food preparation activities. Several PAHs are recognized human carcinogens, and chronic exposure has been associated with increased risks of cancer, cardiovascular disease, and immune dysfunction. However, sensitive and reliable analytical methods for monitoring trace-level PAHs in human plasma remain limited, hindering large-scale human biomonitoring and exposome research.

In this study, we developed and optimized a targeted analytical workflow for the simultaneous quantification of sixteen U.S. EPA priority PAHs in human plasma using a modified QuEChERS extraction combined with gas chromatography–triple quadrupole mass spectrometry (GC-MS/MS). Method performance was evaluated through calibration linearity, recovery, and precision using matrix-matched validation. The validated platform was applied to plasma samples obtained from healthy blood donors across three age groups (adolescents, adults, and elderly; n = 30).

The analytical method demonstrated strong linearity across the concentration range of 0–20 ng/mL (R^2 = 0.963–0.998) with reproducible extraction performance (recovery 48–55%, RSD < 1 0%). All sixteen PAHs were detected in donor plasma, with concentrations ranging from 0.08 to 3.15 ng/mL. Low- and medium-molecular-weight PAHs were the dominant contributors to total PAH burden, consistent with common exposure sources such as traffic emissions, cooking-related combustion, and tobacco smoke. Carcinogenic high-molecular-weight PAHs were detected at lower but quantifiable concentrations. Age-stratified analysis revealed broadly similar PAH distribution patterns across all age groups.

This study establishes a sensitive and practical GC-MS/MS workflow for plasma-based PAH biomonitoring and provides a valuable analytical platform for human exposome investigations. The detection of environmental PAHs in healthy donor plasma underscores the importance of integrating environmental exposure monitoring into public health surveillance and blood safety assessment.

16.2 **3:50 p.m. - 4:10 p.m.** **Abstract 215**

Integrating the chemical exposome into metabolomics via the ExpoLib spectral library and newborn screening programs

PRESENTING AUTHOR: ***Vinicius Verri Hernandes, University of Vienna, Austria***

CO-AUTHORS: *Nick Hetzschold, Thomas Karner-Metz, Maximilian Zeyda, Lukas Wisgrill, Benedikt Warth*

SUB-THEME: Exposome research

Environment and food-related exposure chemicals are often underrepresented in metabolomics workflows due to their relatively low concentrations, limited coverage in mass spectral libraries, and generally low awareness within the metabolomics community. At the same time, there is increasing evidence linking environmental exposure and health effects. Therefore, better capturing the exposome within metabolomics workflows can provide a deeper understanding on the onset, progression and treatment of diseases. We present two contributions in that direction. Firstly, we introduce ExpoLib, a mass spectral library developed in mzmine that includes >200 xenobiotics of toxicological relevance commonly detected in humans, such as bisphenols, phthalates, pesticides, mycotoxins, PFAS chemicals and many others. The library also incorporates biotransformation products, expanding coverage of key metabolic derivatives. High-quality MS/MS spectra are provided across more than ten collision energies, aiming at enhancing the annotation of the external chemical component. Secondly, we describe the application of a novel LC-MS methodology to the screening of these xenobiotics in 750 dried blood

SESSION 16. CHEMICAL EXPOSURES AND BIOACTIVE METABOLITES	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

spot samples from the Austrian Newborn Screening Program. Newborns represent a highly vulnerable population and therefore, implementing a screening program of toxicants in addition to the long-term established newborn screening is of strategic relevance to define baseline exposure levels, investigate potential long-term health outcomes and serve as an early-warning system. Targeted exposomics and untargeted metabolomics were independently performed using the same sample extract. A significant number of xenobiotics were quantitatively reported, with differences observed across four regions of Austria. Detected xenobiotics included personal care products (e.g: parabens), air pollutants (e.g.: cotinine), pharmaceutical drugs (e.g.: acetaminophen and acetaminophen glucuronide), phytoestrogens (e.g.: formononetin) and food processing by-products (e.g.: 5-hydroxymethylfurfural). Untargeted data revealed the enhanced annotation of exposure compounds by including the ExpoLib library into the workflow. Finally, information on key endogenous metabolites were additionally obtained and correlations to environmental exposure are explored.

16.3	4:10 p.m. - 4:30 p.m.	Abstract 265

High-throughput Urine Exposomic Studies for Global Health: Deciphering Cigarette Smoke and Coffee Co-exposures...A Bad Combination?

PRESENTING AUTHOR: ***Philip Britz-McKibbin, McMaster University, Canada***

CO-AUTHORS: *Zachary Kroezen, Biban Gill*

SUB-THEME: Exposome research

Tobacco smoking and coffee consumption are two modifiable lifestyle factors relevant to global health that are also strongly associated with each other. Although moderate coffee consumption alone may confer some health benefits, its combination with tobacco may paradoxically exacerbate tobacco-related harms. To date, observational studies have reported contradictory findings on the causal link between tobacco smoking and coffee intake when relying on self-reports that are prone to bias since sociocultural practices and products consumed vary widely between countries.

Herein, we investigate tobacco smoke and coffee co-exposures in a sub-cohort of the Prospective Urban Rural Epidemiological (PURE) study (n=5,000;14 countries) using high-throughput multisegment capillary electrophoresis-mass spectrometry in positive and negative ion modes. A targeted analysis of the seven urinary nicotine metabolites (i.e., total nicotine equivalent) was used to verify tobacco smoke exposures, whereas nicotine metabolizers were classified based on the nicotine metabolic ratio as an indicator of their nicotine dependence. Untargeted metabolite analyses was also performed to identify specific urinary biomarkers associated with coffee consumption distinct from other caffeinated beverages, including tea and soda. Untargeted profiling identified population-generalizable coffee biomarkers, validated for selectivity, dose-response, association strength, and precision. A panel of urinary biomarkers of coffee intake generalizable to diverse populations in PURE were identified by MS/MS and then validated based on several criteria, including selectivity, association strength, dose-response, and precision.

For the first time, we report striking sex- and regional variations in active/secondhand smoke exposures across country income groups, not reliably captured by self-reports in PURE. Further, fast nicotine-metabolizing female smokers showed the highest coffee intake, exceeding age/BMI-matched males and verified non-smoking females, as confirmed by robust urinary biomarkers of coffee intake. This work may help guide more effective smoking cessation strategies for chronic disease prevention in high-risk female smokers by concomitantly reducing their coffee exposures.

SESSION 16. CHEMICAL EXPOSURES AND BIOACTIVE METABOLITES	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

16.4	4:30 p.m. - 4:50 p.m.	Abstract 136

Untargeted Metabolomics and Type 2 Diabetes Incidence: A Prospective Multi-cohort study in European Populations

PRESENTING AUTHOR: ***Robert van Vorstenbosch, Institute for Risk Assessment Sciences, Utrecht University, Netherlands***

CO-AUTHORS: *Robert van Vorstenbosch, Chrysovalantou Chatziioannou, Nivonirina Robinot, Pekka Keski-Rahkonen, Max Oosterwegel, Ayoung Jeong, Megi Vogli, Natalia Vilor-Tejedor, Regina Pickford, Licia Iacoviello, Simone Costanzo, Marta Manczuk, W.M. Monique Verschuren, Romana Ševčíková, Katerina Coufalikova, Elliot J Price, Jelle Vlaanderen, Nicole Probst-Hensch, Roel Vermeulen, Annette Peters, EXPANSE consortium*

SUB-THEME: Exposome research

Introduction: Type 2 Diabetes (T2D) presents a major global health concern with rapidly increasing prevalence. Where lifestyle and genetic factors are well-established contributors, our understanding of the involvement of the chemical exposome still remains limited.

Aim: The current study explored associations between blood-based metabolome and exposome towards the development of T2D.

Methodology: Within EXPANSE, we analyzed 2365 prospectively collected plasma samples from a nested case-control study across five European adult population-based cohorts (Morgen, Moli-Sani, KORA, Pons and Sapaldia). T2D cases were confirmed for their absence at baseline, with disease presence at follow-up (i.e. based on self-reported diagnosis / medication OR registry confirmed diagnosis OR glycemia levels above diagnostic cutoff). Matched controls (i.e. based on age, sex, and follow-up time) had confirmed absence at follow-up. Untargeted metabolomic profiling was performed using liquid and gas chromatography high resolution mass spectrometry (LC- and GC-HRMS).

Metabolome-Wide association analyses were conducted using conditional logistic regression with false discovery rate correction. In addition, we applied Variable-selection ANOVA Simultaneous Component Analysis (VASCA) to be inclusive towards multivariate signals. The selected set was further explored using Sparse Non-Negative Matrix Underapproximation to estimate mixture scores, which were regressed towards T2D incidence.

Results: LC-HRMS analysis identified 207 metabolomic features significantly associated with incident T2D, of which 45 were annotated (i.e. levels 1-2). These included branched-chain amino acids, ureate and acylcarnitines, consistent with previous observations. Signals potentially reflecting exogenous compounds were also detected. GC-HRMS analysis detected 737 peaks, of which 218 were annotated (level 1), including polycyclic aromatic hydrocarbons , phthalates, persistent organic pollutants , pesticides, cosmetic compounds, and flame retardants.

Conclusion: We identified exogenous and endogenous chemical signals prospectively associated with T2D incidence across diverse European populations. Integrating metabolomics with exposome profiling may provide new insights into environmental drivers of metabolic disease and identify early biomarkers of T2D risk.

SESSION 16. CHEMICAL EXPOSURES AND BIOACTIVE METABOLITES	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

16.5 **4:50 p.m. - 5:10 p.m.** **Abstract 23**

LC–HRMS Metabolomics of Plasma Samples: Diagnosing Interferences, Contaminants and Matrix Effects Using Post-Column Infusion of Stable Isotope-Labelled Standards

PRESENTING AUTHOR: ***Daniel Malheiro, Univerisity of Copenhagen / Cmbio, Denmark***

CO-AUTHORS: *Lea G. Johnsen, Morten Danielsen, Nikoline Juul Nielsen, Jan H. Christensen*

SUB-THEME: Other

Matrix effects (MEs) from complex biological matrices, such as human plasma, can compromise quantitative reliability and comparative analysis in untargeted LC–HRMS metabolomics. This study aimed to systematically establish, monitor, evaluate, and correct MEs via post-column infusion (PCI) of stable isotope-labelled standards (SILSs) in samples prepared using different pretreatment protocols. PCI conditions were optimized with respect to infusion concentration and flow rate to ensure a stable baseline and minimal impact on chromatographic performance. Eight SILSs with diverse physicochemical properties were infused during LC–HRMS analysis of NIST SRM 1950 plasma. Sample pretreatments included protein precipitation (PPT) and phospholipid removal (PLR) using filters with varying solvent volumes.

MEs were quantified by comparing PCI ion signals during injection of plasma extracts or solvent blanks. Homologue series searches and clustering revealed non-plasma contaminants, including polyethylene glycols and fatty amides (oleamide, erucamide). Contaminants were significantly reduced by all PLR washes (p Dilution reduced phospholipid-driven suppression from ~43% (undiluted) to ~4% at 10× dilution. Early-eluting polar compounds in PLR-treated plasma exhibited severe suppression (~88%) that improved only partially (~63%) upon dilution. Large-scale matrix factor (MF) correction was applied using the optimal combination of PCI-SILSs and correction type (mean, median, or minimum) across 335 features. Validation demonstrated significantly improved analytical precision, reducing median RSD from 12.4% to 9.6% ($p = 8\times10^{-}12$) across filtered features. Overall, integrating PCI diagnostics with MF-based correction provides a framework for mitigating MEs in untargeted LC–HRMS.

★ AWARD WINNERS

SESSION 17. DATA ECOSYSTEMS AND KNOWLEDGE SHARING	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

17.1 **3:30 p.m. - 3:50 p.m.** **Abstract 190**

MetabolomicsHub: International Data Exchange and Data Representation Standards for Metabolomics

PRESENTING AUTHOR: ***Callum Martin, EMBL-EBI, United Kingdom***

CO-AUTHORS: *Callum Martin, Ozgur Yurekten, Thomas Payne, Jonathan Hunter, Naveen Raj Kookkal Polliapram, Eoin Fahy, Mano Maurya, Srinivasan Ramachandran, Brian C. DeFelice, Carlos G. Gonzalez, Joshua E. Elias, Nils Hoffmann, Edward Mathias, Yasin El Abiead, Pieter C. Dorrestein, Shankar Subramaniam, Juan Antonio Vizcaíno*

SUB-THEME: Data repositories

With the growth of public metabolomics datasets worldwide, there is an urgent need for standardized, fully interoperable repositories that promote global collaboration. A new international consortium, MetabolomicsHub (https://metabolomicshub.org), has been established to standardize and advance open data practices by creating a unified, FAIR (Findable, Accessible, Interoperable and Reusable)-compliant infrastructure for the discovery, exchange and reuse of public metabolomics data.

Building on successful models such as ProteomeXchange and the International Nucleotide Sequence Database Collaboration, the consortium brings together major repositories, MetaboLights, Metabolomics Workbench and GNPS/MassIVE, alongside researchers, journals, funders and technology vendors.

Initially focused on mass spectrometry datasets, the initiative is implementing a harmonized, graph-enabled common data model aligned with established frameworks (ISA and C2M2), domain ontologies (including MONDO, UBERON, CL, CLO and NCBI taxonomy) and chemical identifiers (ChEBI and RefMet). Repository-specific converters generate standardized, downloadable JSON files compliant with the model, supported by shared utilities for structural and semantic validation and dataset announcement. The extensible architecture supports evolving data types, external integrations and AI-driven research.

Parallel efforts include establishing a shared accession number space (MHDxxx), promoting open standards such as mzTab-M and Universal Spectrum Identifiers (USIs), and developing open-source tools and guidelines for incorporating additional resources into the framework.

A centralized web portal under development will provide unified indexing and access to standardized public datasets, enabling cross-repository discoverability, large-scale meta-analyses, improved experimental reproducibility and enrichment pipelines integrated with platforms such as GNPS2.0 and structureMASST. This collaborative infrastructure lays the groundwork for data-driven discovery across metabolomics datasets and omics layers.

Novel Aspect: First unified, FAIR-compliant infrastructure enabling cross-repository discovery, harmonization and integrated analysis of global public metabolomics datasets.

17.2 **3:50 p.m. - 4:10 p.m.** **Abstract 251**

FASSTrecords and StructureMASST: Unifying Public Metabolomics Data for Worldwide Metabolome Exploration

PRESENTING AUTHOR: ★ ***Yasin El Abiead, BOKU University, Austria***

CO-AUTHORS: *Jeong In Seo, Wilhan Donizete Goncalves Nunes, Ozgur Yurekten, Thomas Payne, Eoin Fahy, Shankar Subramaniam, Juan Antonio Vizcaíno, Nikiforos Alygizakis, Jeremy Carver, Mingxun Wang, Pieter C. Dorrestein*

SUB-THEME: Metabolite and mass spectral databases

Public metabolomics data now spans nearly a million LC–MS/MS raw files across major repositories, but effective reanalysis remains inaccessible to most researchers. The combination of scale, heterogeneity, and computational burden makes it difficult to ask simple biological questions across public data at repository scale. We address this by transforming large, distributed metabolomics resources into a compact, queryable annotation layer that links molecular structures, MS/MS evidence, and harmonized sample metadata.

SESSION 17. DATA ECOSYSTEMS AND KNOWLEDGE SHARING	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

To build this resource, we aggregated MS/MS library spectra from GNPS and MassBankEU and searched them with the FASST algorithm against mz(X)ML raw data deposited in GNPS/MassIVE, MetaboLights, Metabolomics Workbench, and NORMAN/DSFP using a reproducible Nextflow pipeline. All hits passing a cosine threshold of at least 0.7 with at least three matching peaks were stored in a SQLite database together with harmonized metadata from Pan-ReDU. To make repository-scale results broadly accessible, we created StructureMASST, a Streamlit-based web interface that supports BLAST-like searches for structures, substructures, and analogues.

The resulting database, FASSTrecords, contains search results from 1,565,620 library spectra representing 200,258 molecules queried against 920,790 raw data files, yielding 142,538,419 spectral matches, 35% of which are linked to harmonized metadata. Overall, 142,637 library molecules (71%) returned at least one match in public data, highlighting the broad recoverability of known chemical space across repositories. Even when restricting to the top match per raw-data MS/MS scan, 138,051 molecules (69%) remained represented.

FASSTrecords and StructureMASST make repository-scale metabolomics data interpretable and reusable without requiring users to directly process tens of terabytes of raw files. This enables large-scale exploration of molecular distributions across taxa, tissues, and environments and opens new opportunities to study biological patterns, environmental occurrence, and analogue series across the worldwide metabolome.

17.3	4:10 p.m. - 4:30 p.m.	Abstract 63

Mass Spectral Library Network (MERLIN) To Streamline The Generation Of Public Spectral Libraries.

PRESENTING AUTHOR: ***Federico Brigante, Institute Of Organic Chemistry And Biochemistry, Czech Republic***

CO-AUTHORS: *Corinna Brungs, Roman Bushuiev , Filip Jozefov, Tomáš Pluskal*

SUB-THEME: Metabolite and mass spectral databases

Mass Spectral Library Network (MERLIN) To Streamline The Generation Of Public Spectral Libraries.

With an estimated ~10^60 small molecules in the chemical space, assigning a spectrum to its correct structure is inherently challenging. Natural products further complicate this task, as they often occur at low concentrations and feature complex scaffolds and stereochemistry. As a result, confident identification depends on high-quality reference mass spectral libraries. However, the available open libraries present some drawbacks, namely, lack chemical coverage; they provide only MS^2 fragmentation; they are high-priced; or their use is restricted for specific applications. This motivates the creation of an open-source, multi-stage MS^n library in which molecules are fragmented in successive rounds, rather than in a single stage, to reveal deeper structural layers and enhance molecular annotation. Importantly, this approach remains fully compatible with existing MS^2 workflows. The first stage of the MERLIN initiative, the MSnLib project, introduced the Python workflow for data cleanup, sample preparation, acquisition using a flow injection system coupled to an Orbitrap mass analyzer, and data processing for MSn where 163,000 were measured in 2025, and an extra 97,000 compounds will be measured by mid-2026. A comparison of molecular coverage between MERLIN and current public libraries (MoNA, GNPS, Massbank) using a Venn diagram, generated by matching molecules based on their planar structures showed that only 1318 structures are shared out of +200,000 in MERLIN, showing the enormous contribution of unique structures of the initiative. Similarly, a UMAP embedding computed from Tanimoto similarity between Morgan fingerprints highlighted the large difference in coverage of the chemical space of MERLIN compared to the same public repositories. The MERLIN initiative constitutes an enormous community effort to build the largest open-source MSn library up to date.

SESSION 17. DATA ECOSYSTEMS AND KNOWLEDGE SHARING	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

17.4 **4:30 p.m. - 4:50 p.m.** **Abstract 91**

Beyond altruism: How open research maximizes your scientific impact.

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

CO-AUTHORS: *Johannes Rainer, RforMassSpectrometry Community.*

SUB-THEME: Collaborative and open data science

In metabolomics, progress is often hindered by a reliance on opaque software and static archives. These barriers do more than obstruct reproducibility; they force researchers to reinvent the wheel for every project. However, the success of community-driven tools like GNPS, mzmine, xcms, and SIRIUS, all built upon public data and/or collective effort, demonstrates that open development creates superior resources. This presentation argues that adopting such practices is not merely altruistic, but actually a strategic choice to maximize individual scientific impact. By sharing data and building on community foundations, researchers save time: they future proof their own work while contributing to the improvement of the very tools they rely on.

We demonstrate how the RforMassSpectrometry initiative implements these "self-serving" ideals through a modular ecosystem that allows user to consume public resources while facilitating the generation of new, sharable data. Using the Spectra package, researchers can stream and cache raw data directly from repositories like MetaboLights or MassIVE. We highlight how this interoperability bridges programming languages: SpectriPy allows R users to leverage Python libraries like matchms, while RuSirius provides a programmatic interface to SIRIUS 6. To ensure findings are shareable, MsIO and RmzTabM facilitate result exchange via universal formats like mzTab-M. To ensure these technical advances are accessible, the ecosystem includes a dedicated educational focus via Metabonaut. This website provides transparent, executable tutorials using Quarto to encapsulate an entire study, from data loading to final annotation, into a single, reproducible file. Crucially, these standardized files are designed to be shared alongside publications, allowing reviewers and readers to re-run and verify analyses instantly.

Ultimately, adopting this ecosystem is a strategic investment in your own scientific work, ensuring your research is robust, reusable, and achieves maximum scientific impact.

17.5 **4:50 p.m. - 5:10 p.m.** **Abstract 51**

OmniPath Metabo: database and tools for mechanistic modeling and analysis of metabolomics data

PRESENTING AUTHOR: ***Christina Schmidt, University Clinics Heidelberg, Germany***

CO-AUTHORS: *Jonathan Schaul, Yunfan Bai, Jannik Franken, Toby Lawrence, Denes Turei, Julio Saez-Rodriguez*

SUB-THEME: Data repositories

Machine learning–based annotation now substantially increases metabolite identification in untargeted experiments and has outpaced integration with structured prior knowledge. In addition, fragmented databases, inconsistent identifier systems, and multiple identifiers per compound, within and across resources, prevent seamless mapping and mechanistic interpretation. Moreover, most metabolite sets remain restricted to canonical pathways (e.g., KEGG), with limited integration of receptor, transporter, disease, perturbation, and functional context. Together, these limitations hinder mechanistic, metabolite-centered analyses.

We present OmniPath Metabo, a comprehensive, harmonized metabolite-centric prior-knowledge resource covering metabolites, lipids, food-derived compounds, drugs, receptors, transporters, enzymes, reactions, allosteric regulators, and disease associations. OmniPath Metabo resolves cross-database identifiers, tracks ambiguity, and enables dynamic, project-specific construction of context-specific subnetworks. It is built directly from the original resources, harmonized using controlled vocabularies and ontologies, by a modular resource pipeline. The database comprehensively maps identifiers and names to chemical structures and includes built-in cheminformatics by RDKit. OmniPath Metabo is accessible via an interactive web app and an HTTP API at metabo.omnipathdb.org. While 30+ resources are currently included in OmniPath Metabo, more resources are in our development pipeline.

SESSION 17. DATA ECOSYSTEMS AND KNOWLEDGE SHARING	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

From OmniPath Metabo we derive resources for specific applications, including ortho- and allosteric regulation, metabolite–receptor and metabolite–transporter interactions; highly customizable lipid reaction networks; and combined causal networks of signaling and metabolism. This not only provides more up-to-date and customizable knowledge to our multi-omics causal modeling framework COSMOS, but also extends its scope to metabolite-mediated cell-cell communication. Furthermore, we integrate COSMOS to the spatial omics analysis tool MISTy, enabling an integrated and spatially resolved mechanistic modeling of spatial metabolomics and spatial transcriptomics.

By bridging the gap between metabolomics features and database knowledge, OmniPath Metabo transforms fragmented resources into a prior-knowledge solution suitable for mechanistic and functional analysis of multiple omics, enabling the investigation of the regulatory roles of metabolites.

★ AWARD WINNERS

SESSION 18. ENVIRONMENT I	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

18.1 **3:30 p.m. - 3:50 p.m.** **Abstract 96**

Heat, Halogens, and Holobionts: Seasonal Metabolomic and Microbiome Dynamics in the Invasive Red Alga Asparagopsis taxiformis

PRESENTING AUTHOR: ***Tal Luzzatto Knaan, University of Haifa, Israel***

CO-AUTHORS: *Omri Nahor, Hadar Winckler-Aharoni, Nataly Barger, Maya Lalzar, Michael Dubovis, Ivan Plyushchenko, Álvaro Israel*

SUB-THEME: Marine metabolomics

Marine macroalgae are prolific sources of halogenated natural products that shape ecological interactions and offer significant biotechnological value. The invasive red alga Asparagopsis taxiformis produces brominated metabolites with antimicrobial and methane-reducing activity, yet the environmental regulation of this chemical repertoire remains poorly resolved.

We performed a two-year, bi-monthly untargeted UHPLC-HRMS/MS survey of A. taxiformis from two Mediterranean coastal sites to characterize seasonal metabolomic restructuring. Feature-based molecular networking and isotopic pattern analysis resolved halogenated metabolite clusters and their temporal dynamics. Fractionation and antibacterial assays linked LC–MS features to functional activity, while 16S rRNA sequencing enabled integration of epiphytic microbiome composition.

Metabolomic profiles showed strong seasonal differentiation, with seawater temperature explaining the largest share of variance. Warmer periods were associated with expansion of polybrominated chemical space, enrichment of distinct molecular network clusters, and significantly enhanced antibacterial activity. Multiple brominated features displayed temperature-specific abundance patterns and strong bioactivity correlations. Concurrent microbiome shifts indicate coordinated environmental control over microbial assembly and algal secondary metabolism.

These findings demonstrate how longitudinal untargeted metabolomics resolves environmentally driven dynamics in complex halogenated natural product repertoires and provide a framework for climate-responsive marine chemical ecology and targeted natural product discovery.

18.2 **3:50 p.m. - 4:10 p.m.** **Abstract 222**

Biodiversity effects on oak leaf litter volatilome and implications for soil macrofauna

PRESENTING AUTHOR: ★ ***Gabriela A. S. Escaliante, German Centre for Integrative Biodiversity Research (iDiv), Germany***

CO-AUTHORS: *Hannah Koller, Diana Morales-Fonseca, Omer Nevo, María Méndez Camarena, Nicole M. van Dam*

SUB-THEME: Biodiversity

Leaf metabolomes are highly dynamic and respond to both biotic and abiotic stresses. In forest ecosystems, these metabolic responses may also be influenced by the diversity of neighboring tree species, potentially shaping the chemical composition of senesced leaf litter. However, it remains poorly understood to what extent tree species richness influences the volatile composition of decomposing leaf litter and how these changes may affect interactions with decomposer communities. We hypothesized that tree species richness would alter the volatile profiles of Quercus petraea leaf litter across time, possibly due to changes in nutrient availability. Additionally, we hypothesized that such changes could have cascading effects on the species composition of litter-associated macrofauna. To test these hypotheses, we investigated leaf litter volatiles within a biodiversity-ecosystem functioning experiment, sampling plots representing three diversity levels (oak-only, oak +1 species, and oak +3 species). Leaf litter was collected over six consecutive days during early decomposition, alongside macrofauna samples for DNA metabarcoding to characterize macrofauna communities. Volatile compounds were analyzed using solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC-MS). Carbon and nitrogen content were determined using an elemental analyzer, and macrofauna communities were characterized using DNA metabarcoding. Multivariate analyses revealed significant differences in leaf litter volatile profiles among tree diversity levels that remained consistent throughout the sampling week. The C:N ratio increased significantly with tree species richness, and pairwise

SESSION 18. ENVIRONMENT I	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

comparisons showed that all diversity levels differed significantly from each other in both volatile composition and C:N ratios. Tree species richness significantly affected total emissions of terpenoids, fatty acids, and shikimate- and phenylpropanoid-derived compounds. These results demonstrate that tree species richness can shape the chemical dynamics of leaf litter during early decomposition, potentially driven by differences in leaf nitrogen content. Ongoing metabarcoding analyses will further link volatile composition with the structure of litter-associated macrofauna.

18.3 **4:10 p.m. - 4:30 p.m.** **Abstract 204**

Environmental Metabolic Footprinting (EMF): A New Framework for Assessing the Fate and Impact of Complex Biocontrol Natural Extracts in Soil

PRESENTING AUTHOR: ***Cedric Bertrand, Universite De Perpignan, France***

CO-AUTHORS: *Anouar Mejait, Hikmat Ghosson, Robin Cahuzac, Christian Espinoza, Marie-Virginie Salvia*

SUB-THEME: Environmental exposures

In the EU, the market authorization of biocontrol products is governed by the same regulatory framework as synthetic pesticides. A major bottleneck for innovation in this sector is the lack of analytical tools to study and define the environmental persistence of complex natural extracts used as active substances. While standardized OECD guidelines exist for synthetic pesticides—typically composed of a single active molecule—they are ill-suited for complex mixtures. For instance, the half-life (DT50), a key proxy derived from kinetic studies in soil microcosms, is straightforward for single molecules but fails to account for the diverse degradation dynamics of the multiple constituents within a natural organic extract. To address this challenge, we present Environmental Metabolic Footprinting (Patil, 2016), an innovative concept based on monitoring the evolution of soil metabolomic profiles using UPLC-HRMS during microcosm kinetics (Salvia, 2018). We have developed a comprehensive analytical workflow, including standardized soil extraction protocols (Ghosson, 2024), raw data processing pipelines, and bioinformatics tools for soil degradation modeling. Furthermore, we introduce a method to cluster mixture components based on their specific degradation patterns (Mejait, 2025), leading to two new proxies: Dissipation Time and Resilience Time. The latter offers a more integrative perspective: by monitoring the co-extracted soil endometabolome, we can determine the time required for degradation products to vanish while simultaneously assessing the treatment's impact on soil biological activity through comparative analysis of endometabolome of the treated vs. untreated soils. This presentation marks the first international public disclosure of EMF as a untargeted metabolomics framework. By capturing the meta-metabolome—the functional intersection of the xenometabolome (active substances and their derivatives) and the endometabolome (native ecosystem signatures)—EMF transcends classical chemical monitoring and provides, holistic tool to characterize both the environmental trajectory and the ecological footprint of complex mixtures.

Mejait.2025. 10.1016/j.envadv.2025.100665 ; Ghosson.2024. 10.1021/acs.analchem.3c03242 ; Salvia.2018. 10.1007/s11356-017-9600-6 ; Patil.2016. 10.1016/j.scitotenv.2016.05.071

18.4 **4:30 p.m. - 4:50 p.m.** **Abstract 130**

Integrated NMR-MS Metabolomics Reveals Climate-Dependent Neuroprotective Signatures in Mangrove Species

PRESENTING AUTHOR: ***Jordan Kahfi, King Abdullah University of Science and Technology, Saudi Arabia***

CO-AUTHORS: *Galih Kusuma Aji, Dicki Rachman, Susi Kusumaningrum, Abdul-Hamid Emwas, Monika Chodasiewicz*

SUB-THEME: Neurological disorders

Mangrove plants are rich sources of phytopharmaceutical compounds due to their ability to survive under extreme environmental conditions by producing diverse bioactive metabolites. Previous studies have reported that Avicennia marina (AM) and Rhizophora mucronata (RM) possess medicinal activities. However, studies investigating their neuroprotective potential related to Alzheimer's disease remain limited, and comparative analyses of mangrove species from different climatic regions are scarce.

SESSION 18. ENVIRONMENT I	Tuesday, June 23	3:30 p.m. - 5:15 p.m.

This study aimed to compare the metabolome profiles and neuroprotective potentials of AM and RM collected from two contrasting environments: Indonesia (tropical climate) and Saudi Arabia (desert climate). Methanolic crude extracts of AM and RM from both regions were analyzed using Nuclear Magnetic Resonance spectroscopy (NMR) and Mass Spectrometry (MS). Biological activities were evaluated through Total Phenolic Content (TPC), Total Flavonoid Content (TFC), antioxidant assays, cholinesterase inhibition assays (acetylcholinesterase and butyrylcholinesterase), and anti-amyloidogenic activity against amyloid beta 40 (Aβ40). Aβ40 aggregation was assessed using the Thioflavin T fluorescence assay (ThT), confocal microscopy, atomic force microscopy (AFM), and circular dichroism (CD) spectroscopy.

NMR analysis showed that Indonesian AM and RM contained higher levels of sugars, polysaccharides, and aliphatic compounds, whereas Saudi samples were richer in phenolic and aromatic compounds. GC-MS and LC-MS analyses further indicated that Indonesian samples were abundant in sugars and fatty acids, while Saudi samples contained higher levels of flavonoids (hispiduloside, naringenin, quercetin, and epicatechin) and phenolic compounds (chlorogenic acid).

RM Saudi exhibited the highest phenolic and flavonoid contents, antioxidant capacity, and cholinesterase inhibitory activity, followed by AM Saudi, RM Indonesia, and AM Indonesia. Both Saudi mangrove species significantly inhibited Aβ40 fibril formation, as confirmed by ThT, confocal microscopy, AFM, and CD spectroscopy.

These findings warrant further compound elucidation and demonstrate that climatic conditions strongly influence mangrove metabolomes and their neuroprotective potential. It also higlights the importance of environmental factors in natural products and metabolomics research.

18.5	4:50 p.m. - 5:10 p.m.	Abstract 21

Compound-class-based profiling for comprehensive untargeted metabolome analysis

PRESENTING AUTHOR: ***Pilleriin Peets, University of Tartu, Estonia***

CO-AUTHORS: *Meine D. Boer, Bas E. Dutilh, Andreas F. Haas*

SUB-THEME: Marine metabolomics

Identification of chemical features in complex untargeted LC-HRMS datasets remains limited, with fewer than 10% of detected peaks receiving confident structural annotation. To expand the accessible chemical space, computational tools like SIRIUS, combined with CSI:FingerID and CANOPUS, enable the prediction of molecular fingerprints and probabilistic ClassyFire compound classes without requiring final structure elucidation. Because many metabolites exhibit structurally diverse or ambiguous characteristics, assigning them to a single compound class is often challenging and misleading. SIRIUS+CANOPUS overcomes this limitation by providing probabilistic annotations across more than 2,700 compound classes, spanning hierarchical levels from broad (e.g., organic compounds, lipids) to highly specific subclasses (e.g., 2-halobenzoic acids, glycosphingolipids).

We utilize these predicted compound-class vectors to characterize the chemical composition of complex environmental samples. Our initial work [1] showed that, across Earth Microbiome Project samples, compound-class vectors reliably distinguish major microbiome types, demonstrating that class-level chemical profiles can reveal ecological differences independent of compound-level identification.

Building on these insights, we extended the same analytical framework to a focused case-study investigating submerged acid lakes in the Caribbean. Here, we integrated compound-class vectors with LC-HRMS peak intensities to compare samples that share similar chemical composition but differ in relative abundance. We identified several compound classes enriched in these acidic environments, including diverse organohalogen classes. While chlorinated and fluorinated compound classes showed minimal differences between acid lakes and surrounding marine waters, iodinated compounds exhibited clear enrichment in acid lakes. A notable finding given the general rarity and narrow distribution of iodinated metabolites. Additionally, the acid-lake samples demonstrated trends toward higher molecular mass and less polar compound classes.

We are now applying this framework to coral-reef systems, with early analyses revealing metabolomic patterns associated with coral stress, bleaching, and thermal exposure.

[1] Peets et al. (2025), *J Cheminform* 17, 82. DOI: 10.1186/s13321-025-01031-2

★ AWARD WINNERS

SESSION 19. ENVIRONMENT II	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

19.1 **11:30 a.m. - 12:00 p.m.** **Abstract 176**

Development and validation of a fecal steroidomics workflow for wildlife endocrine monitoring

PRESENTING AUTHOR: ***Tom Cools, Leibniz Institute for Zoo and Wildlife Research, Germany***

CO-AUTHORS: *Jella Wauters, Rodrigo Serra, Juan Montoya, Irene Diez Guttierrez, Beate Braun, Lynn Vanhaecke*

SUB-THEME: Non-targeted analysis in biomonitoring and environmental monitoring

Endocrine monitoring has substantially supported wildlife conservation programs such as the Iberian Lynx Ex Situ Breeding Program, which helped recover the Iberian lynx from near extinction. However, access to blood samples in wild species is often limited. Fecal samples offer a readily obtainable, non-invasive alternative but species-specific steroid metabolism produces distinct fecal metabolite patterns, limiting the reliability of conventional immunoassays that depend on antibody cross-reactivity. Therefore, we optimized and validated a fecal processing workflow for application on a previously developed UHPLC-Q-Orbitrap-HRMS platform enabling general (un)targeted steroidomic biomonitoring in wildlife species. Method development and validation was based on pooled female Iberian lynx samples obtained ≥3 weeks pre-estrus or ≥3 weeks postpartum. Targeted screening identified 33 endogenous steroid compounds based on accurate m/z and retention time, including androgens, estrogens, progestagens, and glucocorticoids. Of these, 31 demonstrated excellent linearity (R^2 > 0.99), of which 24 and 23 compounds showed good intra- and inter-day precision, respectively (CV 0.90). The method was subsequently applied to fecal samples from male lynx and from females in anestrus and late pregnancy (n = 3 individuals and 6 samples per group) for further biological validation. Targeted analysis revealed elevated levels of nine different progestagens in all female samples, including anestrus, consistent with the presence of persistent corpora lutea in this species, with more stable signal intensities observed during late pregnancy. Additionally, PGFM, a known pregnancy marker, was exclusively detected in late pregnancy samples. As expected, male samples showed substantially lower levels of progestagens and estrogens compared to females. Overall, these results confirm the analytical and biological validity of the optimized fecal processing workflow and enable broader application of the MS-based wildlife endocrine monitoring platform for conservation research.

19.2 **12:00 p.m. - 12:20 p.m.** **Abstract 295**

Selective sequestration and differential tissue distribution of xenobiotics in the poison frog Phyllobates terribilis: A quantitative HPLC-MS/MS study

PRESENTING AUTHOR: ★ ***Mabel Gonzalez, Stanford University, United States***

CO-AUTHORS: *Anne Wampler, Katelyn Santa Maria, Leigh Henderson, June Winters, Aurora Alvarez-Buylla, Justin Du Bois, Lauren O'Connell*

SUB-THEME: Xenobiotics and nutrient metabolism

The ability of certain organisms to sequester exogenous chemical compounds from their environment is a sophisticated physiological adaptation. This study investigates the uptake and distribution of a diverse array of xenobiotics, ranging from potent neurotoxins to common alkaloids, to elucidate the mechanisms of chemical defense and physiological storage in Phyllobates terribilis. In nature, this frog is able to ingest, survive and sequester one of the most potent neurotoxins in nature, batrachotoxin (BTX), which makes it the most toxic vertebrate on earth; yet the underlying physiological mechanisms remain poorly understood. To unravel these processes, we designed three controlled feeding treatments: BTX only, a synthetic analog (truncated BTX, tBTX), and a "toxic cocktail" (TC) of ten xenobiotics—including a 10% dose of the BTX and tBTX used in the other trials. Tissues (intestine, liver, skin, plasma, and brain) were analyzed using HPLC-MS/MS and compared to tissues of frogs fed with vehicle control. Quantitative results confimed the presence of diverse xenobiotics across all target tissues. Principal Component Analysis (PCA) demonstrated that skin and liver tissues maintain distinct chemical signatures based on the treatment group, with the first two principal components accounting for over 40% of the variance. While low-potency alkaloids like decahydroquinoline (DHQ) were widely distributed, other xenobiotics were not accumulated and the sequestration of high-potency xenobiotics, specifically BTX (m/z 539.3112) and tBTX (m/z 471.2485), preferentially occured in the skin. Notably, these chemical shifts were accompanied by significant changes to measurable behavioral traits such as boldness. This study provides a comprehensive quantitative map of xenobiotic sequestration in P. terribilis. The data suggest a

★ AWARD WINNERS

SESSION 19. ENVIRONMENT II	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

highly selective physiological mechanism for the transport and storage of some specific alkaloids, distinguishing between different structural analogs. These results advance our understanding of how organisms manage a complex "chemical library" of acquired defenses.

19.3 **12:20 p.m - 12:35 p.m.** **Abstract 234**

Untargeted DI-HRMS Metabolomics Reveals UV-A-Induced Metabolic Plasticity in High-Andean Lichen Species

PRESENTING AUTHOR: ★ ***Erika Lizet Milagros Calla Quispe, Pontificia Universidad Católica del Perú, Peru***

SUB-THEME: Environmental exposures

Lichens are complex symbiotic systems formed by fungi and photosynthetic partners that enable survival in environments exposed to multiple environmental stressors. In high-Andean ecosystems, particularly in the central Andes of Peru, organisms experience intense ultraviolet radiation (UV) due to high altitude and reduced atmospheric filtering. Despite their ecological importance, the metabolomic diversity of Andean lichens and their biochemical responses to abiotic stress remain poorly characterized. This study aimed to investigate the influence of UV-A radiation on the metabolic expression of lichen species collected from high-Andean Peruvian environments. Lichen thalli were exposed to controlled UV-A radiation for three consecutive days in a growth chamber under standardized environmental conditions. Untargeted metabolomic profiling of thalli extracts, including both UV-A-stressed and non-stressed samples, was conducted using direct-infusion high-resolution mass spectrometry (DI-HRMS/HESI) to capture stress-induced metabolic fingerprints. Comparative analysis revealed significant shifts in molecular feature profiles across lichen species following UV-A exposure. Several metabolites detected in stressed samples were absent or present at different abundances in non-stressed thalli, suggesting activation of stress-responsive biosynthetic pathways potentially associated with photoprotection and oxidative stress mitigation. These findings highlight the metabolic plasticity of Andean lichens under extreme environmental conditions and demonstrate the potential of DI-HRMS-based metabolomics for rapid characterization of chemical responses to environmental stress. This work provides one of the first untargeted metabolomic explorations of UV-induced metabolic responses in Andean lichens, contributing to the understanding of chemical adaptation mechanisms in high-altitude ecosystems.

19.4 **12:35 p.m. - 12:55 p.m.** **Abstract 132**

PFAS Exposure Modulates Mitochondrial Susceptibility in Steatotic Hepatocytes

PRESENTING AUTHOR: ★ ***João Marcos Barbosa, Örebro University, Sweden***

CO-AUTHORS: *Andi Alijagic, Anh Hoang Nguyen, Victor Castro-Alves, Lorane Valgueblasse, Matej Orešič, Tuulia Hyötyläinen*

SUB-THEME: PFAS

Per- and polyfluoroalkyl substances (PFAS) are highly persistent environmental pollutants that bioaccumulate in humans and are linked to metabolic diseases, such as metabolic dysfunction-associated steatotic liver disease (MASLD). Mitochondria, as central regulators of energy and lipid metabolism, are key subcellular targets of PFAS toxicity, yet PFAS-induced alterations in mitochondria-enriched fractions under clinically relevant steatotic conditions remain underexplored. This study aimed to determine how steatosis modulates PFAS-induced mitochondrial metabolic changes in human hepatocytes at the organelle level. Human HepaRG hepatocytes were exposed to a PFAS mixture under non-steatotic and steatotic conditions. The PFAS mixture (47.8% PFOS, 28.7% PFOA, 12.1% PFHxS, 6.3% PFDA, 5.1% PFNA; 0.1–2.8 µM) reflected concentrations and profiles commonly reported in human epidemiological studies. Mitochondria-enriched cellular fractions and the extracellular secretome were isolated and extracted using MeOH:MTBE:IPA (20:15:15, v/v) to enable integrated exposomics, metabolomics, and lipidomics with comprehensive coverage, followed by liquid chromatography–high-resolution mass spectrometry (LC-HRMS) analysis. Functional mitochondrial assays were performed to assess effects on respiratory capacity. PFAS and steatosis induced both distinct and combined effects on metabolism, including lipid accumulation in mitochondria-rich fractions consistent with increased mitochondria–lipid droplet contact, disturbances in cardiolipin levels, and elevated excretion of acylcarnitines. PFAS-induced perturbations of mitochondrial metabolism were markedly exacerbated in steatotic

★ AWARD WINNERS

SESSION 19. ENVIRONMENT II	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

hepatocytes. Pathway analysis indicated disruptions in lipid metabolism and inflammation-related pathways, including leukotriene metabolism and squalene and cholesterol biosynthesis, revealing a shared metabolic signature along the PFAS-steatosis axis. Mitochondrial function assays showed that PFAS reduced respiratory capacity in both conditions, whereas steatosis alone increased baseline mitochondrial activity but nearly abolished spare respiratory capacity, particularly in combination with PFAS. Together, these findings demonstrate that steatosis amplifies PFAS-induced mitochondrial dysfunction in human hepatocytes, increasing the susceptibility of steatotic liver to PFAS exposure and indicating a mechanistic link through which PFAS may worsen MASLD progression.

19.5	12:55 p.m. - 1:15 p.m.	Abstract 184

Seasonal Metabolite Profiling of Thyone aurea from Saldanha Bay, South Africa

PRESENTING AUTHOR: ★ ***Cassandra Upton, University of South Africa, South Africa***

CO-AUTHORS: *Cassandra Upton, Gerhard Prinsloo, Paul Anton Steenkamp, Moses Okpeku*

SUB-THEME: Marine metabolomics

The pursuit of natural therapeutic drugs has fuelled interest in metabolomics, particularly marine metabolomics, with sea cucumbers standing out for their high nutritional value and potential for bioactive metabolites. However, limited research, especially in Southern Africa, has resulted in significant knowledge gaps regarding the value of lesser-known species, contributing to the exploitation of select high-value species due to rising market demand for holothurian products. This study aimed to address these gaps by characterising the metabolic profile of three body tissue extracts–body wall, gonad, and gut/mesentery–from Thyone aurea, an endemic species found along the Western Coast of Southern Africa (33°03'S, 17°55' E), using untargeted 1H-NMR metabolomics and full-scan UPLC-QTOF-MS/MS. The results revealed a diverse array of amino acids, sugars, and organic acids, with the gut/mesentery tissue and summer month extracts exhibiting the highest metabolic potential. While some compounds may be integral to sea cucumber physiology, the origin of others remains unclear, warranting further research into holothurian metabolic pathways and the potential impact of dietary sources and microbial activity on the metabolome. Therefore, this study emphasises the importance of understanding metabolic responses to environmental changes, which could offer valuable insights to support conservation and sustainable cultivation strategies. Moreover, these findings underscore the need to explore lesser-known species as alternative resources in cultivation strategies to promote sustainable holothurian resources for commercial, pharmaceutical, and nutritional applications. Overall, this research enhances our understanding of sea cucumber biology and underscores the importance of incorporating a broader range of species in scientific research.

SESSION 20. CANCER METABOLOMICS	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

20.1 **11:30 a.m. - 12:00 p.m.** **Abstract 364**

Uncovering the Heterogeneity of the Human Metabolome

PRESENTING AUTHOR: ***Rafael Montenegro Burke, University Of Toronto, Canada***

CO-AUTHORS: *Jeremy K. Chan, Nicholas Ly, Olivia Taverniti, William Gwynne, Brandon Lieng, Sophia Alonzi, Verne Urquhart-Cox, Andrew T. Quaile*

SUB-THEME: Cellular metabolism

In recent years, significant efforts have been focussed into understanding metabolic reprograming in cancer with the hope of discerning context-specific biology that is exploitable for either for diagnosis or treatment. While some generalized phenomena such as the 'Warburg Effect' have been identified, the metabolic landscape of cancer is highly heterogeneous, as tumors from the same sub-type can exhibit vastly different metabolic profiles, which can influence disease progression and response to treatments.

As research begins to study how generalized treatments fail for specific patients and cancers, we need to understand what makes specific cancer lineages unique, in order to identify potential vulnerabilities with a greater level of precision. While other 'omics fields have quantified the expression of genes, transcripts and proteins of hundreds of tissue and cell types, our understanding of the metabolic composition of human cells remains rudimentary, and only a limited number of highly targeted metabolite maps of cancer cell lines currently exist.

Our work focuses on addressing this limitation by systematically mapping the metabolomes of a broad range of human cell lines including patient derived cancer cell lines using large scale LC-MS-based metabolomics and lipidomics analyses. With hundreds of mapped metabolites, these maps have yielded valuable insights into the metabolites that are highly specific to individual cell types and diseases (i.e. biomarkers) as well as cancer-selective metabolic vulnerabilities and 'choke-points' for specific cancer lineages, leveraging existing drugs and chemical inhibitors with wide therapeutic windows.

20.2 **12:00 p.m. - 12:20 p.m.** **Abstract 241**

Metabolomic Landscape of IPMN cyst fluid reveals signatures of pancreatic cancer progression

PRESENTING AUTHOR: ***Eduardo Sommella, University of Salerno, Italy***

CO-AUTHORS: *Vicky Caponigro, Paolo Riccardo Camisa, Benedetta Ferrara, Giulio Belfiori, Marco Schiavo Lena, Lorenzo Piemonti, Massimo Falconi, Pietro Campiglia, Stefano Crippa*

SUB-THEME: Cancer

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy often diagnosed at advanced stages. While most PDACs arise from pancreatic intraepithelial neoplasms (PanINs), approximately 5–10% originate from intraductal papillary mucinous neoplasms (IPMNs), cystic precursor lesions frequently detected in the general population. IPMNs represent a valuable model to investigate early pancreatic carcinogenesis and risk assessment. However, distinguishing low-risk from high-risk lesions remains challenging due to the complex and multistep nature of IPMN progression. Here we applied a workflow combining untargeted metabolomics and lipidomics to characterize cyst fluid from 65 surgically resected IPMN patients spanning the progression spectrum, including low-grade dysplasia (LG), high-grade dysplasia (HG), and invasive cancer (IC). Polar metabolites were profiled using HILIC-HRMS, while lipids were analyzed by RP-UHPLC-TIMS, enabling broad coverage of the cyst fluid metabolome and lipidome.

Unsupervised analyses revealed partial overlap among groups but also a consistent progression-related trend, suggesting that biologically meaningful metabolic information is present but diluted in the multi-class setting. Supervised modelling using PLS-DA combined with multiple feature-selection strategies and data fusion improved classification performance and enabled robust identification of progression-associated metabolic signatures. In the LGD vs IC comparison, VIP-based models, achieved ~78% test accuracy with balanced sensitivity and specificity. In the clinically relevant LGD vs HGD+IC comparison, accuracy remained comparable (≈70–76%), with feature selection improving robustness relative to full-variable models. From a biological perspective, several acylcarnitines were increased in HG and IC lesions, consistent with enhanced fatty-acid oxidation and OXPHOS activity, suggesting metabolic reprogramming in early pancreatic tumorigenesis. Lipidomics

SESSION 20. CANCER METABOLOMICS	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

revealed increased ether-linked phosphatidylcholines (PC-O) and sphingomyelins (SM), lipid classes involved in membrane remodeling and tumor progression. Overall, cyst fluid phenotyping provides a clinically informative readout of metabolic reprogramming associated with IPMN progression and represents a promising strategy to support risk stratification and surveillance of patients with pancreatic cystic lesions.

20.3 **12:20 p.m - 12:35 p.m.** **Abstract 126**

Metabolomic signatures of low-insulinemic and anti-inflammatory dietary patterns in relation to colorectal cancer risk across global populations

PRESENTING AUTHOR: ***Fred Tabung, Ohio State University, United States***

CO-AUTHORS: *Ni Shi, Rachel Hoobler, Matthew Lee, Lei Wang, Rie Kishida, Li Na Ang, Rhea Harewood, Demetrius Albanes, Marc J. Gunter, Seow Wei Jie, Johanna W. Lampe, Erikka Loftfield, JoAnn E. Manson, Cristina Menni, Steven C. Moore, Marian L. Neuhouser, Marjorie McCullough, Eric Orwoll, Kathryn M. Rexrode, Jerome I. Rotter, Rachael Stolzenberg-Solomon, Ying Wang, Alexis C. Wood, Danxia Yu, Amanda E. Toland, Fred K. Tabung, Mary C. Playdon.*

SUB-THEME: Cancer

Objective: Diet is a key modifiable risk factor for colorectal cancer (CRC), partly through effects on insulin regulation and inflammation. We applied a polymetabolomics approach to characterize metabolomic profiles of biologically defined dietary patterns (reverse Empirical Dietary Index for Hyperinsulinemia (rEDIH), reverse Empirical Dietary Inflammatory Pattern (rEDIP), and the Healthy Eating Index-2020 (HEI-2020), and examined their associations with CRC risk.

Methods: We harmonized semi-targeted metabolomics data across 14 nested studies within 11 prospective cohorts in the United States, Europe, and Asia, participating in the Consortium of Metabolomics Studies (COMETS). Polymetabolite profiles were derived using elastic net regression within studies and meta-analyzed. Associations between polymetabolite scores and incident CRC were evaluated in nested case-control analyses and combined using inverse-variance-weighted meta-analysis.

Results: Among 26,770 participants with 9,825 measured metabolites, each dietary pattern exhibited a distinct metabolic signature. Higher rEDIH scores reflected lower branched-chain amino acids and acylcarnitines and higher coffee/polyphenol-, and gut microbial-related metabolites, consistent with improved insulin sensitivity. The rEDIP profile emphasized polyphenol-rich beverages, microbial metabolites, and inflammatory lipid and bile acid pathways. HEI-2020 captured broader dietary quality, including antioxidants, omega-3 fatty acids, and micronutrient biomarkers. Participants in the highest versus lowest quintile of rEDIH, rEDIP, and HEI-2020 profile scores had 16-23% lower CRC risk, with rEDIP showing the most consistent associations.

Conclusion: Polymetabolite profiling revealed complementary metabolic dimensions of dietary patterns related to insulin regulation, inflammation, and overall dietary quality, with anti-inflammatory dietary signatures most strongly associated with reduced CRC risk, highlighting inflammation-related metabolic pathways as potential targets for dietary prevention of CRC.

20.4 **12:35 p.m. - 12:55 p.m.** **Abstract 200**

Metabolomic and proteomic profiling in an underrepresented population reveals novel molecular risk factors for gastric cancer

PRESENTING AUTHOR: ***Xiang Shu, Memorial Sloan Kettering Cancer Center, United States***

CO-AUTHORS: *Ying Liang, Brittany Small, Jie Wu, Eunji Choi, Qiuyin Cai, Monika Laszkowska, Wei Zheng, Irene Orlow, Xiao-ou Shu*

SUB-THEME: Cancer

Background Gastric cancer (GC) remains a significant health burden worldwide. Although several risk factors for GC such as Helicobacter pylori (H. pylori), have been recognized, additional mechanisms implicated in GC development remain to be elucidated. In addition, studies focusing on racial/ethnic minority populations, such as African Americans, is scarce.

★ AWARD WINNERS

SESSION 20. CANCER METABOLOMICS	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

Methods We conducted a nested case-control study within the Southern Community Cohort Study and generated untargeted metabolomic profiling using pre-diagnostic blood samples from 93 incident GC cases and 184 matched controls. We also generated unbiased proteomics data in a subset of 80 case-control pairs. Batch effects were corrected, and biomarkers with more than 30% missing values were excluded from downstream analyses. We performed multivariable conditional logistic regression to identify risk-associated biomarkers. We additionally developed metabolomic signatures of dietary sodium/potassium intake and proteomic aging clocks following established approaches and assessed their associations with GC risk.

Results Seventeen metabolites were nominally associated with GC risk (P Conclusions This study highlights low fruit intake, a high sodium-to-potassium intake ratio, and intestinal aging as key risk factors for GC development in a predominantly African American population.

20.5	12:55 p.m. - 1:15 p.m.	Abstract 199

Targeting the K-RAS4B/PDE6δ Complex Reveals Differential Lipid Metabolic Responses in Pancreatic Cancer Cells

PRESENTING AUTHOR: ★ ***Sandra Delfín-Azuara, CINVESTAV, Mexico***

CO-AUTHORS: *Aldo Moreno-Ulloa, Miguel Vargas*

SUB-THEME: Pharmacometabolomics

Pancreatic ductal adenocarcinoma (PDAC), a highly lethal cancer, requires therapies with improved efficacy–toxicity profiles. Cancer metabolic reprogramming, partly orchestrated by oncogenic KRAS4B, drives tumor progression and chemoresistance; thus, metabolomic responses to KRAS4B-targeting agents may uncover actionable vulnerabilities. We previously reported two antineoplastic compounds, C14 and its analogue P8 (KRAS4B–PDE6δ complex stabilizers), which display preferential cytotoxic activity in PDAC cells versus non-cancerous controls. Here, we applied nano–liquid chromatography–mass spectrometry–based untargeted metabolomics combined with chemoinformatics to characterize acute metabolic remodeling in non-tumorigenic KRAS wild-type cells (hTERT-HPNE, ARPE-19) and PDAC cell lines (Mia PaCa-2, PANC-1, Capan-1, BxPC-3). The indirect KRAS inhibitor deltarasin served as a reference compound. Time-response evaluation identified 1 h at IC_{50} as the earliest point showing metabolic effects without visible cell death, as determined by viability and morphology assessments, minimizing apoptosis-associated confounders.

Deltarasin and P8 induced broader metabolic perturbations across cell lines than C14, although responses were heterogeneous. Cancer cells consistently exhibited greater metabolic disruption than non-tumor cells. Chemical class-level analysis indicated that P8 prominently remodeled lipid metabolism together with increases in short peptide–like features consistent with protein turnover products, whereas deltarasin effects were predominantly lipid-centered and more globally distributed. Lipid enrichment analysis suggested a higher probability of perturbations in membrane-associated components, particularly glycerophospholipids and cholesterol-related lipids, for C14 and P8, while deltarasin additionally pointed to potential alterations in intrinsic membrane properties such as curvature. Manually curated pathway mapping converged on lipid metabolic routes, including the mevalonate pathway, the Kennedy pathway, the Lands' cycle, and broader phospholipid metabolism.

Collectively, these findings indicate that early pharmacological stabilization of the KRAS4B–PDE6δ complex rapidly impacts lipid homeostasis in PDAC models. Because increased lipid availability is linked to tumor progression and chemoresistance, the observed reduction in specific lipid classes by C14 and P8 supports further mechanistic investigation and highlights their potential as resensitizing agents.

SESSION 21. NUTRIMETABOLOMICS	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

21.1 **11:30 a.m. - 12:00 p.m.** **Abstract 373**

Decoding Variability in Dietary Response: A Metabolic Phenotyping Approach for Personalised Nutrition

PRESENTING AUTHOR: ***Isabel Garcia Perez, Imperial College Healthcare NHS Trust, United Kingdom***

CO-AUTHORS: *Vally Wu Yiwei , Joram M Posma, Melpomeni Kasapi, Elaine Holmes, Gary Frost, Isabel Garcia-Perez*

SUB-THEME: Precision health

Habitual consumption of poor-quality diets is a major contributor to non-communicable diseases (NCDs), driving the global adoption of population-level dietary guidelines. However, individuals exhibit substantial variability in their metabolic responses to dietary interventions, limiting the effectiveness of uniform recommendations. This challenge is compounded by reliance on self-reported dietary intake, which often fails to capture true exposure and metabolic impact. Metabolic phenotyping, shaped by genetics, environment, lifestyle, diet, and the gut microbiome, offers a promising framework to address this complexity.

We investigated inter-individual variability in the urinary metabolome in response to controlled dietary interventions in healthy individuals and those at risk of cardiovascular disease (CVD) within an inpatient setting. Diet-responsive metabolites were identified and mapped to metabolic pathways to assess individual-level effects, alongside a metabolic entropy approach to visualise personalised response patterns.

We developed the Global Metabolic Impact Score (GMIS), a novel metric derived from high-resolution nuclear magnetic resonance (NMR) spectroscopy that quantifies global changes in the urinary metabolome by capturing variation in signal density and intensity across the full spectral profile.

Despite controlled conditions, participants exhibited distinct and quantifiable metabolic responses. While pathway-based analyses revealed variability in metabolite-specific responses, they relied on a priori annotation. In contrast, GMIS captured inter-individual variability directly from the global metabolomic profile and was strongly associated with improvements in lipid-related cardiometabolic risk factors.

These approaches were integrated into MetaboMeals, a digital platform designed to deliver metabolically informed, personalised dietary recommendations.

21.2 **12:00 p.m. - 12:20 p.m.** **Abstract 50**

An Integrated Untargeted and Targeted LC-MS Metabolomics Approach to Characterise the Systemic Effects of S-Methyl Cysteine Sulfoxide in Diet-Induced Metabolic Syndrome

PRESENTING AUTHOR: ***Armaghan Shafaei, Edith Cowan University, Australia***

CO-AUTHORS: *Armaghan Shafaei, Hayley Abbiss, Caroline Hill, Lois Balmer, Lauren Blekkenhorst*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

S-methyl cysteine sulfoxide (SMCSO) is an organosulfur compound abundant in cruciferous and allium vegetables, whose intake is associated with reduced cardiometabolic risk. Although previous animal studies report hypocholesterolemic, anti-diabetic, and antioxidant effects, the systemic metabolic impact of SMCSO in diet-induced metabolic syndrome (MetS) has not been characterised using untargeted metabolomics. This study aimed to characterise dose-dependent metabolic alterations induced by SMCSO and identify metabolic pathways potentially underlying its protective effects using a mouse model of MetS.

Forty-five male C57BL/6 mice were randomised to five groups: (1) normal diet (ND); (2) high-fat diet (HFD); or HFD supplemented with SMCSO at 60, 170, or 350 mg kg^{-1} body weight (oral gavage, 5 days/week) for 12 weeks. Plasma samples collected at baseline, 6, and 12 weeks were analysed using untargeted liquid chromatography mass spectrometry (LC-MS) metabolomics (C18 column, positive ion mode). Data were processed and subjected to quality control correction prior to multivariate and univariate statistical analyses, including PCA and two-way ANOVA with Benjamini-Hochberg false discovery rate adjustment. Pathway enrichment analysis was performed in MetaboAnalyst 5.0 using Mus musculus KEGG pathways.

SESSION 21. NUTRIMETABOLOMICS	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

HFD feeding induced significant weight gain, impaired glucose tolerance, and adverse lipid changes ($p<0.05$), confirming MetS development. Untargeted metabolomics detected 2,997 features after QC correction filtering, and 114 were identified. PCA demonstrated clear separation between ND and HFD groups, with partial dose-responsive metabolic shifts in SMCSO-treated mice. Five metabolites significantly differed between HFD and SMCSO groups (FDR-adjusted $p<0.05$). Although three pathways were nominally enriched, none remained significant after multiple testing correction (FDR>0.8), suggesting coordinated metabolic modulation rather than isolated pathway disruption. To validate these findings, a targeted LC-MS assay quantifying 30 metabolites from implicated pathways was developed and validated (linearity, precision, accuracy, LOD/LOQ, and stability). Quantitative confirmation of these metabolic alterations and their mechanistic interpretation will be presented at the conference.

21.3	12:20 p.m - 12:35 p.m.	Abstract 305

High throughput ex vivo stable isotope tracing using tissue homogenates to model in vivo metabolism

PRESENTING AUTHOR: ***Shauni Loopmans, KU Leuven - VIB, Belgium***

CO-AUTHORS: *Sam De Craemer, Bart Ghesquière*

SUB-THEME: Cellular metabolism

Stable isotope (e.g. 2H, 13C) tracing is a powerful tool for mapping metabolic pathway activity, yet its application remains predominantly in vitro, where nutrient composition and isotopic replacement can be tightly controlled. However, resolving the metabolic mechanisms underlying health and disease requires physiologically relevant in vivo data, which remains technically challenging in mice and ethically constrained in humans. Moreover, performing multiple isotope tracings typically requires separate animals for each tracer, rapidly increasing animal numbers. To overcome these limitations, we aimed to develop a high-throughput ex vivo tracer metabolomics workflow that enables multi-nutrient tracing using a single tissue sample.

Freshly collected mouse kidney, liver, and heart tissues were homogenized using gentleMACS and incubated with U-^{13}C-glucose, U-^{13}C-glutamine, or U-^{13}C-palmitate. Time-course labeling was quantified to determine pathway-specific steady-state incorporation. The workflow was also evaluated for compatibility with small-molecule inhibitors, using 2-deoxy-D-glucose as a proof-of-principle.

Our results show that glucose and glutamine tracings reached isotopic steady state in glycolytic and TCA cycle intermediates after 8-12 hours, whereas palmitate oxidation achieved steady state within 2-4 hours. Organ-specific nutrient utilization patterns were recapitulated: kidneys showed higher glucose-derived transamination to alanine than liver or heart; liver retained its characteristic glycogen-related and gluconeogenic signatures; and hearts displayed the highest fatty acid oxidation from U-^{13}C-palmitate. Pharmacological inhibition with 2-deoxy-D-glucose significantly reduced glucose incorporation, demonstrating functional compatibility with drug screening.

Our work introduces a robust ex vivo stable isotope tracing platform that enables multiplexed nutrient tracing from a single tissue sample while preserving physiologically relevant metabolic characteristics. This methodology reduces animal usage, expands the feasibility of multi-nutrient metabolic studies, and provides a scalable platform for mechanistic and pharmacological investigations in metabolomics.

★ AWARD WINNERS

SESSION 21. NUTRIMETABOLOMICS	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

21.4 **12:35 p.m. - 12:55 p.m.** **Abstract 25**

Nontargeted metabolomics reveals changes in plasma metabolome after an oat-rich low-gluten diet with candidate biomarkers of oat consumption

PRESENTING AUTHOR: ★ ***Enni Mannila, University of Turku, Finland***

CO-AUTHORS: *Hany Ahmed, Ville Koistinen, Anna Kårlund, Marjukka Kolehmainen, Kaisa Linderborg, Kati Hanhineva*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Low-gluten diets (LGDs) are increasingly popular but may lack fiber that is essential for gut and overall health. Oats provide an important fiber source in LGDs and improve cholesterol status, yet the overall metabolic impact is unclear. The aim was to compare plasma metabolites of volunteers with increased cardiometabolic risk who followed either oat- or rice-rich LGD for 6 weeks, and to identify potential biomarkers of oat consumption.

Participants (n = 69) were allocated in parallel groups of oat or rice and completed a 6-week randomized clinical trial. Rice, that is used in a similar way as oats in LGDs, acted as a control. Fasting plasma samples and dietary information were collected before and after the intervention. Plasma metabolites were analyzed with a nontargeted metabolomics approach using a high-resolution UHPLC-qTOF-MS platform with both positive and negative ion modes and using RP and HILIC chromatographies. Raw data were preprocessed with the notame workflow. Statistical significance was assessed with linear mixed-models and features of q

Metabolites showing time-treatment interactions included increases in an unknown phospholipid 509.2578@9.32; 2,6-dihydroxybenzoic acid, medium-chain fatty acids and linoleic acid containing phosphatidylcholines, and decreases in polyunsaturated fatty acids, amino acids and medium-chain acylcarnitines during the oat diet. During the rice diet, trimethyluric acid, caffeine and its metabolites increased, while circulating polyunsaturated fatty acids containing phosphatidylcholines decreased. The unknown phospholipid 509.2578@9.32 and 2,6-dihydroxybenzoic acid increased with oat intake and decreased with rice (q

Our study provides insights into the plasma metabolome after an oat-rich LGD in participants with increased cardiometabolic risk, reflecting endogenous metabolic responses to the dietary intervention. The changes in metabolome after oat consumption suggest possible improvements in cardiometabolic health. Additionally, we identified candidate plasma biomarkers of oat consumption contributing to the on-going research on cereal biomarkers.

21.5 **12:55 p.m. - 1:15 p.m.** **Abstract 87**

Lipidomic Fingerprinting Reveals Bioactive Signatures of Human Milk Extracellular Vesicles

PRESENTING AUTHOR: ***Julia Kuligowski, Health Research Inst. La Fe, Spain***

CO-AUTHORS: *Jose Luis Moreno-Casillas, Laura Ripoll-Seguer, Emmanuel Rumba Matano, Nadezhda Nikiforova, Marta Estivalis-Alapont, Isabel Ten-Doménech, Isabel Vicente-Pérez, Anna Parra-Llorca, Danuta Dudzik, Guillermo Quintás, María Cernada, María Gormaz*

SUB-THEME: Pregnancy, maternal and neonatal health

Introduction and Aims: Human milk (HM) protects against necrotizing enterocolitis (NEC), a severe inflammatory disease affecting preterm infants. Human milk extracellular vesicles (HMEVs) are lipid bilayer–enclosed nanoparticles resistant to digestion, enabling delivery of bioactive cargo to the infant gut. We aim to develop a nutritional supplement based on HMEVs isolated from pasteurized donor HM for NEC prevention. Here, we present lipidomic characterization of HMEVs and evaluation of their functionality in a 3D in-vitro gut model.

Materials and Methods: HM samples from mothers of preterm infants with NEC and matched controls (N=20) were collected. A pooled pasteurized donor HM batch was used for in-vitro experiments. HMEVs were isolated by size exclusion chromatography and/or ultracentrifugation. Vesicles were characterized by ATR-FTIR, TRPS, TEM, Western blot, and ExoView®. Lipidomics included targeted/semi-targeted oxylipin profiling (UPLC-MS/MS) and untargeted LC-qTOF-MS analysis. Functional

SESSION 21. NUTRIMETABOLOMICS	Wednesday, June 24	11:30 a.m. - 1:15 p.m.

assays were performed in a 3D Caco-2 gut model (MIMETAS®) assessing tight junction proteins, cytokine release, cytotoxicity (LDH), and cellular metabolomic responses.

Results: HMEVs displayed a mean size of 106 nm and high purity ($2.1\pm0.2\times10^{14}$ particles/g protein). Seven oxylipins were quantified, including 12,13-DiHOME, 9,10-DiHOME, 14-HDHA, and multiple EETs, consistent with inflammatory regulation. Untargeted lipidomics annotated 671 features, mainly triacylglycerols (29%), glycerophospholipids (16%), and phosphosphingolipids (11%). No significant lipidomic differences were observed between NEC and control groups. In vitro, HMEVs were non-cytotoxic, enhanced epithelial barrier integrity (↑E-cadherin, ↑occludin-1), reduced TNF-α levels, and induced distinct metabolic pathway modulation.

Conclusions: HMEVs exhibit a bioactive lipidome enriched in inflammatory-regulatory mediators and promote epithelial barrier function in vitro. These findings support their translational potential as a lipid-based nutritional strategy for NEC prevention in preterm infants.

POSTER ABSTRACTS

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1000 A Searching for Epilipids in Lipostar: how many strategies?

PRESENTING AUTHOR: ***Stefano Bonciarelli, Mass Spec Analytica SL, Spain***

CO-AUTHORS: *Paolo Tiberi, Zhixu Ni, Gabriele Lombardi Bendoula, Bruna B. Neves, Rosario Maria Domingues, Maria Fedorova, Laura Goracci*

SUB-THEME: Data processing & software tools

Recent breakthroughs in analytical platforms for lipidomics have paved the way for advanced mass spectrometry (MS) strategies, enabling the detection of previously unknown, low-abundance lipid species. This evolution has catalyzed the rise of epilipidomics—a rapidly expanding field dedicated to the study of enzymatically and non-enzymatically modified lipids. Epilipids are formed by the introduction of new functional groups (e.g., hydroxy, epoxy, or nitro) into native lipid molecules. Far from being mere metabolic byproducts, these species act as critical biological mediators, playing pivotal roles in both physiological homeostasis and various pathological processes. While specific classes, such as oxylipins, have been extensively characterized over the past decades, our understanding of the full "epilipidome" remains fragmented due to significant experimental and computational challenges. To address these limitations, this work summarizes innovative strategies within the Lipostar framework designed to streamline epilipid discovery, identification, and structural characterization and bridge the gap between raw MS data and functional biochemical insights.

Specifically, a spectral library of 100 oxylipin standards was recently converted into Lipostar database format, enabling their high-confidence identification in complex biological matrices. Beyond free oxylipins, Lipostar provides an untargeted strategy to detect complex oxidized lipids through a synergistic connection with LPPtiger. This untargeted workflow first employs a high-throughput cheminformatic approach in Lipostar to achieve annotation at the molecular species level; subsequently, these data are processed by LPPtiger to pinpoint specific modification types. Finally, to enlarge the search of the epilipidome space, a semi-targeted approach was developed in Lipostar to annotate nitrated lipids. In this regard, a virtual library containing 2,200 structures of modified glycerophospholipids (PC, PE, PS, and PI) was generated. Based on experimental evidence, specific fragmentation rules were established to construct a corresponding spectral library.

Overall, this integrated workflow represents a further step toward the systematic profiling of the epilipidome in clinical and biological research.

1001 B MetaboFlow-graph: A Graph-Structured Representation for Executable Metabolomics Workflows

PRESENTING AUTHOR: ***Madeleine van Zuylen, Tufts University, United States***

CO-AUTHORS: *Professor Soha Hassoun, Ash Sze*

SUB-THEME: Data processing & software tools

The rapid rise of AI for Science has enabled automated reasoning, planning, and execution across complex scientific domains, with multi-agent based systems increasingly capable of orchestrating end-to-end workflows. However, metabolomics has lagged behind this transformation. Despite the availability of powerful tools for data processing, annotation, and analysis, workflows remain fragmented. A key barrier to end-to-end automation and agent-based execution of metabolomics workflows is the absence of structured, machine-readable representations of workflows. Workflow knowledge is often unpublished, and if it is, descriptions are embedded in unstructured text, informal protocols, and fragmented scripts, where critical details such as workflow steps, tool parameter settings, data dependencies, and execution order are implicit or missing. Despite substantial efforts in standardization and workflow tooling, prior approaches stop short of representing metabolomics workflows as structured, machine-readable programs, limiting reproducibility and preventing end-to-end execution by AI agents.

We address this limitation by introducing a structured graph-based schema that we refer to as metaboFlow-graph. Workflows are encoded as hierarchical compositional graphs, where nodes represent operations at multiple levels of abstraction, ranging from high-level conceptual steps to fully specified execution units with defined parameters, while edges capture both dataflow and control dependencies. This representation formalizes the transformation of data across tools, while preserving execution order, parameterization, and tool interoperability. To support both machine execution and human interpretability, we serialize the graph into an XML-based format, metaboFlow-XML, that provides a visualizable, standardized, portable specification of the workflow. We demonstrate the utility of this representation on metabolomics workflows for representative published studies, showing that our graph representation can capture complex, multi-step pipelines and enable their end-to-end execution in a structured and reproducible manner. In addition to reproducibility, our work establishes a foundation for

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

treating metabolomics workflows as executable, interoperable computational objects, enabling their direct use by AI agents for automated, end-to-end analysis.

1002 A Precursor Inference Is Not Binary: Uncertainty Aware Assignment with Scanning DIA

PRESENTING AUTHOR: ***Gordana Ivosev, SCIEX, Canada***

CO-AUTHORS: *Gordana Ivosev, Lyle Burton, Adam Lau, Jinal Patel, Eva Duchoslav, Pradeep Narayanaswamy*

SUB-THEME: Data processing & software tools

Accurate precursor inference remains an unsolved problem in DIA untargeted LC-MS metabolomics. Even “clean” DDA with ~1 Da Q1 isolation windows is not unambiguous and co-elution and chimeric isolation are common. While DIA begins with a wider Q1 window, the chromatographic peak shape and, for scanning DIA, the Q1 dimension provides orthogonal evidence that can be exploited to reduce ambiguity and improve precursor inference.

We treat precursor–fragment assignment as a probabilistic inference problem with explicit uncertainty and confidence, rather than using a single fixed precursor m/z. We use those uncertainties together with MS2 spectra annotation to assess the result uncertainty in addition to the traditional match score.

Scanning DIA may be implemented with narrow isolation windows at high scan speed, enhancing effective Q1 selectivity while acquiring MS/MS for all detectable precursors. We combine the high mass accuracy of precursor and fragment spectra with LC and Q1 information to infer precursor-fragment links and precursor-specific uncertainty intervals.

To demonstrate the method, we acquired samples of metabolites extracted from NIST 1950 human plasma spiked with a standard compound mixture; these were analysed using HPLC coupled to a SCIEX research breadboard mass spectrometer using a 12-min gradient and CID fragmentation. Acquisition included DDA and scanning DIA. Data was processed using research tools developed to support uncertainty propagation from data extraction to final annotation.

This uncertainty-aware inference improves feature detection and increases the robustness of spectral and database matching. Comprehensive fragmentation and spectra correlation support rational grouping of related features (adducts and in-source fragments), improving interpretability beyond MS1-centric workflows. Compared with fixed tolerances, propagating the precursor-specific uncertainty reduces ambiguous matches and improves confidence in untargeted analysis of complex matrices. Together, these results show that precursor inference is a coupled acquisition–inference–annotation problem and that explicit uncertainty modeling is key to reproducible, high-confidence DIA untargeted metabolomics.

1003 B GetReliableLipids: an R package for multi-criteria assessments of MS-DIAL lipid annotations

PRESENTING AUTHOR: ***Gianfranco Frigerio, San Raffaele Scientific Institute, Italy***

CO-AUTHORS: *Chiara Ansermino, Radmila Pavlovic*

SUB-THEME: Data processing & software tools

Reliable lipid annotation remains a challenge in untargeted lipidomics. MS-DIAL is one of the most implemented open-source software within the lipidomic community, and it typically generates large feature tables out of a lipidomics LC-M/SMS experiment that still contain many biologically implausible annotations. To address this issue, we developed GetReliableLipids, an R package designed to evaluate the reliability of lipid annotations obtained from MS-DIAL through a transparent, reproducible, and customisable workflow.

The package first imports MS-DIAL output tables and performs initial steps including detection of internal standards and removal of unknowns and features lacking MS/MS information. An highlight of this package is a rule-based evaluation of the lipid name assigned in MS-DIAL: using a user-editable cut-off table, GetReliableLipids assesses whether annotations are biochemically plausible according to lipid ontology and structural constraints, such as exclusion of odd-numbered chains and definition of acceptable ranges for carbon number per chain, degree of unsaturation, and oxygen content.

The package then evaluates annotation reliability from chromatographic behaviour: in particular, retention time cutoffs can be set according to the analytical conditions used, and additional checks verify whether lipids sharing comparable structural characteristics show coherent retention time ordering. Orthogonal evidence is further incorporated by assessing adduct consistency across parallel negative-and positive-ionisation datasets. Finally, GetReliableLipids evaluates lipid reliability based on MS/MS spectra verifying presence of characteristic fragment ions and fragmentation patterns known for specific lipid classes.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

By integrating structural, chromatographic, ionisation, and fragmentation-based checks within a single framework, GetReliableLipids is a practical tool for getting an insight of which lipids annotated with MS-DIAL are reliable, prior to downstream statistical and biological interpretation.

1004 A OmicSurf: An Online Tool for Identifying Metabolite Predictors of Patients Survival

PRESENTING AUTHOR: ***Eric Law, The Children's Hospital at Westmead, Australia***

CO-AUTHORS: *Martin Chow*

SUB-THEME: Data processing & software tools

Introduction: A metabolome-wide approach to evaluate features for association with prognostication using Kaplan–Meier (KM) survival analysis is not widely available in an accessible, web-based form. To address this gap, we developed an online platform - OmicSurf, that performs KM survival analysis across all imported metabolomic features, producing statistical metrics and interactive visualizations to facilitate marker discovery and interpretation.

Methods: The application accepts omics data arranged in a conventional table: one row per case with a panel of feature abundances plus survival time and event status. For each feature, the platform determines an optimal cutoff and then performs KM survival analysis to compute survival curves, log-rank p-values, and hazard ratios (HRs) with confidence intervals. Results are summarized in tabular and graphical forms, and individual-feature pages display the selected cutoffs, KM plots, p-values, HRs, and sample distributions. For details: https://omicsurf.org/

Results: We validated the system using an example metabolomics dataset. All features were analyzed automatically; for each feature the platform reported the optimal cutoff, KM survival curves, log-rank p-values, and HRs. To aid the interpretation, we implemented several interactive visualizations: a volcano-style plot that maps hazard ratio vs -log10(p-value), sortable result tables, and clickable feature nodes that link to detailed KM plots and statistics. These interfaces allow rapid prioritization of candidate prognostic metabolites and inspection of individual survival curves.

Conclusion: While various in-house tools exist for biomarker-survival association, to our knowledge this is the first web-based platform that integrates omics with automated, comprehensive KM analysis across all imported features and provides multiple interactive visual aids. Identifying prognostic metabolites offers a distinct perspective from typical biomarker discovery, highlighting features that reflect underlying pathophysiology and potential therapeutic checkpoints. The platform is adaptable to other omics (e.g. genomics, protenomics, etc.) and supports rapid translation of large-scale datasets into clinically and biologically meaningful survival insights.

1005 B LipidRT: Improving Lipid Annotations through Retention Time Modeling in Lipidomics

PRESENTING AUTHOR: ***Agustín Lahoz Rodríguez, Fundacion Para La Investfundacio Del Hospital La Fe, Spain***

CO-AUTHORS: *Celia Regal Marques, María Isabel Alcoriza-Balaguer, Marta Roca, Juan Carlos García-Cañaveras*

SUB-THEME: Data processing & software tools

Liquid chromatography (LC) coupled with high-resolution mass spectrometry (HRMS) is one of the most widely used analytical platforms in untargeted lipidomics. Lipid annotation commonly relies on MS/MS spectral matching or fragmentation-based rules; however, current workflows still produce false identifications due to incorrect adduct assignment, in-source fragmentation, and limited structural evidence. To address this challenge, we developed LipidRT, a new R package that improves lipid annotation by incorporating retention time (RT) as an orthogonal variable. LipidRT operates in two complementary stages. First, an automated filtering strategy removes unreliable annotations by analyzing lipid classes prone to in-source fragmentation. Clustering based on RT, carbon number (CN), and double bond content (DB) identifies overlapping RT distributions between related lipid classes, retaining only consistent groups. Species deviating from expected RT trends are subsequently removed, generating a refined set of high-confidence annotations. In the second stage, RT behavior is modeled for each lipid class as a function of CN and DB, enabling prediction of RT values for new lipid candidates derived from m/z measurements. New annotations are proposed only when experimental m/z values and predicted RT strongly agree. An interactive Shiny application was also developed to facilitate visualization and expert curation of results. Evaluation across multiple datasets showed that the workflow removed ~20% of inconsistent annotations, while RT-based prediction generated an additional 15% of plausible lipid identifications. Although the total number of annotations remained similar, confidence and reliability improved substantially, demonstrating that LipidRT provides a robust and user-friendly framework for lipid annotation using combined m/z and RT information.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1006 A Mapping Chemical Space: An Open Repository of 1.1 Million MS/MS Spectra Across 185,000 Compounds

PRESENTING AUTHOR: ***Christoph Krettler, Enveda, United States***

CO-AUTHORS: *Tobias Kind, Erik DeBloois, James Taylor, Pelle Simpson, August Allen, David Healey*

SUB-THEME: Data repositories

Small molecule structure elucidation from mass spectra remains a major challenge despite advances in machine learning. A key bottleneck is limited open training data. Existing repositories such as MoNA, GNPS, and MassSpecGym together contain spectra for roughly 80,000 unique compounds, representing only a tiny fraction of chemical space and far fewer entries than proteomics datasets. To address this gap, we introduce the Enveda-180k MS/MS library, comprising 185,024 structurally diverse compounds profiled across multiple collision energies in both ion modes, totaling 1,163,051 spectra. This library more than doubles the number of molecules available in open MS/MS resources and provides a foundation for improved machine learning approaches to small molecule identification.

Compounds were sourced from the Enamine HLL-200 diversity library, reconstituted to 100 µM in methanol:water, and stored in 384-well plates at −20 °C. Samples were pooled in groups of 30 to reduce isobaric overlap, producing 6,800 mixtures alongside controls. LC-MS/MS profiling used an H-Class Bio UPLC coupled to Bruker timsTOF Pro2 and timsTOF HT systems in positive and negative HESI mode at 20, 40, and 60 eV. Acoustic liquid handling enabled high-throughput preparation. Spectra were extracted by targeting common adducts, and composite spectra were generated by merging collision energy data.

To assess coverage, we deduplicated open-access databases by InChIKey, yielding 84,191 distinct structures. Enveda-180k contributes 185,024 structures with only 2,400 overlapping, increasing the total to 266,815 publicly available compounds with MS/MS data. Using Morgan fingerprints and minimum spanning tree embeddings, we observe a substantial expansion of chemical diversity relative to existing libraries. This resource is expected to significantly improve structure identification in LC-MS/MS workflows and advance machine learning for tandem mass spectrometry. All authors are employees or contractors of Enveda. This dataset also enables benchmarking across models and supports future methods development in data-scarce regimes while improving reproducibility and accessibility globally.

1007 B MetaboLights - Scaling Open and FAIR Metabolomics Data Worldwide

PRESENTING AUTHOR: ***Thomas Payne, EMBL-EBI, United Kingdom***

CO-AUTHORS: *Thomas Payne, Noemi Tejera, Jonathan Hunter, Mark Williams, Ibrahim Karaman, Felix Xavier Amaladoss, Callum Martin, Naveen Raj Kookkal Polliapram, Ozgur Yurekten, Juan Antonio Vizcaino*

SUB-THEME: Data repositories

MetaboLights (https://www.ebi.ac.uk/metabolights) is a global, cross-species and cross-technique database for metabolomics and related fields such as lipidomics and exposomics, hosting raw data, metadata, metabolite structures and reference spectra. MetaboLights is the recommended metabolomics repository for a number of leading journals and ELIXIR.

MetaboLights applies community standards, ontologies and expert curation to ensure data quality, support reanalysis and align with the FAIR (Findable, Accessible, Interoperable, Reusable) principles. The team collaborates internationally to advance data discovery, integration, and standardization across metabolomics and multi-omics research through initiatives such as the mQACC, OECD OORF and HUPO-PSI.

MetaboLights has continued to experience significant growth in the number and diversity of submitted studies: a record number of 438 complete studies were accessioned during the first 6 months in 2025 (with a total number of 1467 ongoing). As of January 2026, data hosted in MetaboLights (>325 TB in total) comprise 7397 different organism/organism parts, with user accounts from across 146 countries. This growth however presents challenges and the MetaboLights team spent much of the last year evolving the resource with a new simplified submission workflow (‘Provisional’ - ‘Private’ - ‘Public’), which reduces reliance on manual curation and assigns permanent identifiers (MTBLSxxx) only after submitted datasets are complete and compliant. Other recent developments include the new study validation framework (based on Open Policy Agent) and the implementation of automated metadata updates (‘fixes’), a new revision mechanism for public studies, open-source libraries (‘metabolights-utils’ on PyPI) and updated documentation (MkDocs).

As a key point, in 2025, MetaboLights, Metabolomics Workbench and GNPS/MassIVE started to coordinate the new international consortium MetabolomicsHub (https://metabolomicshub.org), aiming to bring the main metabolomics data resources together with the aim to standardize open and FAIR data practices within the metabolomics field worldwide.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1008 A Machine learning-based metabolomics for drug discovery

PRESENTING AUTHOR: ***Phuong Y MAI, Insitut Pasteur, France***

CO-AUTHORS: *Cédric Thépenier, Christophe Zimmer, Ivo Gomperts-Boneca*

SUB-THEME: Machine Learning and AI

Antimicrobial resistance has emerged as a global health emergency, necessitating the discovery of antibiotics with novel modes of action (MoA). However, determining the MoA of candidate compounds remains a critical bottleneck in antibiotic development. To address this challenge, we employed high-throughput metabolomics using Escherichia coli as a model organism, capable of processing over 400 metabolite samples daily. This approach generates metabolic fingerprints characteristic of specific MoAs, providing mechanistic insights even in the absence of observable growth inhibition. To handle massive and complex metabolomics datasets, we apply machine learning algorithms to extract drug-specific features and develop computational mass spectrometry pipelines to predict compound targets. Preliminary validation using metabolite profiles from E. coli exposed to 22 antibiotics representing diverse MoAs demonstrated accurate classification of antibiotic classes and drug concentrations. We are now integrating high-content imaging with metabolomics to capture complementary morphological and biochemical drug responses, thereby improving model prediction accuracy.

1010 A Transfer Learning for Metabolomics: Using Large Unlabeled LC-HRMS Datasets to Improve Classification Performance in Small-Scale Studies

PRESENTING AUTHOR: ***Lisa M. Menacher, Linköping University, Sweden***

CO-AUTHORS: *Liam J. Ward, Henrik Green, Oleg Sysoev*

SUB-THEME: Machine Learning and AI

Deep learning is a powerful tool for analyzing LC-HRMS-based metabolomics, yet its broader application remains challenging due to the lack of large, annotated training datasets. Self-supervised learning addresses this limitation by directly learning meaningful latent representations from unlabeled data, which can subsequently be used to improve the predictive performance of downstream tasks through knowledge transfer. Building on this approach, we investigated whether self-supervised learning followed by transfer learning improves classification performance in small-scale metabolomics studies. Specifically, we developed an autoencoder to learn latent representations from profile-mode LC-HRMS data without prior feature extraction or metadata.

Autoencoders are a class of self-supervised models that learn to compress input data into a low-dimensional, informative space. As profile-mode LC-HRMS data is extremely large, we employed a patch-wise autoencoder to improve computational efficiency. Thus, each sample was divided into several patches, and each patch was processed individually. Furthermore, the raw LC-HRMS data was modeled as a discrete set of points in a three-dimensional space defined by retention time, mass-to-charge ratio, and intensity. This point cloud representation enabled efficient training of the autoencoder through parameter-sharing.

The patch-wise point cloud autoencoder achieves a Chamfer distance of 0.01, indicating good reconstruction quality from the latent space, which was also confirmed through visual inspection of the reconstructed patches. This is especially impressive as the autoencoder compresses an input patch to approximately 2% of its original size. We also evaluated the usefulness of the learnt representations for metabolomics-based classification tasks using forensic cause-of-death screening as a case study. Our results show that self-supervised pretraining with 270 femoral blood samples improves the predictive performance on the test set by 6% compared to training the model from scratch with 70 samples. Lastly, the proposed patch-wise processing offers new opportunities for post-hoc biological interpretation of deep learning models in metabolomics.

1011 B Retention Time Prediction by Learning Thermodynamic Parameters with Graph Neural Networks

PRESENTING AUTHOR: ***Marynka Ulaszewska, Thermo Fisher Scientific, Italy***

CO-AUTHORS: *Gábor Zsemlye, Michal Gramblička, Juraj Lutišan, Vytautas Tamošiūnas, Marynka Ulaszewska*

SUB-THEME: Machine Learning and AI

Retention time (RT) in liquid chromatography (LC) is increasingly important in metabolomics and small-molecule workflows because it complements mass spectrometry, supports targeted method development, and accelerates compound identification. However, RT is highly sensitive to experimental conditions, limiting transferability between laboratories and methods. Existing machine-learning (ML) RT prediction models often share the same limitation: they predict RT only for

conditions matching the training data and do not account for chromatographic system parameters such as dead volume or gradient delay time.

We present a novel ML architecture for RT prediction trained and validated on a large dataset. The dataset comprises 2,293 compounds measured under 90 experimental conditions, including varying temperatures, pH levels, eluents (acetonitrile and methanol), three gradient profiles, and two C18 columns. RT values were extracted and quality-checked using an automated in-house workflow.

The model combines a Graph Attention Network (GAT) with molecular descriptors, column parameters, and experimental-condition vectors. Instead of directly predicting RT, the network predicts hidden parameters of a chromatographic model, which are then used to calculate RT. RT prediction errors are backpropagated through the full pipeline to optimize model performance.

This physics-informed strategy enables condition-aware RT prediction and extrapolation beyond the training conditions. The model can generate RTs for arbitrary gradient programs, including non-linear gradients, different column dimensions, flow rates, dead volumes, and gradient delay times, as well as temperatures within the trained range, while remaining compatible with the trained columns, pH, and eluents. Across validation experiments, the approach achieved a mean absolute error (MAE) of 0.93 min with state-of-the-art accuracy and broad generalizability.

1012 A MetExplore 3: a web solution for network-based interpretation of metabolomics data

PRESENTING AUTHOR: ***Ludovic Cottret, INRAE, France***

CO-AUTHORS: *Ludovic Cottret, Marion Liotier, Jean-Clément Gallardo, Florence Vinson, Nabil Zoubai, Nathalie Poupin, Clément Frainay, Fabien Jourdan*

SUB-THEME: Metabolic networks

Introduction: Metabolomics experiments routinely generate lists of metabolites (metabolic profiles), but transforming these results into biologically interpretable metabolic mechanisms remains challenging. Existing workflows or web solutions often rely on disconnected tools for identifier search, pathway enrichment, network extraction, visualization, and dissemination of results.

Aim: We developed MetExplore 3 to provide an integrated web platform for the exploration, contextualization, visualization, and sharing of graphical representation of metabolomics data mapping within genome-scale metabolic networks.

Methods: MetExplore 3 is an open access web server based on modern web components that provides interactive tools for metabolic network analysis and visualization. The platform includes published curated genome-scale metabolic networks for several organisms and also allows users to import their own metabolic networks from SBML files. A dedicated metabolite identification module maps experimental metabolites onto metabolic networks using ChEBI identifiers. Once mapped, metabolites can be analyzed through pathway overrepresentation approaches and used to extract biologically contextualized subnetworks. These subnetworks can then be interactively visualized, edited, and shared through reproducible graphical representations. In addition to the graphical interface, MetExplore 3 provides an API enabling access to data structures and analytical methods from external environments and programming languages, facilitating integration into custom bioinformatics workflows.

Results: MetExplore 3 enables users to move from metabolite identification to interpretable and shareable metabolic network representations within a unified environment, providing an alternative to widely used pathway enrichment interpretations. The platform integrates metabolite mapping, pathway overrepresentation, subnetwork extraction, and interactive visualization into a unique web solution. The ability to save and share curated network layouts facilitates collaborative interpretation of metabolomics data.

Conclusion: By integrating analytical, visualization, and sharing functionalities within the same web solution, MetExplore 3 helps bridge the gap between metabolite lists and biologically meaningful network interpretations.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1013 B Comparability of LC-MS/MS-based non-targeted Metabolomics of Dissolved Organic Matter across Laboratories

PRESENTING AUTHOR: ***Bruno Costa, University of São Paulo, Brazil***

CO-AUTHORS: *Jarmo-Charles Kalinski, Tilman Schramm, Lihini I. Aluwihare, Carsten Simon, Jeffrey Hawkes, Daniel Petras*

SUB-THEME: Metabolite and mass spectral databases

The marine exometabolome (often termed dissolved organic matter, DOM) is a heterogeneous mixture of organic molecules that shapes aquatic biogeochemistry. To address this complexity, non-targeted liquid chromatography–tandem high-resolution mass spectrometry (LC–MS/MS) is increasingly used for structure-resolved analysis. However, one question still limits data reuse: when identical samples are analyzed in different laboratories, do we detect the same molecules and chemical trends? To test this at community scale, we conducted a ring trial with 24 laboratories across the globe, in which identical algal and DOM extracts were mixed at defined ratios, spiked with standards, and analyzed under standardized chromatography and MS/MS settings. We processed all data through a unified, open-source workflow: raw files were converted to mzML, deposited in MassIVE, and processed in GNPS using both classical (CMN) and feature-based molecular networking (FBMN), followed by standardized cleanup and multivariate analysis. Our results showed that platforms of similar mass spectrometer types, run with aligned parameters, reproduced similar chemical trends. The strongest agreement came from high-intensity features, which were consistently detected and comparably interpreted across labs, enabling coherent cross-laboratory multivariate separation of sample types. In contrast, low-intensity features showed greater detection variability, consistent with subsampling bias in data-dependent acquisition (DDA). In complex matrices such as DOM, DDA targets only a small intensity-biased subset of ions for fragmentation, and differences in instrument scan speed/duty cycle can shift which features receive MS/MS coverage. Therefore, cross-laboratory DOM comparisons can yield converging qualitative conclusions, but current feature-level overlap remains limited, particularly for low-intensity ions and when instruments differ in acquisition behavior or method standardization. For robust cross-study meta-analyses and long-term time series, we recommend a harmonized analytical workflow covering sample preparation and LC–MS/MS acquisition, ideally with shared quality-control samples, together with open sharing of raw and processed data and rich contextual metadata.

For more details: https://doi.org/10.1021/acs.est.5c12691E

1014 A Computational Strategies to Expand the Known Chemical Space: Revealing Hidden Ergot Alkaloids and Human Metabolites through class-Resolved Spectral Libraries and Computational Workflows

PRESENTING AUTHOR: ***Federico Padilla Gonzalez, Wageningen Food Safety Research, Netherlands***

CO-AUTHORS: *Katja van Dongen, Karsten Beekmann*

SUB-THEME: Metabolite and mass spectral databases

Mass spectrometry–based metabolomics depends on spectral libraries for compound annotation; however, current repositories cover only a limited fraction of the chemical space and are rarely organized in a "class-resolved" manner. This limitation constrains annotation performance, particularly in fields like food safety and environmental monitoring. To address this gap, we developed a class-resolved spectral library for food-relevant compounds, organized into coherent chemical and use classes to enable targeted dereplication, class-informed annotation, and systematic expansion of underrepresented compound groups/classes. Two complementary case studies—ergot alkaloids and human fecal metabolites—demonstrate the applicability of this approach to both exogenous toxicants and endogenous metabolites.

A dedicated ergot alkaloid sub-library was constructed and applied to dereplicate 151 fungi sclerotia samples collected across five continents, spanning approximately 150 years (since 1876). Data processing (MZmine), targeted filtering (MassQL), in-house scripts, and feature-based molecular networking enabled class-specific analysis. Following stringent filtering for diagnostic ergopeptine fragments, more than 500 mass features were classified as potential ergot alkaloids, displaying host-associated distribution patterns. Manual inspection revealed alkaloids absent from existing libraries, including putatively novel compounds (i.e., Ergogaline, Ergosedmine derivatives) and a candidate at m/z 604.3494 [M+H]+, likely representing a previously undescribed ergocryptine homologue, and demonstrating a large diversity of unknown ergot alkaloids with potential implications for risk assessment and regulatory monitoring.

To expand class-informed annotation, a complementary strategy was applied to build the “human fecal metabolome spectral library” based on literature and database mining. Literature mining yielded a curated inventory of ~7,000 metabolites previously reported in human feces. Systematic filtering and harmonization of open-access spectral libraries (GNPS, Massbank, MSnLib, MoNA) resulted in the Fecal Metabolome Spectral Library containing ~1,400 experimental MS/MS spectra,

highlighting the gap between reported occurrence and spectral representation. Together, these studies show that "class-resolved" libraries integrated with computational workflows effectively expand annotated chemical space across toxicological and human metabolomics applications.

1015 B The MZ2Struct database: mapping m/z to substructures for the annotation of small molecules with mass spectrometry

PRESENTING AUTHOR: ***Xavier Domingo-Almenara, Universitat Rovira i Virgili, Spain***

CO-AUTHORS: *Aleksandr Zubov, Adrià Olomí, Antoni Del Pino, Núria Canela*

SUB-THEME: Metabolite and mass spectral databases

The difficulty in annotating MS/MS data remains a significant impediment. Tools and resources that address this problem from different perspectives and facilitate metabolite annotation are needed. There are tools to annotate MS/MS via machine learning-based interpretation, such as SIRIUS, or via molecular networking, such as GNPS. The annotation of specific fragments with a database of substructures' m/z values could complement these strategies. However, only a few non-public resources enable searching for a specific m/z to provide a list of potential substructures. Here, we present MZ2Struct, a new library and online resource for identifying potential substructures of queried m/z values from ion peaks observed in experimental MS/MS data.

Our methods yielded a list of more than 30K unique m/z values and structure pairs after the analysis of more than 35K spectra from the GNPS library. The library contains a heterogeneous set of representative substructures found in small molecules, mostly between 40 and 300 Da. The freely available library can be easily queried via a web interface, where m/z from ion peaks can be searched. Our results demonstrate that our tool can be used as a complementary strategy to obtain a wider number of potential substructures for a given peak, which is of special relevance in cases where other methods fail to suggest the right structure or when the true structure is absent in libraries.

1016 A Systematic comparison of Collision-Induced Dissociation (CID) and Electron-Induced Dissociation (EID) spectra across multiple collision energies reveals valuable complementary fragmentation information specific to chemical classes in LC–MS/MS metabolomics

PRESENTING AUTHOR: ***Andres Rogelio Martinez Bilesio, Friedrich Schiller University of Jena, Germany***

CO-AUTHORS: *Sebastian Böcker*

SUB-THEME: Metabolite and mass spectral databases

Electron-induced dissociation (EID) has emerged as a promising alternative fragmentation mechanism to conventional collision-induced dissociation (CID) in LC-MS/MS metabolomics [1-3], yet its contribution to structurally interpretable and non-redundant spectral information remains insufficiently characterized. In this work, we performed a large-scale comparative analysis of MS/MS spectra from nearly 3,000 standard compounds using the MultiMS2 multi-modal, multi-energy spectral library [4].

Spectra acquired under CID at 20 eV and 40 eV were systematically compared with EID spectra recorded at 12 eV, 16 eV, and 24 eV, across positive and negative ionization modes and multiple adduct types. Peak-to-peak comparisons were conducted allowing ±H mass differences to identify shared and technique-exclusive fragments. For EID-exclusive peaks, we quantified the fraction of total explained intensity attributable to fragments consistent with valid precursor subformulae, including searches against both monomeric and dimeric precursor compositions, while unexplained signals were classified as noise. Additional comparisons between [M+Na]+ and [M+H]+ EID spectra were performed to investigate adduct-dependent fragmentation behavior.

Our results demonstrate that EID consistently provides additional, non-redundant fragment information that is chemically interpretable and complementary to CID. Statistical analyses reveal that specific metabolite classes exhibit significantly higher proportions of EID-exclusive explained intensity, indicating class-dependent benefits of EID fragmentation. Furthermore, distinct metabolite classes preferentially express interpretable EID information at different kinetic energies, highlighting the importance of multi-energy acquisition strategies.

These findings support the integration of EID as a complementary fragmentation modality to enhance metabolite annotation in LC-MS/MS workflows, and establish a foundation for the development of computational methodologies that explicitly incorporate EID-derived MS/MS information into metabolite annotation workflows. Future work will focus on designing

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

algorithmic frameworks and spectral interpretation strategies capable of leveraging EID fragmentation patterns to improve structural annotation accuracy and expand the analytical capabilities of LC-MS/MS metabolomics.

[1] Kurizaki, Anal.Chem. 2025

[2] Calabrese, Anal.Bioanal.Chem. 2024

[3] Wu. Analyst. 2025

[4] MultiMS2-GitHub

1017 B Integrated spectral networking, motif assignment and automated data enrichment enables annotation of novel anticancer Myxothiazol A analogs

PRESENTING AUTHOR: ***Luís Feitosa, Universidade de São Paulo, Brazil***

CO-AUTHORS: *Renan da Silva de Oliveira, Elthon Gois Ferreira, Paula Christine Jimenez, Leticia Veras Costa-Lotufo, Norberto Peporine Lopes*

SUB-THEME: Metabolite identification/annotation

Access to the metabolome of Brazil's vast biodiversity is essential for prospecting molecules with promising biological activities. In this context, HPLC-ESI-MS/MS analysis promotes the separation and structural characterization of molecules in samples with high chemical complexity. Moreover, cheminformatic resources can be associated with MS/MS data to suggest the identity of compounds (annotation) related to a bioactivity. This work presents two computational tools aimed at annotating structural motifs and enriching taxonomic and bioactivity information. PuzzleFrags MS tool performs the annotation of substructures from an in-house database, suggesting structural motifs for metabolites not annotated by spectral libraries. MetaboLens tool allows the search of chemical data in taxa and bioassay results of compounds in open access databases, and association of these information with experimental MS/MS. Biological models for this study were bacteria from the genus Myxococcus isolated from soil samples collected in a mangrove in Praia Grande/SP, Brazil. Extracts of the isolated strains presented remarkable cytotoxicity against colorectal cancer cells (HCT-116) and human fibrosarcoma (HT-1080). The 16S rRNA gene sequences showed that strains BRX002 and BRX014 are closely related to M. fulvus. Analysis of extracts by HPLC-ESI-MS/MS followed by manual interpretation of MS/MS spectra and chemical data of M. fulvus from MetaboLens allowed the annotation of the major constituent of BRX002 and BRX014 as Myxothiazol A. The fragmentation of Myxothiazol A includes losses of methanol, NH3, CO groups and a diagnostic ion for the bithiazol moiety, and this pattern was used by the PuzzleFrags for further annotations. Subsequently, the spectral network enriched with MetaboLens results suggested that Myxothiazol A, as well as bithiazolic analogues annotated by PuzzleFrags, may be metabolites accountable for the observed cytotoxicity. The analogs are currently being isolated for structural elucidation. Together, these results demonstrate the potential of PuzzleFrags and MetaboLens for annotating novel analogs when spectral references are absent.

1018 A Annotating small molecules from massive affinity selection libraries

PRESENTING AUTHOR: ***Nils Haupt, Friedrich Schiller University Jena, Germany***

CO-AUTHORS: *Edith van der Nol, Martin A. Hoffmann, Martin Engler-Lukajewski, Marcus Ludwig, Markus Fleischauer, Sebastian Pomplun, Sebastian Böcker*

SUB-THEME: Metabolite identification/annotation

Discovering new bioactive molecules that bind to a specific drug target requires the screening of millions of compounds. In contrast to traditional high-throughput methods, affinity selection-based strategies enable the rapid screening of entire compound libraries by incubating the screening library with the receptor of interest and then isolating only those molecules that bind to the receptor. The main challenge remains in identifying these isolated hit compounds, which is most commonly achieved by initially linking each molecule to a unique DNA barcode. Although those DNA-encoded libraries facilitate the identification of these hit compounds through sequencing, they suffer from the fundamental drawbacks of limited synthesis complexity and the potential interference of the attached tags during an affinity selection experiment. Here, we focus on combinatorial molecule libraries coupled with affinity selection-mass spectrometry (AS-MS), a tag-free alternative that relies solely on MS to identify the isolated hit compounds. Because these screening libraries may contain millions of different compounds, tandem mass spectrometry (MS/MS) has to be utilized as the monoisotopic mass of the molecules will most likely be insufficient for identification. Therefore, we developed a computational workflow for annotating the acquired MS/MS spectra with potential hit compounds of the library. First, this workflow filters out all spectra that do not match a library molecule, either due to a non-matching precursor mass or fragmentation pattern. Then, the annotation of the remaining

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

MS/MS spectra is accomplished by combinatorially fragmenting each candidate structure, using the resulting fragments to assign each candidate the likelihood of producing the observed spectrum, and ranking all candidates accordingly. To show the feasibility of our approach, we synthesized four combinatorial libraries and performed affinity selections against the two oncology targets carbonic anhydrase IX and flap endonuclease 1, resulting in the identification of nanomolar binders and inhibitors for both targets.

1019 B From spectra to certainty: end-to-end workflows for confident metabolite identification

PRESENTING AUTHOR: ***Maciej Bromirski, Thermo Fisher Scientific, Poland***

CO-AUTHORS: *Michał Kaczmarek; Bashar Amer; Rahul Deshpande; Susan S. Bird*

SUB-THEME: Metabolite identification/annotation

Metabolite identification remains a major bottleneck in untargeted LC–MS metabolomics, where feature lists can be inflated by adducts/isotopes, contaminants, and in-source fragmentation. We present an end-to-end workflow that increases identification confidence by combining (i) softer ion transfer to preserve intact precursors, (ii) blank-aware iterative MS/MS acquisition to prioritize sample-derived spectra, and (iii) multi-evidence informatics that reports why an annotation is trusted. Plasma and plant extracts were analyzed on a modified Orbitrap hybrid mass spectrometer using enhanced dynamic range scan mode and iterative deep-scan MS/MS. Sample reference injections were used to build the inclusion list, while solvent/matrix blanks were used to build the exclusion list; both were iteratively updated across replicate injections. Data processing integrated feature detection; isotope/adduct grouping; automated in-source-fragment recognition; MS/MS library matching and similarity search; and structure-consistency scoring, with outputs mapped to established confidence frameworks. Softer ion transfer reduced unintended MS1 fragmentation in a plant matrix (phenylalanine fragment-to-precursor ratio 51%→35% compared to legacy configuration), improving precursor integrity for downstream matching and formula support. Iterative, blank-aware acquisition increased MSI level 2 annotations by 57% versus conventional triplicate DDA by expanding unique precursor MS/MS coverage while deprioritizing background ions. For small-molecule annotation in biological matrices, we demonstrate how database-only searching can yield hundreds of structural candidates for a single formula (e.g., 421 candidates for C27H44O), motivating the necessity of orthogonal evidence such as curated MS/MS library matches, similarity searches, and structure-based fragment coverage scoring. Where reference spectra are absent, in-silico fragmentation and class prediction supported Level 3–4 reporting. Additionally, in a challenging positional isomeric case, an AI/ML confidence score separated a true hit (91) from near-misses (28) despite high legacy similarity scores. Collectively, this workflow reduces false positives, improves reproducibility, and enables defensible reporting aligned with commonly used small-molecule annotation confidence frameworks (Metabolomics Standards Initiative, and Schymanski-style levels).

1020 A Multi-Omic Stratification of Nephropathy Using Targeted High-Coverage Mass Spectrometry

PRESENTING AUTHOR: ***Brittany Lee, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Jake T Kline, Amal M Kamal, Amy K Carfango, Philip M. Remes, Cristina C. Jacob, Lilian Heil, Eleonora Forte, Romain Huguet, Lorenzo Gallon, Luca Fornelli*

SUB-THEME: Multi-omics/Integrative omics

Introduction: Human plasma serves as a critical liquid biopsy for understanding systemic disease. We demonstrate the utility of a quadrupole-linear ion trap for the interrogation of the proteome, metabolome, and lipidome. This approach bridges the gap between discovery-phase coverage and targeted quantitative precision. Specifically, we analyzed plasma from patients with various nephropathies, including focal segmental glomerulosclerosis (FSGS), to evaluate whether integrated multi-omics enhances disease state discrimination and mechanistic insight compared to single-omics analysis.

Methods: Plasma from 10 patient groups (8 kidney diseases plus controls) was analyzed via Parallel Reaction Monitoring (PRM) on a Stellar mass spectrometer. Proteomic libraries (~500 protein groups) were constructed using automated sample preparation and extracellular vesicle enrichment. Metabolomics targeted ~200 analytes, while a newly developed high coverage lipidomics workflow targeted ~1,000 precursors (>2,300 species). Data integration and statistical modeling (PCA, PLS-DA, UMAP) were performed using a custom R-based multi-omics suite.

Results: The targeted approach yielded high reproducibility, with >80% of peptides showing CVs

Conclusion: This study establishes a robust multi-omics framework using a nominal mass instrument. By combining deep proteomics and metabolomics and lipidomics, we enabled superior patient stratification. This scalable workflow provides a path for longitudinal monitoring of kidney disease progression and therapeutic response.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1021 B Temperature control enhances lactic acid bacteria abundance and lactic acid production during coffee fermentation

PRESENTING AUTHOR: ***Matheus Gomes, Federal University of Uberlandia, Brazil***

CO-AUTHORS: *Lívia Carneiro Fidélis Silva, Gisele Xavier Ribeiro Costa, Marcelo Antônio Duarte Cruz, Luiza Manuela Alves Braga Cardoso, Pedro Luiz Lima Bertarini, Laurence Rodrigues do Amaral, Líbia Diniz Santos*

SUB-THEME: Multi-omics/Integrative omics

Controlled fermentation strategies have increasingly been applied to enhance the quality and sensory complexity of specialty coffees. Among organic acids, lactic acid plays a relevant role in promoting balanced acidity, sweetness perception, and improved mouthfeel. Understanding the relationship between microbial succession and metabolite production during coffee fermentation is essential for the development of reproducible, quality-oriented processing protocols. In this study, auto-induced anaerobic fermentations were performed in 200 L bioreactors for 72 h under two conditions: without temperature control and with temperature maintained at 27°C. Samples were collected every 24 h, in triplicate, for microbial and metabolite analyses. Bacterial community composition was characterized by 16S rRNA gene-based metataxonomic analysis, and lactic acid concentrations were determined by high-performance liquid chromatography (HPLC). The relative abundance of lactic acid bacteria (LAB) increased markedly throughout fermentation, particularly under temperature-controlled conditions. In the 27°C treatment, LAB abundance increased from 1.64% at 0 h to 85.56% at 72 h, whereas in the non-controlled fermentation it reached 68.58% at the same time point. This microbial succession was accompanied by a progressive increase in lactic acid concentration. At 72 h, lactic acid levels reached 46.09 mg g^{-1} in the controlled fermentation, compared with 28.23 mg g^{-1} in the non-controlled condition. Similar trends were observed at 24 h and 48 h, further supporting the effect of temperature regulation on microbial growth and metabolic output. These findings demonstrate a strong association between LAB proliferation and lactic acid accumulation during auto-induced anaerobic coffee fermentation and identify temperature control as a key factor influencing microbiome dynamics and metabolite production. The integration of metataxonomic profiling with targeted metabolite analysis expands the current understanding of microbiome–metabolome interactions in specialty coffee processing.

1022 A Application of NMR Metabolomics and Machine Learning Approaches for the Characterization of Dual PGE_2/LTB_4 Inhibitors in Brazilian Lauraceae Species

PRESENTING AUTHOR: ***Miller Santos Ferreira, University of Alfenas, Brazil***

CO-AUTHORS: *Albert Katchborian-Neto, Danielle F. Dias, João Henrique Lago, Daniela A. Chagas-Paula, Mario-Ferreira Conceição Santos, RuAngelie Edrada-Ebel, Marisi G. Soares*

SUB-THEME: Multi-omics/Integrative omics

Chronic inflammation contributes to diseases such as cancer, autoimmune and cardiovascular disorders, Alzheimer's disease, and COVID-19. It is mediated by cytokines that activate transcription factors (e.g., NF-κB), inducing COX-2 and LOX-5 expression and promoting the biosynthesis of PGE_2 and LTB_4. The Lauraceae family is known for producing anti-inflammatory natural products. In this study, we employed NMR-based metabolomics integrated with multivariate analysis and machine learning to characterize dual bioactive markers in ten Lauraceae species from the Brazilian Atlantic Forest. Spectral data were processed using MestReNova (v14.2.3), SIMCA-P, and KNIME (v5.1). KNIME enabled implementation of advanced statistical tools including heatmaps for pattern visualization, ROC AUC for evaluating predictive model performance, and Benjamini-Hochberg correction to control false discovery rates in multiple comparisons. *Ex vivo* assays confirmed dual inhibition of PGE_2 and LTB_4. PCA revealed moderate sample separation ($R^2X = 0.742$), while OPLS-DA showed strong group discrimination ($R^2Y = 0.908$, $Q^2 = 0.927$), with validation by CV-ANOVA ($p = 3.57 \times 10^{-19}$). S-plots and loadings identified relevant chemical shifts (0.6–3.0 ppm and 6.0–9.5 ppm), suggestive of methyl, methylene, and aromatic protons. Based on these patterns and known Lauraceae constituents, these may reflect glycosylated flavonoids and alkaloid structures. Metabolites with high VIP scores and significant p- and q-values ($q < 0.05$) were characterized as potential predictive markers. These statistical methods enhanced confidence in biomarker prioritization and interpretation. Altogether, this study demonstrates the power of integrating NMR metabolomics with statistical and machine learning tools. The Lauraceae species investigated show strong potential for future development of dual-action anti-inflammatory agents.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1023 B Multi-omics Profiling of Prebiotic, Polyphenol, Protein, and Probiotic Responses in a Human Gut Ex Vivo Model: A Pilot Study

PRESENTING AUTHOR: ***Marlene Jara Portocarrero, CMBio, Denmark***

CO-AUTHORS: *Marlene Jara, Kristian Jensen Pedersen, Jonathan Samson, Cynthia Vesterager, Rasmus Wollenberg, Chloe Moritz, Renata Blatnik, Pierre-Olivier Schmit, Aurélien Baudot, Pieter Van den Abbele, Morten Danielsen, Lea Johnsen*

SUB-THEME: Multi-omics/Integrative omics

Introduction: Prebiotics, probiotics, and bioactive food compounds are increasingly used to modulate gut microbial activity, but their effects depend on the substrate, strain, and microbial community. Ex vivo gut models provide a controlled framework to investigate these responses under human gut-like conditions. Here, we applied untargeted metabolomics and metaproteomics to characterize the functional response of a stool-derived microbial community exposed to selected prebiotics, naringenin, whey protein, and Bifidobacterium longum using the SIFR® technology platform (Systemic Intestinal Fermentation Research).

Methods: Stool from a single donor was cultivated and exposed to naringenin, inulin, 2'-fucosyllactose, pectin, resistant dextrin, whey protein, and a combined inulin, whey protein, and B. longum treatment. Bacterial pellets and spent media were collected at 0, 6, and 24 h. Spent media metabolite profiles were assessed by untargeted metabolomics and processed in-house. Microbial proteome and protein-based taxonomic profiles were assessed by metaproteomics. DDA data were analyzed using MSFragger v4.2 within FragPipe v23.0, and peptide identifications were performed against a donor-specific database constructed from metagenome-assembled genomes.

Results: The largest metabolite response relative to the non-stimulated control was observed after 6 h in the combined inulin, whey protein, and B. longum treatment, with 22 increased and 15 decreased metabolites using fold-change thresholds of >5 and <0.2. Other treatments showed overall metabolite accumulation in spent media over time, consistent with microbial byproduct production and release. Increased p-hydroxyphenyllactic acid, indole-3-lactic acid, and 3-phenyllactic acid suggested aromatic amino acid metabolism associated with lactic acid bacterial activity. Metaproteomics supported treatment-associated changes in the active microbial community and functional protein profiles.

Conclusions: This proof-of-concept pilot study shows that combined metabolomics and metaproteomics can link extracellular metabolite changes with active microbial contributors, supporting functional characterization of prebiotic and probiotic modes of action and connecting microbiota shifts to functional phenotypes in an ex vivo model.

1024 A Metabolomics and genomics integration reveals signatures associated with early sexual development in Nelore heifers

PRESENTING AUTHOR: ***Vladimir Elias, Genika Multiomics Solutions, Brazil***

CO-AUTHORS: *Brennda Goncalves Lima, Vanusa Alquino Rabelo, Pietro Sampaio Baruselli, Bárbara Prestes de Castro Souza, Vladimir Oliveira Elias, Rafael dos Santos Bezerra, Bruna Lima Chechin Catussi*

SUB-THEME: Multi-omics/Integrative omics

This pilot study investigated integrated genomic and metabolomic signatures associated with divergent sexual development in Nelore heifers. A total of 115 heifers (13.8 ± 0.2 months of age and 300.4 ± 5.01 kg of body weight) were subjected to a P4/E2-based FTAI protocol. Heifers that did not conceive were resynchronized and subsequently exposed to natural mating. A subset of 20 heifers was selected for this pilot study based on extreme reproductive outcomes: early-maturing (EM; n=10), defined as heifers that conceived at the first FTAI and maintained pregnancy, and late-maturing (LM; n=10), defined as heifers that failed to conceive after two FTAI attempts followed by natural mating exposure. Serum samples for metabolomic analysis were collected 10 days before synchronization. Untargeted metabolomic analysis was performed by Q-TOF LC-MS. Genomic data were obtained from high-density SNP array genotyping and integrated with metabolomic data using Data Integration Analysis for Biomarker discovery using Latent components (DIABLO). DIABLO analysis revealed a clear separation between EM and LM heifers, indicating coordinated genomic and metabolic signatures associated with divergent reproductive phenotypes. The integrated model identified discriminant SNPs and metabolites contributing to group separation. Among the most relevant metabolites, succinate, PE 18:1, PI 48:6, taurocholic acid, sphingolipid-related metabolites, and other lipid- and energy metabolism-associated compounds were highlighted. Notably, succinate consistently emerged as an important discriminant feature across analyses, suggesting alterations in energy metabolism between groups. Correlation network analysis demonstrated structured associations between selected SNP markers and metabolites involved in mitochondrial metabolism, lipid remodeling, and cellular signaling pathways. In conclusion, these findings suggest that early sexual development in Nelore heifers is associated with integrated genomic and metabolic signatures involving energy and lipid

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

metabolism. This pilot study supports the application of multi-omics approaches to improve the understanding of biological mechanisms underlying reproductive development in cattle.

1025 B Nested cross-validated machine learning identifies urinary S-adenosylhomocysteine as a key metabolite linked to mortality and inflammatory signalling in severe COVID-19

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

CO-AUTHORS: *Marc-Emmanuel Dumas, Peter J M Openshaw, Matthew K Siggins*

SUB-THEME: Multi-omics/Integrative omics

Severe COVID-19 is characterised by substantial alterations in metabolic and immune function, yet the specific metabolites linking these changes to clinical outcomes remain incompletely understood. To address this, we analysed urine LC–MS metabolomics from hospitalised participants in the UK ISARIC-4C cohort to identify metabolites associated with mortality and to examine their relationships with the host inflammatory response. Using this approach, urinary S-adenosylhomocysteine (SAH) emerged as the primary metabolite associated with mortality and showed robust associations with inflammatory signalling.

To robustly identify outcome-associated metabolites, we applied Random Forest models with nested cross-validation, ensuring robust and unbiased model evaluation while minimising the risk of overfitting. Model interpretability was assessed using SHAP (Shapley additive explanations), which quantified the contribution of each metabolite to mortality prediction across outer cross-validation folds.

Across outer cross-validation folds, SAH consistently emerged as the strongest predictor of mortality, with SHAP contributions substantially larger than those of any other metabolite. Longitudinal analysis revealed clear separation between survivors and non-survivors. Patients who later died showed persistently elevated urinary SAH levels, particularly during the early phase following hospital admission.

We next examined the relationship between metabolites and inflammatory signalling. Several top predictive metabolites were significantly associated with key cytokines, including IL-6, TNF, and IL-10, suggesting coordinated metabolic and immune responses during acute infection. SAH showed particularly strong associations with inflammatory markers, pointing to a potential link between methylation-related metabolism and inflammatory dysregulation in severe COVID-19.

These results identify urinary SAH as a central metabolite associated with COVID-19 mortality. The consistent signal across nested cross-validation, longitudinal trajectories, and cytokine associations suggests that SAH may link metabolic disruption with inflammatory responses in acute disease. Ongoing mediation and causal network analyses will further clarify potential mechanistic connections between metabolism, immune activation, and clinical outcomes.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1050 A Untargeted metabolomics for the assessment of cognitive resilience-associated metabolites in human serum

PRESENTING AUTHOR: ***Lurian Caetano David, UFG (Brazil); UCSD (USA), Brazil***

CO-AUTHORS: *Toon A. W. Scheurink, Jeong In Seo, Lurian Caetano David, Crystal Wang, Deyvis Solis, Jasmine Zemlin, Jaclyn Bergstrom, Pieter C. Dorrestein, Ipsita Mohanty, Anthony Molina*.

SUB-THEME: Aging research

An important characteristic of healthy aging is the ability to maintain cognitive performance throughout the aging process, known as cognitive resilience. In this study, an untargeted LC-MS/MS metabolomics approach was used to analyze serum samples collected from 237 participants in a 28-year follow-up of cognition in the Rancho Bernardo Study (RBS) of Healthy Aging. Longitudinal data from this cohort were assessed to filter for age-matched samples among the participants. Comparison of single-visit samples with respect to cognitive resilience was performed using an sPLS regression model, which selected 3080 features based on the selected sparsity parameters. Glycerophospholipids, small peptides, fatty esters, and oligopeptides accounted for more than 50% of all NPC superclasses predicted by SIRIUS using the CANOPUS module for our selected metabolites. Feature-based molecular networking in GNPS was applied to the dataset, revealing a big cluster of MS/MS matches to newly described N-acylcarnitines derived from short- to very long-chain fatty acids, and indicating that the majority (83%) were negatively correlated with cognitive resilience. A similar negative association was observed in another subnetwork of glutamine-conjugated fatty acids and in hippuric acid conjugates, with some well-characterized and others putative matches. Some phosphocholines (e.g., lysophosphatidylcholine) were positively associated with cognitive resilience, likely due to their roles in cell membranes. MassQL queries were also performed to search for glucuronide conjugates, a major detoxification pathway, and 129 matches were found, 81,4% of which (i.e., DCA glucuronide) were associated negatively with cognitive resilience. From a xenobiotic perspective, queries into the metabolites of the antihypertensive drugs atenolol and metoprolol revealed distinct trends in metabolic pathways associated with cognitive resilience profiles. Diet-derived compounds, piperine and lutein, were more frequently detected in participants with high cognitive resilience. MASST was applied to a repository-scale search, presenting insights into risk factors and reinforcing the importance of microbial metabolites in cognitive function.

1051 B Development of a methodological framework for studying metabolic aging in Nothobranchius furzeri

PRESENTING AUTHOR: ***Jean-charles Portais, University of Toulouse, France***

CO-AUTHORS: *Ambroise Jezequel, Léana Porcher-Bibes, Anais Seguela, Séverine Ethuin, Caroline Nevoit, Yannick Jeanson, Spiro Khoury, Audrey Carrière, Jean-Philippe Pradère, Jean-Charles Portais*

SUB-THEME: Aging research

Aging is associated with multi-organ metabolic dysregulation, the mechanisms of which remain poorly understood. To address this, we developed an approach in the annual fish Nothobranchius furzeri (lifespan ~7 months), including a systematic comparison of two isotopic tracing methods: water immersion and intraperitoneal injection. This approach uses ^{13}C-labeled compounds (i.e glucose) to track metabolic fluxes in real time. Two protocols were evaluated: substrate infusion in water (continuous exposure) and intraperitoneal injection (single bolus). Water immersion allows for gradual and homogeneous tracer incorporation, reflecting a steady-state condition, while injection provides high temporal resolution to study early metabolic dynamics. Mass spectrometry analysis of ^{13}C incorporation into key intermediates (glycolysis, TCA cycle, pentose phosphate pathway, etc) captures inter-organ exchanges, such as lactate transport between liver and muscle. Cross-analysis of isotopic tracing data reveals tissue-specific metabolic features, such as low glucose dependence in the liver, in contrast to high dependence in the brain. The comparison of the two tracing methods showed that water immersion enables better detection of long-term fluxes, whereas injection is more suitable for studying acute metabolic responses. Methodological validation revealed age-related differences in ^{13}C-glucose incorporation, with an increase in the brain and a decrease in the liver. This methodology opens new avenues for studying aging or metabolic diseases in Nothobranchius furzeri as a valuable biological model of natural aging. It can be extended to other substrates (amino acids, lipids) or models (zebrafish) and could be integrated with other omics approaches (metabolomics, transcriptomics, proteomics) in order to decode the metabolic crossroads of aging.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1052 A Early detection of brain alterations using immunofluorescence and MALDI-TOF in an APP/PS1 mouse model

PRESENTING AUTHOR: ***Natalia Hernandez, Universidad de Concepción, Chile***

CO-AUTHORS: *Natalia Hernandez-Urzúa, Catalina Bravo, Jessica Panes, Loreto San Martin, Sebastian Riquelme-Riffo, Daniela Nova-Baza, Andy J. Perez, Claudia Mardones, Jorge Fuentealba, Lia Olivares-Caro*

SUB-THEME: Aging research

Alzheimer's disease (AD) is characterized by molecular alterations that precede the onset of overt pathology; however, spatially resolved changes at early stages are not yet fully characterized. The aim of this study was to evaluate early brain changes associated with AD using a 3-month-old APP/PS1 murine model. Multichannel immunofluorescence analyses were performed to detect IL-6, P2X4R, and PGC-1α. In addition, mass spectrometry imaging (MSI) by MALDI-TOF was conducted on coronal brain sections (10 µm) mounted on ITO slides using 2,5-dihydroxybenzoic acid (DHB) as matrix. Region of interest (ROI) analysis by immunofluorescence showed a significant increase in IL-6 and P2X4R in cortex and hippocampus ($p < 0.001$), along with the presence of $IL\text{-}6^{+}/P2X4R^{+}$ inflammatory clusters distributed across cortical and subcortical regions, indicating early activation of neuroinflammatory pathways, while PGC-1α was found to be increased in APP/PS1 mice, suggesting a compensatory response associated with metabolic dysfunction. For MSI analysis, serial sections were analyzed in triplicate and the data were processed using SCiLS and MetaboAnalyst 6.0 through multivariate analyses (PCA) and volcano plot ($p < 0.05$; $FC \geq 1.5$). MSI results revealed metabolic changes associated with ROI between both groups, with alterations in cortex, hippocampus, thalamus, hypothalamus, and amygdaloid complex, including changes in the ion m/z 856.557 ± 0.205 Da, putatively assigned to phosphatidylcholine PC (40:6). Overall, these results demonstrate that the combination of immunofluorescence and MALDI-MSI enables the detection of early alterations in AD, providing information for the identification of associated metabolites and the study of early disease mechanisms.

FONDECYT3240171,FONDECYT1230625 ,FONDEQUIPEQM220055

1053 B Biomolecular Characterisation of Surgical Frailty Across Emergency and Elective Surgery

PRESENTING AUTHOR: ***Nicholas Rattray, University of Strathclyde, United Kingdom***

CO-AUTHORS: *Hwei Jene Ng, Tara Quasim, Gillian Farrell, Patricia Kelly, Catriona M. Douglas, Rhona Hurley, Ana Talbot, Jessica A. Lasky-Su, Thomas Jackson, Niharika Duggal, David I. Broadhurst, Susan J. Moug, Lynda Wild, Ilaria Bellantuono, Jayan George*

SUB-THEME: Aging research

Frailty reflects diminished physiological reserve and is a major determinant of adverse outcomes following surgery, particularly in older adults. Despite widespread clinical use, existing frailty assessment tools remain largely subjective and do not capture the molecular heterogeneity that underpins vulnerability to surgical stress. There is therefore a pressing need for objective, biomarker-based approaches to improve perioperative risk stratification and to better characterise surgical frailty, including frailty that is unmasked or accelerated by operative intervention.

Here, we present integrated metabolomic and proteomic analyses from three clinical trials investigating surgical frailty across contrasting operative contexts: two independent emergency colorectal surgery cohorts and one elective breast cancer surgery cohort with post-operative follow-up. Together, these studies enable evaluation of biomolecular responses to both acute, high-stress surgery and planned, lower-risk surgical intervention, providing complementary insight into mechanisms of frailty and recovery.

Plasma samples collected pre- and post-operatively were analysed using LC-MS-based untargeted metabolomics alongside a discovery proteomic platform. Multivariate and pathway-level analyses were applied to identify molecular signatures associated with baseline frailty, post-surgical frailty progression, complications, delayed recovery, and short-term mortality. Across emergency colorectal cohorts, frailty was associated with pronounced disruption in pathways linked to energy metabolism, amino acid turnover, inflammation, immune activation, lipid remodelling, and oxidative stress, with several alterations persisting or worsening following surgery. In contrast, the elective breast cancer cohort revealed subtler but reproducible molecular changes associated with recovery trajectory and functional resilience.

Integration of metabolomic and proteomic data demonstrate that surgical frailty is underpinned by biologically meaningful and partially shared molecular signatures across procedures, supporting the development of objective, translational tools to guide precision perioperative care.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1054 A Metabolomics biomarkers of brain health: Early dysregulation of branched-chain amino acids, lysine, and creatine pathways in middle-aged adults

PRESENTING AUTHOR: ***Cristina Andres-Lacueva, University of Barcelona - CIBERFES, Spain***

CO-AUTHORS: *Meryl Cruz, Miriam Martínez-Huélamo, Gabrielle Cattaneo, Alejandro Sánchez-Pla, Javier Solana-Sánchez, Olga Jauregui, Antonio Miñarro, Anna Guadall, David S. Wishart, Yamilé López-Hernández, Álvaro Pascual-Leone, Tomás Meroño, David Bartrés-Faz, Cristina Andrés-Lacueva*

SUB-THEME: Aging research

Aim and Background. Brain health is a multidimensional state across several functional domains susceptible to be differently altered during aging. Decline in these brain domains may be determined by cellular and molecular imbalances affecting metabolic processes. We aimed to define brain health domains using a comprehensive lifestyle and brain health evaluation to identify specific metabolomic signatures in a cohort study of middle-aged adults.

Methodology. We included 826 participants from the Barcelona Brain Health Innitiative study who underwent a complete lifestyle, clinical and brain health evaluation (including a complete neuropsychological and personality profile evaluation, neurofilament light-chain (NfL) quantification, and brain-MRI tests) at baseline and after a one-year follow-up. To obtain operational definitions of brain domains we integrated brain health data with lifestyle and clinical variables (namely, age, BMI, blood pressure and lipids, physical exercise, sleep, nutrition, social interactions, and vital plan) into a Multiple Factor Analysis to obtain dimensions, and cluster individuals. Plasma metabolomics was assessed using a clinical biomarkers targeted assay including up to 721 metabolites. Results of correlations and linear regression analyses at baseline were validated using timepoint 2 data. Four distinct dimensions corresponding to brain health domains were identified

Conclusion: We have found a common metabolomic signature of brain aging and metabolic health impairment related with dysregulation of BCAAs and lysine metabolism. Furthermore, a novel association between creatine metabolism and hippocampal volume was identified. These metabolomic signatures may serve as early biomarkers to detect individuals at higher risk for brain health impairment during midlife.

1055 B Chemical Metabolomics – New Chemical Biology Tools to Explore Microbiome Metabolism

PRESENTING AUTHOR: ***Leo Martin, Uppsala university- BMC, Sweden***

CO-AUTHORS: *W. Lin, I. Tsiara, A. Torregrosa, W. Haberek, V. Dovhalyuk, D. Globisch*

SUB-THEME: Biomarker validation and clinical translation

Metabolites produced by the gut microbiome play a crucial and diverse role on host physiology, which are detectable in a wide range of biological samples. Mass spectrometric metabolomics is the method of choice for identification and quantification of these metabolites. Advanced methods at the interface of chemistry and biology coupled with metabolomics analysis are required but still limited. We have therefore developed a unique and multifunctional chemoselective probe with synthetic 13C/12C isotopically labelled analogues that allows for comparative and quantitative analysis of metabolites in human samples at low concentrations.2 Coupled to magnetic beads, this method allows the straightforward chemoselective extraction of metabolites from human samples.[2-6] This isolation procedure of specific classes of metabolites from sample matrices led to significantly increased mass spectrometric sensitivity by sixth orders of magnitude and facilitates the detection of metabolites at femtomole quantities. The chemoselective probe was applied for analysis of amine-, carboxylate-, thiol-, or carbonyl-containing metabolites in human fecal, urine and plasma samples. Currently, we are applying these for diverse biological and medical research questions to discover bioactive metabolites and biomarkers. The success of this large-scale application of this methodology results in further validation of this method and encourages the general use of this method in metabolomics studies.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1056 A Mass spectrometry Evaluation of Fabry Disease-Related Biomarkers in a Gene Therapy Clinical Trial Cohort

PRESENTING AUTHOR: ***Christiane Auray-Blais, Université de Sherbrooke, Canada***

CO-AUTHORS: *Christiane Auray-Blais, Pamela Lavoie, Tristan Martineau, C. Anthony Rupar, Dwayne L. Barber, Armand Keating, Ronan Foley, Aneal Khan, Michael L. West, Jeffrey A. Medin.*

SUB-THEME: Biomarker validation and clinical translation

Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by a mutation in the GLA gene that impairs the catabolism of glycosphingolipids. Currently approved treatments for FD include enzyme replacement therapy (ERT) and pharmacological chaperone therapy, both of which aim to reduce substrate accumulation. Ex vivo gene therapy (GT) represents another promising therapeutic approach with the potential for long-term disease correction. In 2016, the world's first GT clinical trial for FD was launched in Canada, with the primary objective of assessing the safety and toxicity of lentivirus α-galactosidase A transduced autologous CD34+ cells in FD adult males with classical mutations. Biomarker analyses were conducted as an essential component of the evaluation of this novel therapy using LC-MS/MS. The aim of this project was to assess a comprehensive 5-year profile of FD-related biomarkers in participants enrolled in this GT clinical trial. All participants (n=5) received ERT at baseline. ERT was discontinued one month prior to GT and resumed one-month post-GT, except for one participant who chose not to resume ERT. For participants who met predefined clinical endpoints, ERT was paused after a minimum of 6 months following GT. Plasma and urinary Gb3 and lyso-Gb3 isoforms and analogues were repeatedly measured by LC-MS/MS over a 5-year surveillance period. Our results show that urine and plasma lyso-Gb3 and analogues, as well as urine Gb3 levels, were reduced following GT and during ERT treatment compared to baseline (ERT only). Three participants discontinued ERT at some point after GT. Two of them had lower urine and plasma lyso-Gb3 and analogue levels relative to baseline. An increase in urine Gb3 was observed upon ERT discontinuation post-GT. In conclusion, this evaluation of a glycosphingolipid biomarker profile proved valuable in monitoring the biochemical response to GT in this cohort of five male patients with FD.

1057 B Urinary Acylcarnitine Signature as a Metabolic Indicator of the Transition from UTI to Urosepsis

PRESENTING AUTHOR: ***Paweł Wityk, Medical University of Gdansk, Poland***

CO-AUTHORS: *Wiktoria Brzezińska, Beata Krawczyk, Joanna, Raczak-Gutknecht, Mariusz Siemiński, Jacek Szypenbejl, Jan Łuczkiewicz, Michał Markuszewski*

SUB-THEME: Biomarker validation and clinical translation

Urosepsis accounts for approximately 25% of all sepsis cases and remains associated with high mortality, largely due to delayed recognition of the transition from localized urinary tract infection (UTI) to systemic inflammatory dysregulation. Current diagnostic strategies based on clinical scores (qSOFA/SOFA), blood cultures, C-reactive protein, and procalcitonin lack sufficient specificity and speed to reliably distinguish early urosepsis from uncomplicated UTI.

To address this unmet clinical need, we applied an untargeted LC-MS-based lipidomics approach to characterize urinary metabolic alterations in patients with E. coli-induced UTI and urosepsis. Urine samples were analyzed using high-resolution LC-MS (ZenoTOF 7600), followed by multivariate and univariate statistical analyses, including PCA, PLS-DA, Volcano plots, ROC curve analysis, and FDR correction.

We identified a consistent and statistically significant decrease in selected medium-chain acylcarnitines in urosepsis compared with UTI, particularly CAR 8:0, CAR 9:0, CAR 10:0, CAR 11:0, and CAR 12:0 (Mann-Whitney $p = 0.001$-0.013). These alterations contrast with plasma acylcarnitine accumulation reported in systemic sepsis and suggest a disease-specific mechanism involving mitochondrial β-oxidation impairment, altered renal clearance, and tubular reabsorption changes.

Based on these findings, we developed the UTI-to-Sepsis Index (USI), defined as the ratio of urinary acylcarnitine signal intensity to free creatinine concentration. The USI serves as a non-invasive quantitative marker of mitochondrial–renal metabolic coupling. A decrease in USI values characterizes progression toward urosepsis, whereas normalization accompanies recovery. Mechanistically, reduced urinary acylcarnitines likely reflect mitochondrial dysfunction, inflammatory metabolic reprogramming, and lipid remodeling associated with systemic infection.

Urine-based lipid profiling combined with the USI provides a rapid, low-cost, and repeatable strategy for early differentiation of UTI and urosepsis, supporting risk stratification and therapy monitoring.

This research was funded by the Science for The Society project (NdS-II/SP/0438/2024/01, PW).

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1058 A Sensitive and robust quantitation of serum bile acids using a novel kit workflow on Stellar MS to study Crohn's disease

PRESENTING AUTHOR: ***Giorgis Isaac, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Cynthia M. Grim, Bashar Amer, Will Thompson, Scott Mellors, James Campbell, Cristina C. Jacob, Scott M. Peterman, Susan S. Bird*

SUB-THEME: Biomarker validation and clinical translation

Accurate quantitation of bile acids in serum is analytically challenging due to low endogenous concentrations, structural similarity among isomers, and matrix effects. Moreover, practical pain-points such as queue building, sample preparation, and data curation for chromatographic isomers decrease throughput and increase the potential for analyst bias. These challenges are particularly relevant in human cohort studies such as Crohn's disease, where subtle but biologically meaningful alterations in bile acid composition may reflect disease-associated dysregulation of gut-liver metabolism. Standardized, reproducible workflows with reduced human touch-points are essential to support robust cohort-scale analyses.

Serum samples from individuals with Crohn's disease and matched healthy controls were analyzed using a novel quantitative workflow (MoveKit™ BA) which incorporates sample prep, internal standards, reference materials, and software. Chromatographic separation was performed using reversed-phase liquid chromatography coupled to a quadrupole ion trap mass spectrometer (Stellar MS platform). A targeted MS^2 acquisition strategy combining collision-induced dissociation (CID) and higher-energy collisional dissociation (HCD) enabled selective detection and improved discrimination of structurally related bile acids. Quantitative data processing was performed using reference materials along with low and high QC standards.

Automated acceptance criteria for sensitivity, retention time and resolution were enabled in the MoveApp™ software before initializing data collection on samples. The method demonstrated excellent linearity and reproducibility, with low coefficients of variation. Sensitivity supported low single-digit nM detection of bile acids in serum. Targeted MS^2 acquisition reduced matrix interference relative to MS^1-only approaches, improving quantitative accuracy and selectivity.

Application of this workflow revealed distinct differences in bile acid concentrations and overall profiles between Crohn's disease patients and healthy controls, consistent with reported alterations in bile acid metabolism in inflammatory bowel disease.

Together, these results demonstrate that integrating standardized sample preparation with selective MS^2 acquisition provides a robust platform for sensitive serum bile acid quantitation in clinical metabolomics studies.

1059 B Metabolomics of Ocotea spp. reveals alkaloid signatures linked to promising antiproliferative and apoptosis induction in breast cancer cells.

PRESENTING AUTHOR: ***Fernanda Brito Leite Dornelas, Federal University of Juiz de Fora, Brazil***

CO-AUTHORS: *Fernanda Brito Leite Dornelas, Wanderleya Toledo dos Santos Faulhaber, Matheus Fernandes Alves, Albert Katchborian-Neto, Clarissa Ferreira Cunha, Michael Murgu, Fernanda Maria Pinto Vilela, Daniela Aparecida Chagas-Paula, Ana Claudia Chagas de Paula*.

SUB-THEME: Cancer

Natural products are an important source of anticancer agents, however, comprehensive metabolomic profiling of bioactive extracts across many genera remains scarce. This study integrated untargeted metabolomics with biological evaluations to identify cytotoxic Ocotea spp. extracts and annotate metabolites associated with antiproliferative activity in MCF-7 breast cancer cells. Fifty-six Ocotea spp. leaf extracts were screened using the MTT assay (48 h). Metabolomic profiling was performed by UPLC-HRMS in ESI+ and ESI− modes. Data were normalized, log-transformed, and Pareto-scaled prior to multivariate analysis. PCA and OPLS-DA were applied to identify discriminant features, and metabolites with VIP > 3 and |Log2 fold-change| > 5 were considered influential. Putative annotation was conducted using MS^1/MS^2 data and spectral library comparison according to Metabolomics Standard Initiative (MSI) criteria. Among the extracts, only Ocotea villosa demonstrated significant cytotoxicity (IC_{50} = 100 µg/mL; SI = 1.3). Flow cytometry revealed increased early and late apoptotic populations, accompanied by CASP9 upregulation, indicating activation of the intrinsic apoptotic pathway, a promising mechanism of action. Besides, the extract reduced cell migration in the scratch assay and significantly inhibited colony formation in the clonogenic assay. PCA showed clear metabolic discrimination of O. villosa relative to inactive species. OPLS-DA models demonstrated robust goodness-of-fit and predictive ability (ESI+: R^2 = 0.978, Q^2 = 0.849; ESI−: R^2 = 0.986, Q^2 =

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

0.815). Twenty-seven metabolites were associated with the cytotoxic profile, predominantly isoquinoline-derived alkaloids. Nuciferine and related aporphines were annotated at MSI Level 2 and exhibited high VIP scores. The enrichment of nuciferine-type alkaloids provides a chemical basis for the observed bioactivity and highlights untargeted metabolomics as a strategic tool for anticancer discovery studies.

1060 A — Formaldehyde fixation products in FFPE samples recover pathway coverage in gastric cancer samples

PRESENTING AUTHOR: ★ ***Maximiliano Ramm, Universidad de Concepción, Chile***

CO-AUTHORS: *David Martínez, Alexander Brevis, Tania Higueras, Lía Olivares-Caro, Carolina Delgado, Claudia Mardones & Luis Bustamante*

SUB-THEME: Cancer

Metabolomics is a powerful tool for understanding cancer pathophysiology. Using archival formalin-fixed, paraffin-embedded (FFPE) tissue biopsies for metabolomic analysis remains challenging because fixation and embedding processes lead to metabolite loss and degradation. In this work we developed, evaluated, and applied sample preparation methods for polar metabolome extraction from FFPE gastric biopsies and their subsequent anlysis through HILIC-MS. The method tested tissue sample quantity, deparaffinization, and solvent re-extraction to maximize metabolite recovery. The optimized process preserved 39% of metabolic features compared to fresh-frozen (FF) samples. Applied to a cohort of 16 patients, paired cancerous and non-cancerous tissue from both FF and FFPE samples were analyzed. Results show relevant metabolic alterations, including significant changes in amino acid metabolism, were maintained in FFPE samples. Fixation-derived amino acids in FFPE samples were identified for the first time. Cycled fixation products of arginine, histidine and valine; and methylated glutamate in FFPE samples reflect metabolic alterations in FF cancerous tissue. This approach enables large-scale, retrospective metabolomic studies using extensive FFPE biobanks.

1061 B — Metabolomic Analysis of Breast Cancer in Colombian Patients: Exploring Molecular Signatures in Different Subtypes and Stages

PRESENTING AUTHOR: ***Lizeth Dayana León Carreño, Universidad de los Andes, Colombia***

CO-AUTHORS: *Daniel Pardo-Rodriguez, Andrea Del Pilar Hernandez-Rodriguez, Juliana Ramírez-Prieto, Gabriela López-Molina, Ana G. Claros, Daniela Cortes-Guerra, Julian Alberto-Camargo, Wilson Rubiano-Forero, Adrian Sandoval-Hernandez, Mónica P. Cala, Alejandro Ondo-Mendez*

SUB-THEME: Cancer

Breast cancer (BC) is a neoplasm characterized by high heterogeneity and is influenced by intrinsic molecular subtypes and clinical stage, aspects that remain underexplored in the Colombian population. This study aimed to characterize metabolic alterations associated with subtypes and disease progression in a group of newly diagnosed, treatment-naive Colombian women using an untargeted metabolomics approach. Samples were analyzed using a multiplatform approach, LC-QTOF-MS and GC-QTOF-MS, along with amino acid profiling. Analysis across biological subtypes showed elevated levels of acylcarnitines and overexpression of free fatty acids in the Luminal B subtype compared to the other subtypes. It also presented increased levels of carbohydrates and essential glycolytic intermediates, suggesting that this subtype may adopt a hybrid metabolic phenotype characterized by increased glycolytic flux and fatty acid oxidation. Tumor, Node, and Metastasis (TNM) staging analysis revealed progressive metabolic reprogramming of BC. In advanced stages, a sustained increase in phosphatidylcholines and a decrease in lysophosphatidylcholines were observed, reflecting lipid alterations associated with key roles in tumor progression. In early stages (I-II), plasma metabolites with high discriminatory power were identified, such as glutamic acid, ribose, and glycerol, which are associated with dysfunctions in energy and carbohydrate metabolism. These results highlight metabolomics as a promising tool for the early diagnosis, clinical follow-up, and molecular characterization of BC.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1062 A — Untargeted Metabolomics for the Identification of Common Pan-Cancer Metabolic Signatures across Lung, Colorectal, and Ovarian Cancers

PRESENTING AUTHOR: ***Efstathia Vouzaxaki, Uppsala University, Sweden***

CO-AUTHORS: *Tsiara Ioanna, Sjöblom Tobias, Globisch Daniel*

SUB-THEME: Cancer

Cancer remains the leading cause of mortality worldwide, with lung, colorectal, and ovarian malignancies posing significant clinical challenges. The discovery of cancer type-specific biomarkers could facilitate early detection and support personalized treatment strategies. While these cancers arise from distinct genetic drivers, they often converge on shared metabolic pathways to sustain oncogenesis. Identifying metabolites that are consistently dysregulated across multiple cancer types could lead to the development of universal diagnostic markers. Harnessing the complexity of these metabolomic datasets requires robust biostatistical and computational tools to extract biologically meaningful insights.

An untargeted metabolomics approach was applied to analyze plasma samples from a large-scale cohort (N=710), comprising patients across four different clinical stages of lung, colorectal, and ovarian cancers, alongside non-cancerous controls. UHPLC-MS/MS analysis was utilized to capture a broad metabolic profile and to identify key metabolic alterations that represent candidate biomarkers for diverse clinically relevant comparisons. We also performed advanced biostatistical tools and a Receiver Operating Characteristic (ROC)-based biomarker discovery approach with values up to 0.95 to identify a “pan-cancer” metabolic fingerprint.

Furthermore, this study assessed the clinical relevance of specific metabolites identified as potential biomarkers, aiming to both validate these findings within these current patient cohorts and to contribute to a broader understanding of metabolism in cancer diagnosis. Some of the most promising metabolites our analysis revealed are 3-hydroxypyridine sulfate, S-propeny-L-cysteine, and lysoPC(20:3) as part of distinct early and late-stage signatures. Shared features across cancers suggest common metabolic stress and inflammation pathways, while cancer-specific metabolites reflect tissue-specific biological mechanisms and, in some cases, diet or microbiome-derived exposures. Our pan-cancer approach expands on the number of candidate blood-based cancer biomarkers and additionally demonstrates a deeper understanding of universal cancer metabolism, providing a biological framework for integrating metabolomics with microbiome and genomic data in future validation studies.

1064 A — Precision Metabolomics for Early Lung Cancer Detection: A Translational Validation Framework Across Diverse Populations

PRESENTING AUTHOR: ***Yamile Lopez Hernandez, University of Alberta, Canada***

CO-AUTHORS: *Lun Zhang, Jiamin Zheng, Mathew Jhonson, Rupasri Manual, Nancy Shirley Garcia, Dorsa Yahya Rayat, Abby Kropielnicki, Scott Mackay, Juan Jose Oropeza Valdez, William Lockwood, David S. Wishart*

SUB-THEME: Cancer

Background: Early detection of Non-Small Cell Lung Cancer remains a critical unmet need, as current screening approaches are limited by invasiveness, cost, and restricted accessibility. We aimed to develop and validate plasma metabolite biomarkers for early-stage lung cancer detection across diverse populations using quantitative metabolomics. Methods: Quantitative targeted plasma metabolomics was performed. The discovery study included 216 plasma samples from lung cancer patients and healthy controls. A second validation study analyzed 896 samples from a Quebec cohort, including 622 lung cancer patients and 274 controls with other lung diseases. A third validation study evaluated 410 individuals from a Chinese cohort, including healthy controls and early- and late-stage lung cancer cases. A fourth ongoing multicenter study is being conducted across Alberta and British Columbia, including 145 Stage I NSCLC patients and 300 individuals enrolled through a lung cancer detection program, incorporating decentralized sampling via novel plasma separator devices. Results: The discovery cohort identified five plasma metabolites distinguishing early-stage lung cancer from controls with AUROCs >0.90. In Quebec, a refined 10-metabolite panel achieved AUROCs >0.91 for Stage I–II detection. In the Chinese cohort, key metabolites including lactate, pyruvate, fumarate, tryptophan, and α-ketoglutarate showed strong diagnostic performance with AUROCs >0.92 and consistent cross-population reproducibility despite genetic and environmental differences. Preliminary data from Alberta and British Columbia confirm strong biomarker robustness in 145 Stage I NSCLC patients and 300 screened individuals. The ongoing fourth study integrates decentralized sampling through an affordable at-home plasma collection kit enabling mail-in lung cancer screening. This platform supports scalable population screening and provides a low-burden alternative to centralized, invasive diagnostic pathways currently used in clinical practice. Conclusions: These

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

findings establish a robust and reproducible plasma metabolomic signature for early NSCLC detection across diverse populations, supporting its translational potential within scalable screening frameworks integrating decentralized sampling.

1066 A Characterizing Tumor Heterogeneity in Chilean Gastric Cancer Cohort via Multiplatform Metabolomics

PRESENTING AUTHOR: ***Luis Bustamante, Universidad De Concepción, Chile***

CO-AUTHORS: *Maximiliano Ramm, David Martínez, Aracelly Quiroz, Carolina Delgado, Claudia Mardones, Mónica Cala, Luis Bustamante*

SUB-THEME: Cancer

Gastric cancer exhibits high incidence and mortality rates in Chile, primarily due to delayed diagnosis and insufficient patient stratification. Untargeted metabolomics provides a robust approach for elucidating alterations in metabolites and lipids associated with gastric cancer. The primary objective of this study is to comprehensively investigate metabolomic and lipidomic changes in cancer tissue and relate these findings to clinical and histological parameters.

Fresh gastric biopsy samples were collected from patients undergoing gastrectomy, following informed consent approved by a Certified Research Ethics Committee (IORG0007794). Cancerous (CT) and non-cancerous tissue (nCT) from the same gastrectomy were analyzed using three analytical platforms: GC-QTOF, HILIC-MS, and RPLC-MS.

Multi-factor analysis (MFA) of the data revealed separation between CT and nCT in dimension 2, and a scattered distribution of CT in dimension 1, indicating that metabolic profiles may reflect cancer heterogeneity. Integrating clinical and histological data with metabolomics enabled the annotation of potential biomarkers associated with invasion grade (TG and HexCer) and differentiation grade (sugars and glucose phosphate).

Unsupervised k-means clustering (k=2) in the principal component analysis (PCA) space of CT samples enabled stratification of the cohort into two metabotypes. Cluster 1 was defined by significant accumulation of amino acids, biosynthetic precursors, phospholipids (PC, PE, PI, PE-O), sphingolipids (Cer and HexCer), and cholesteryl esters. In contrast, Cluster 2 displayed a metabolic profile indicative of fermentative and degradative metabolism, with elevated levels of lactic acid, pyruvic acid, glucose, and dicarboxylic acids such as adipic and aminoadipic acid, as well as a pronounced increase in glycerolipids (DG and TG).

The clinical significance of these clusters remains to be determined, as they may enrich molecular stratification or treatment response.

1067 B Multi-LC-MS Platform Plasma Lipidomic Profiling of BRCA1/2 Mutation Carriers and Breast Cancer Risk: A Retrospective Komen Tissue Bank Study

PRESENTING AUTHOR: ***Elisabeth Schwiebert, Georgia Institute of Technology, United States***

CO-AUTHORS: *Katherine J. Kenney, Rana German, Samuel G. Moore, Rahul R. Deshpande, Bashar Amer, David A. Gaul, Susan S. Bird, Jaeyeon Kim, Facundo M. Fernández*

SUB-THEME: Cancer

Breast cancer (BC) remains the most common cancer among women worldwide with survival strongly influenced by stage at diagnosis and genetic risk. Individuals carrying germline BRCA1/2 mutations face significantly elevated lifetime BC risk and often undergo intensified monitoring or prophylactic interventions. While clinically effective, these strategies highlight gaps in understanding early biological mechanisms underlying BRCA1/2-associated cancer risk. Emerging evidence implicates dysregulation of steroid and lipid metabolism in early tumorigenesis with circulating lipid alterations detectable prior to diagnosis. We hypothesized that progesterone-related steroid and lipid dysregulation produces distinct metabolomic signatures preceding BC development.

Plasma samples (n = 210) and metadata were obtained from the Komen Tissue Bank under IRB-approved protocols. The cohort included non-diseased women at sample collection, BRCA1/2-mutation carriers, individuals who later developed breast or ovarian cancer, and matched controls (age, BMI, menopausal status). Lipids were extracted using 2-isopropanol containing isotopically labeled standards and analyzed by non-targeted RP-UHPLC-MS on an ExplorisTM 240 and a modified Orbitrap tribrid mass spectrometer, enabling complementary semi-quantitative profiling and enhanced structural elucidation. Lipid annotations were assigned using HCD data from the ExplorisTM platform, with additional CID, MSn, and UVPD fragmentation on the tribrid instrument, refining sn-1/sn-2 fatty acyl positions. Spectra were processed in Compound Discoverer v3.5 with LipidSearch, mzCloud, and mzVault libraries.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Across 543 and 837 high-confidence lipid annotations from the two platforms, multiple lipid subclasses—including ceramides, hexosylceramides, lysophosphatidylcholines, lysophosphatidylethanolamines, and sphingomyelins—trended toward eventual BC development in BRCA1/2-mutation carriers and non-carriers. Pathway analysis when comparing BRCA1/2-mutation carriers and controls and women who later developed BC versus controls revealed enrichment of glycerophospholipid metabolism ($p = 0.0154$, $p = 0.0093$ respectively) and sphingolipid metabolism ($p = 0.0502$, $p = 0.0192$ respectively). These findings highlight the utility of multi-platform lipidomics and advanced fragmentation strategies for resolving pathway-level lipid alterations preceding clinical BC diagnosis.

1068 A A Decision-support Scheme for Clinical Implementation of Lipidomic Classifiers to Guide Cancer Care

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

CO-AUTHORS: *Dara Vlaminck, Alexa Fiorante, Michael Woolman, Francis Talbot, David Munoz, Farshad Nassiri, Gelareh Zadeh, Sunit Das, Howard Ginsberg*

SUB-THEME: Cancer

Several 'ambient' mass spectrometry methods leverage alterations in tissue lipidome – correlated to cancer states – to rapidly diagnose cancer types. However, as the complexity of lipidomic classifiers and the number of cancer classes in differential diagnosis grows, novel approaches in decision science are required to reduce the clinically burdensome misclassifications. Multiclass predictions on large, often imbalanced datasets are facilitated by hierarchical "decision-trees" that reduce the number of classes that must be simultaneously compared, further allow customization of machine learning approaches at each 'tree' node. A 'data-driven' decision-tree - based on unsupervised spectral similarities- logically regress the decision confidence across tree branches and enables the users to scale back the depth of diagnosis to 'higher' nodes to match a desired level of confidence in prediction threshold. This offers several advantages. First, the simultaneous display of classification results and associated probabilities allows clinicians to implicitly incorporate a 'cost function' based on expected clinical burden of misclassification. Second, assessing the "flatness" of classifier model's 'learning curve' at each node provides yet another confidence metric to be used in decision-support. This 'adaptive' decision-making approach addresses limitations of low confidence in weak predictions and/or classifier models, providing a better assessment of the pathology classification depth warranted for robust patient care. Using a dataset of 10,225 mass spectra from 1,052 central nervous system (CNS) cancer patients collected with ambient picosecond infrared laser mass spectrometry, we propose lipidomic classifier models (across fatty acid and phospholipid signals) layered onto an adaptive decision-tree alongside a software for the display of confidence-based decision paths for differential diagnosis. Integrating this platform with machine learning approaches has resulted in > 90% sensitivity and specificity for the detection of 24 prognostically important CNS cancer types. This enables integrated molecular and morphometric diagnosis using a single MS instrumental based on analyzing tissue lipids in

1069 B Combining metabolomics with cellular imaging to explore iron metabolism and ferroptosis in cancer cells

PRESENTING AUTHOR: ***James McCullagh, University Of Oxford, United Kingdom***

CO-AUTHORS: *Ziming Wang, Oliver Cashmore Antia Valle Tojeiro, Xinming Ni, Paige Norton-Edwards, Yiquan Chen, Rachel Williams, Imogen Thomas, Ingvild Hvinden, John Sidda, John Walsby-Tickle, Sidda Chiara Bardella, Tom Cadoux-Hudson, Christopher Schofield & James McCullagh*

SUB-THEME: Cancer

The oncometabolite 2-hydroxyglutarate (2-HG) accumulates in cancer cells expressing mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2) where it inhibits enzymes regulating transcription and metabolism, however, its precise role in tumorigenesis remains unclear. Resistance to clinically-approved mutant IDH inhibitors has been reported, revealing a need for new therapeutic approaches. The aim of this study was to combine advances in metabolomics methods, and high-resolution cellular imaging, to explore the susceptibility of mutant IDH1/2 cells to ferroptosis and its impact on the metabolome.

We used isogenic LN18 glioblastoma cells and compared IDH1/2 mutant/wild-type strains with/without induction of ferroptosis using a range of pharmacological approaches. We performed untargeted metabolomics, including anion-exchange chromatography-coupled to high-resolution orbitrap MS (AEC-MS/MS) to focus specifically on primary metabolic pathways (including redox metabolism, energy transduction and amino acid metabolism). Intracellular fluorescence imaging was also performed to reveal the spatial distribution of selected proteins and metabolites.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Our results revealed profound changes in 2-HG abundance and associated changes in redox metabolism, including intriguing links to iron metabolism in mutant IDH1 cancer cells. When we induced ferroptosis (by inhibiting glutathione production), there was a significant increase in the susceptibility to cell death in mutant IDH1 cells (compared to isogenic wild-type IDH cells) and AEC-MS/MS analysis revealed associated metabolic changes. These results were overlaid with fluorescence imaging showing the distribution of Fe2+ within IDH mutant cells.

Our study reveals metabolic changes in mutant IDH1 cancer cells that extend well beyond the production of 2-hydroxyglutate and AEC-MS/MS metabolomics results shows altered iron-metabolism and antioxidant pathways. This study provides evidence to support ferroptosis as a novel therapeutic target in mutant IDH1 cancer cells.

1070 A From GlycA to Glycan Structure: Integrated NMR and LC-MS Glycomics Improve Cancer Detection in Patients with Non-Specific Symptoms

PRESENTING AUTHOR: ***Daniel Anthony, University Of Oxford, United Kingdom***

CO-AUTHORS: *Tereza Kacerova, Abi G. Yates, James R. Larkin, Boris Shulgin, Jack J. J. J. Miller, Philippa L. Harris Gleave, Sebastian de Jel, Jack Cheeseman, Georgia Elgood-Hunt, The SCAN consortium, Eric Schiffer, Daniel I. R. Spencer, Suzie Anthony, Daniel C. Anthony.*

SUB-THEME: Cancer

Early cancer diagnosis in patients presenting with non-specific symptoms remains challenging due to the limited discriminatory power of routine investigations. Within the Oxfordshire Suspected CANcer (SCAN) pathway, prior work demonstrated that serum 1H-NMR metabolomics can identify cancer with high accuracy. SCAN2 evaluated whether integrating NMR with high-resolution LC-MS glycomics improves discrimination in a clinically heterogeneous, multimorbid population.

Serum from 369 SCAN patients (59 cancers) was analysed using AXINON®-lipoFIT®-NMR metabolomics and HPLC–MS glycomics. The NMR analysis identified GlycA as a prominent cancer-associated signal; to resolve this composite inflammatory resonance, high-resolution LC–MS glycomics was performed to characterise the underlying glycan structures. LC-MS glycomics provided structural resolution of specific bi- and tri-antennary glycan species contributing to this inflammatory axis, enabling direct molecular deconvolution of the GlycA signal. Machine-learning random forest classifiers were trained using repeated cross-validation to predict cancer status, with performance assessed by ROC analysis of pooled out-of-fold predictions.

To account for competing diagnoses within this multimorbid pathway, a second classifier modelling alternative non-cancer conditions was developed. Rather than relying on a single cancer probability score, mean predicted probabilities from both models were jointly projected into a two-dimensional probability space. This dual-probability framework preserved strict separation of training and test data and enabled contextualisation of cancer risk relative to comorbidity burden within a shared diagnostic landscape.

Integration of glycomics with metabolomics improved discrimination, achieving an AUC of 0.88 in a refined cohort excluding dominant comorbidities. Cancer-associated glycans, including FA2G2S1, FA2BG1, and M5A1G1S1, differentiated cases and provided structural context to the GlycA-driven inflammatory signature. A metastatic-disease classifier achieved an AUC of 0.80, and projection-based classification reached 89.8% accuracy.

These findings validate the SCAN1 metabolomic signature in a more clinically complex cohort and demonstrate that resolving the composite GlycA signal into LC–MS-defined glycan structures enhances cancer detection within multimorbid diagnostic pathways.

1072 A Metabolomics highlights linkage between cardiovascular risk factors, sphingolipid and one-carbon metabolism in clinically healthy adults

PRESENTING AUTHOR: ***Mengjiang Huang, UC Davis, United States***

CO-AUTHORS: *Debra K M Tacad, Tong Shen, Kevin Hyunh, Nancy L. Keim, Peter J. Meikle, Brian J. Bennett, John W. Newman*

SUB-THEME: Cardiovascular diseases

Background: Cardiovascular disease (CVD) risk persists despite therapeutic cholesterol reduction, suggesting parallel metabolomic shifts during early atherogenesis. Understanding metabolome-wide associations during atherogenic initiation will improve our understanding of these events. Objectives: Investigate metabolomic-lipidomic signatures of LDLc variation in a population without diagnosed CVD. Methods: Plasma metabolomics obtained from a clinically healthy cross-sectional

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

cohort (n =362; Clinical Trials.gov ID: NCT02367287) using Biocrates MxP Quant 500 kits were complemented with expanded lipidomic coverage for a sex/age-matched subset with LDLc difference >60 mg/dL (n =38). Cohort partial least squares regressions against LDLc levels and high vs low LDLc group partial least squares discriminate analyses were performed. Metabolomic-lipidomic findings were mapped onto metabolic pathways. Results: Plasma lipids showed the largest LDLc-associated changes. Sphingolipids and cholesteryl esters were higher, and multiple ether-linked phosphatidylcholines were lower as LDLc levels increased. While subtle, higher alanine levels and lower serine and glycine levels were seen as LDLc levels increased. High-resolution lipidomics further highlighted elevations in triglycerides and deoxyceramides with elevated LDLc. When adjusted for alanine concentrations, deoxyceramide concentrations were higher as serine levels declined (p =0.0001), and the deoxyceramide:ceramide ratios were higher in the high LDLc group (p =0.038). Lower serine levels were also accompanied by parallel changes in glycine (p=0.0004), and anti-parallel changes in transsulfuration pathway metabolites particularly when adjusted for ceramide levels. Notably, the alanine:serine ratio was more strongly associated with fasting triglycerides (r = 0.38; p <0.0001) than LDLc[MH1] . Conclusions: An increased metabolic demand for serine to support sphingolipid synthesis, and possibly the transsulfuration pathway, is associated with elevations in cytotoxic deoxyceramides and reductions in glycine availability for one-carbon metabolism. These changes were strongly associated with fasting triglycerides, a key marker of residual CVD risk.

1073 B Preclinical Assessment of the Bioavailability of Avocado Fatty Alcohol Esters: Impact on biochemical markers, targeted and un-targeted lipidomic profiles, and gut microbiome.

PRESENTING AUTHOR: ***Francisco Velazquez, Instituto Tecnológico y de Estudios Superiores de Monterrey, Mexico***

CO-AUTHORS: *Perla Ramos-Parra, Shirley Mora-Godínez, Jorge Fragoso-Medina, Dariana Rodríguez-Sánchez, Noemí García-Ramírez, Gerardo García-Rivas, and Carmen Hernández-Brenes*

SUB-THEME: Cardiovascular diseases

Avocado Fatty Alcohols (AFAs) are lipophilic molecules predominantly found in avocado pulp and seed, with health-promoting and antimicrobial properties. However, their bioavailability and metabolic transformations remain poorly understood.

This study evaluated the in vivo bioavailability of AFAs and their impact on lipid metabolism, biochemical variables, and the gut microbiome using targeted AFA quantification and untargeted lipidomics in a preclinical model. Specifically, we assessed blood and tissue quantification of AFAs, differential lipidomics, and microbiome changes following daily AFA ester supplementation for four weeks.

Male C57BL/6 mice received a 30-day supplementation with a purified avocado extract added to drinking water at three doses (15, 25, and 150 mg AFAs/kg body weight; n = 4 per group), and a vehicle control. Tissue samples were analyzed using LC-MS/MS-for targeted AFAs quantification and LC-QTOF-MS/MS for untargeted lipidomics. Physiological and histological variables, plasma or tissue biochemical markers, and fecal microbiota (16S rRNA gene sequencing) were assessed.

Non-significant changes were observed in body weight, metabolic markers, or inflammatory parameters across treatments.

Targeted lipidomics confirmed hepatic bioavailability of AFAs following supplementation. Plasma AFAs levels varied by dose, with higher concentrations in the low- and medium-dose groups. AcO-avocadene was the predominant species, while avocadenin and avocadiene B were detected at lower concentrations.

Untargeted lipidomics revealed treatment-dependent lipid differences. In heart tissue, 439 lipid features (100 annotated) differed significantly across groups (Kruskal–Wallis, $p < 0.05$), while blood samples showed 59 significant features, including 11 annotated lipid species.

Preliminary microbiota analysis indicated that the high-dose intervention altered the relative abundance of Bacteroidota and shifted the Firmicutes/Bacteroidetes ratio, affecting microbial families associated with metabolic health, including *Lachnospiraceae* and *Bacteroidaceae*.

Overall, these findings demonstrate that avocado-derived AFAs are bioavailable *in vivo* and modulate hepatic lipid profiles and gut microbiota without metabolic dysfunction, supporting their potential as functional lipid components.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1074 A Integrative Metabolomic and Microbiome Profiling Identifies Candidate Biomarkers and Pathological Mechanisms Associated with Coronary Artery Disease

PRESENTING AUTHOR: ***Carmen Ludwig-Papst, biocrates life sciences gmbh, Austria***

CO-AUTHORS: *Enrique Ozcariz, Gabriel Vignolle, Iqra Yousaf, Maximilian Tscharre, Franz Xaver Roithinger, Guido Dallmann, Carmen Ludwig-Papst, Alice Limonciel, Christa Nöhammer, Klemens Vierlinger*

SUB-THEME: Cardiovascular diseases

Coronary artery disease (CAD) is a leading cause of mortality worldwide. CAD is caused by the formation of atherosclerotic plaque in the lumen of coronary arteries. Stenosis refers to a significant narrowing of the arterial lumen by advanced atherosclerotic plaque formation, resulting in impaired blood flow and reduced oxygen supply to the myocardium. Sclerosis refers to atherosclerotic changes and plaque deposits within the vessel wall, leading to thickening and hardening of the arteries, but not necessarily causing a flow-limiting narrowing. Stenosis and sclerosis are often mixed up in diagnosis, particularly at early stages. Thus, identifying distinct biomarkers associated with the development of coronary stenosis and sclerosis is crucial for guiding precise and early therapeutic strategies. A total of 48 plasma samples from patients with stenosis (N = 23), sclerosis (N=12) and disease controls (N=13) were analyzed. Metabolites and lipids were targeted and quantified using the Quant 1000 kit from biocrates. Next generation sequencing was used to generate microbiome data by mapping microbial reads using an in-house built pipeline to obtain microbe abundances. Several models were applied to integrate the two omics and explain group variance: Multi-Omics Factor Analysis (MOFA) infers latent factors that explain variance, Group Aggregation via UMAP Data Integration (GAUDI) identifies clusters through non-linear dimensionality reduction, canonical correlation analysis (CCA) maximizes correlation between the omics and was used to evaluate the associations between single metabolites and microbiome strains. Our analysis found three multi-omics clusters: one dominated by stenosis, one dominated by disease controls and one including patients from all the groups. The multi-omic interaction network showed two modules: one made of triglycerides (TG) with associations to Capnocytophaga and Cryptococcus, and another associating phospholipids with Corynebacterium, Citrobacter and Clostridium, among others. Combining analyses with different integration strategies, distinct profiles for both microbiome and metabolome were identified for all groups.

1075 B Humic acid supplementation promotes tissue-specific metabolomic alterations in mice

PRESENTING AUTHOR: ***Alan Machado, Universidade do Estado de Minas Gerais, Brazil***

CO-AUTHORS: *Eric Francelino Andrade, Maria Luiza Nonato Salvador, Lúcia Pinheiro Santos Pimenta, Elizabeth Luciana Marinho Miguel, Thays Cristina dos Santos, Luciano José Pereira, Leonardo Barros Dobbss*

SUB-THEME: Cellular metabolism

Periodontal disease and estrogen deficiency are associated with increased oxidative stress, systemic inflammation, and metabolic alterations. In this context, humic acids (HA), obtained from biomass, exhibit antioxidant and immunomodulatory properties capable of reducing oxidative damage and attenuating bone loss in experimental models. Previous studies from our group demonstrated that HA treatment reduced alveolar bone loss, modulated inflammatory cytokines, and improved systemic and tissue redox parameters. However, the metabolic alterations associated with these effects remain poorly understood. Therefore, this study aimed to evaluate, by 1H NMR, the metabolome of liver, kidney, and gastrocnemius muscle from mice treated with HA. After approval by the ethics committee (CEUA/UFLA—protocol 3643011123), twelve female C57BL/6 mice were distributed into groups treated with saline solution or HA (80 mg/kg), administered daily by gavage for 28 days. After euthanasia, liver, kidneys, and gastrocnemius muscle were collected, frozen in liquid nitrogen, stored at −80 °C, and lyophilized. Metabolites were extracted using phosphate buffer in D2O containing TSP-d4 and methanol-d4. 1H NMR spectra were acquired using a Bruker Avance Neo 600 MHz spectrometer and converted into data matrices using 0.04 ppm bins. Multivariate analyses were performed by PCA and sPLS-DA using the MetaboAnalyst platform. PCA revealed clear tissue separation, with PC1 and PC2 explaining 45.5% and 21.6% of the total variance, respectively, although no clear separation between treatments was observed. Individual sPLS-DA analyses demonstrated a tendency for discrimination, especially when comparing kidney and gastrocnemius muscle metabolomes. The spectral regions contributing most to discrimination were mainly concentrated in the ranges of 0.7–1.5 ppm and 6.2–9.8 ppm. The results indicate that HA administration induces tissue-specific metabolomic modulation, particularly in kidney and gastrocnemius muscle. The authors thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, APQ-01460-25).

METABOLOMICS AND LIPIDOMICS IN HEALTH AND DISEASE

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1076 A Comparative lipidomic profiling of PEX3, PEX14 and PEX11β knockouts defines isoform-specific peroxisomal lipid fingerprints

PRESENTING AUTHOR: ***Mansha Soni, Max Planck Institute and Ruhr University Bochum, Germany***

CO-AUTHORS: *Mansha Soni, Samantha Jeng, Wolfgang Schliebs, Ralf Edmann, Frank Schulz*

SUB-THEME: Cellular metabolism

Peroxisomes are single-membrane-bound organelles of eukaryotic cells essential for various metabolic functions, primarily lipid metabolism and the detoxification of reactive oxygen species. Disrupted peroxisomal function results in peroxisomal biogenesis disorders such as Zellweger Spectrum Disorder (ZSD), highlighting the critical role of individual peroxins in maintaining metabolic homeostasis. Beyond rare genetic disorders, the role of peroxisomes is increasingly recognized in cancer research, where tumor cells exploit peroxisomal pathways to facilitate oncogenesis, yet the lipid metabolic consequences of specific peroxin dysfunction remain poorly defined.

To address this, we performed untargeted lipidomic analysis on peroxin knockout (PEX KO) T-REx293 cell lines, revealing distinct lipid fingerprints that diverge from wild-type in a peroxin-specific manner. PEX3 KO (biogenesis defect) and PEX14 KO (matrix import defect) cell lines are highly enriched in very-long-chain fatty acids (VLCFAs) and possess lower levels of ether- and plasmalogen-linked phospholipids. While they differ in the same lipid analytes, PEX3 KO cells represent more drastic shifts from the wild-type profile than PEX14 KO cells. In contrast, PEX11β KO (division defect) cells have a lipid profile that closely matches that of the wild-type, but have much higher levels of several lipid analytes, most notably several ceramide and triacylglycerol (TG) species. These unique metabolic adaptations are paralleled by the cell lines' divergent responses to ferroptosis induction by GPX4 inhibition, as well as glucose-6-phosphate dehydrogenase (G6PD) inhibition, suggesting peroxin-specific differences in redox stress and NADPH source reliance. These findings suggest that peroxisome-targeted therapies must be peroxin-specific, as biogenesis inhibition may inadvertently trigger protective adaptations that appropriately targeted approaches could avoid.

1077 B Metabolomics uncovers alterations in neurochemical and energy metabolism induced by DMT and 5-MeO-DMT in neuroblastoma cells

PRESENTING AUTHOR: ***Alessandra Sussulini, University of Campinas (Unicamp), Brazil***

CO-AUTHORS: *Uedson da Silva das Neves, Alexandre Leite Rodrigues de Oliveira, Alessandra Sussulini*

SUB-THEME: Cellular metabolism

Recently, psychoactive compounds such as N,N-dimethyltryptamine (DMT) and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) have attracted increasing scientific interest as potential therapeutic agents for neurodegenerative diseases due to their reported neuroprotective and neuroplasticity-inducing effects. Despite their structural similarity to serotonin, the molecular mechanisms underlying their distinct cellular responses remain poorly understood. In this context, this study aimed to investigate the metabolic alterations induced by these alkaloids in human neuroblastoma cells (SH-SY5Y). Cytotoxicity was evaluated using a resazurin assay after 24 h of exposure to DMT (0.39-50 µM) and 5-MeO-DMT (0.35-45 µM). As no significant effects on cell viability were observed, a concentration of 5 µM was selected for untargeted LC-MS metabolomics analysis. Cells were seeded at 14,545 cells/cm2 and divided into three groups (n = 6): control, DMT-treated, and 5-MeO-DMT-treated, with a 24 h exposure period. Following metabolic quenching and biphasic extraction using MeOH/MTBE, the polar fraction was analyzed by LC-MS in both positive and negative electrospray ionization modes. Data processing and annotation were performed using MS-DIAL and GNPS2. In positive mode, more than 2,500 features were detected, of which approximately 600 were annotated based on spectral databases. PLS-DA and Random Forest classification models demonstrated clear discrimination among experimental groups. Key discriminant features included the parent compounds and their metabolites, such as N-methyltryptamine and indole-3-glyoxylic acid, as well as metabolites associated with cellular energy metabolism and neurochemical pathways, including creatine, adenine, N-acetylarginine, and phosphocholine. Overall, these findings indicate neurochemical and metabolic reprogramming in response to DMT and 5-MeO-DMT exposure. Ongoing lipidomic analyses and studies in differentiated SH-SY5Y cells are expected to further elucidate these effects. Together, these results provide new insights into the metabolic mechanisms underlying the action of these alkaloids and support the therapeutic potential of these compounds in neurodegenerative disorders.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1078 A Metabolomics reveals the effects of neuronal differentiation and full-spectrum cannabis oil in neural tumor cell lines

PRESENTING AUTHOR: ***Gabriel Ravaglia, Universidade Estadual de Campinas, Brazil***

CO-AUTHORS: *Alessandra Sussulini, Murilo de Carvalho*

SUB-THEME: Cellular metabolism

Full-spectrum cannabis oil contains a complex mixture of phytocannabinoids, terpenes, and flavonoids that modulate neural cell metabolism through the endocannabinoid system. We performed an untargeted metabolomics study to characterize the compartment-specific metabolic responses of U87MG glioblastoma and SH-SY5Y neuroblastoma cells exposed to full-spectrum oil (2 µg/mL CBD equivalent). Intracellular and extracellular extracts were collected from treated and untreated controls. SH-SY5Y cells were assayed in both undifferentiated and retinoic-acid-differentiated states, with six biological replicates per group, while U87MG experiments included ten replicates per group. Data were acquired by UHPLC-HRMS using a HILIC column and data-dependent acquisition mode, processed with MZmine, and annotated through GNPS2 and SIRIUS platforms. Statistical selection combined Welch's t-test and Random Forest, and significant differential features identified in both analyses were further evaluated. Distinct metabolic patterns emerged between the cell lines. U87MG cells exhibited pronounced alterations in phosphatidylcholines, lysophosphatidylcholines, phosphatidylethanolamines, phosphosphingolipids, acylcarnitines, and purine nucleosides, suggesting mitochondrial stress, sphingolipid metabolism modulation, and membrane remodeling. In SH-SY5Y cells, enrichment of gamma-glutamyl peptides, N-acyl-alpha amino acids, and the endocannabinoid-like molecule palmitoylethanolamide was observed, consistent with modulation of glutamatergic pathways and alterations in neuronal differentiation-associated metabolism. Compartment-specific partitioning of lipids and amino acid derivatives was evident in both cell lines. These metabolomic signatures are consistent with previously described cannabinoid-associated mechanisms, including mitochondrial dysfunction, autophagic processes, and synaptic metabolic modulation. Overall, our results demonstrate that full-spectrum cannabis oil elicits cell-line- and compartment-specific metabolic responses, providing a multifaceted view of cannabinoid action in neural tumor cells.

1079 B Stable isotope tracer metabolomics reveals that Complex II assembly drives the metabolic adaptation to oxidative phosphorylation dysfunction.

PRESENTING AUTHOR: ***Sheik Nadeem Elahee Doomun, Metabolomics Australia, Australia***

CO-AUTHORS: *Kugapreethan Roopasingam, David de Souza, David A. Stroud*

SUB-THEME: Cellular metabolism

Mitochondrial respiratory chain Complex II is an inner membrane-bound enzyme that directly connects the TCA cycle with oxidative phosphorylation and the production of ATP. Complex II has been suggested to play a crucial role in regulation of cellular energy metabolism in scenarios such as cancer, diabetes, and inflammation due to its bidirectional conversion of succinate to fumarate at catalytic subunit A (also referred as Complex II-F site) and concomitantly reduces ubiquinone (CoQ10) into ubiquinol (CoQ10H2) at the transmembrane quinone reaction site formed by SDHC and SDHD. It was previously reported that mouse tissues exhibited net reversal of Complex II activity and used fumarate as terminal electron acceptor to protect the ubiquinone/ubiquinol pool redox state during Complex III or IV dysfunction. The data presented here built on this by revealing a cell type-specific mechanism triggered by alterations in the ubiquinone/ubiquinol redox state where the binding of fumarate to the Complex II F-site was blocked by excess succinate dehydrogenase assembly factor 2. The measurements of polar metabolites in HEK293T cells with impaired Complex III revealed that succinate accumulated in the knockout cell lines compared to the wild-type whereas lower levels of the most TCA metabolites were observed. Using 13C6-glucose tracer, a detailed analysis of the isotopologue distribution in key TCA metabolites revealed a decrease in isotopologues associated with operation of a canonical cyclic TCA in which carbon backbones cycle multiple times with a concomitant increase in the unlabelled isotopologue of all TCA intermediates. We also followed up a 13C5-glutamine tracer experiment which showed that elevated levels of succinate dehydrogenase assembly factor 2 inhibited both forward and reverse activity of Complex II.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1080 A Oxidative stress disrupts NAD^+ biosynthesis/breakdown balance and cellular bioenergetics

PRESENTING AUTHOR: ***Valentina Ferro, Vrije Universiteit Amsterdam, Netherlands***

CO-AUTHORS: *Nathalie Reuss, Brian van der Kieft, Victoria Pozo Garcia, Johan van Heerden, Sofia Moco*

SUB-THEME: Cellular metabolism

Oxidative stress plays a key role in disrupting cellular metabolism, associated with aging, neurodegeneration and metabolic disease. Nicotinamide adenine dinucleotide (NAD^+) and its reduced form (NADH) are essential coenzymes that maintain redox balance and support glycolysis, fatty acid oxidation, the tricarboxylic acid (TCA) cycle, and the electron transport chain (ETC). Most studies have focused on using precursors to boost NAD(H) synthesis, while the fate of NAD+ breakdown metabolites has been largely neglected. Some of these catabolites have been identified as potential biomarkers and uremic toxins in chronic kidney disease. Understanding how oxidative stress affects central metabolism and bioenergetics, beyond NAD+ biosynthesis, may reveal disease mechanisms and advance potential therapies.

The aim of this study is to investigate the metabolic effects on central and redox (NAD+ metabolome) metabolism, induced by oxidative stress (by administration of menadione) in a human cell system. To achieve this, a dedicated hydrophilic interaction chromatography (HILIC) coupled with high resolution multiple reaction monitoring (MRMHR) mass spectrometry was implemented, covering a total of 27 NAD+ metabolites and catabolites. Central metabolism was assessed using nuclear magnetic resonance (NMR) spectroscopy, including 20 amino acids, TCA cycle intermediates, and other metabolites.

Menadione generates reactive oxygen species (ROS) via redox cycling within the mitochondrial complex I. Notably, NAD(P)+ decreased, while nicotinamide (NAM) and methyl-NAM concentrations increased, highlighting the importance of monitoring the full spectrum of NAD+ metabolism. Most amino acids accumulated, under menadione treatment, suggesting protein degradation or reduced utilization due to impaired TCA cycle activity. Simultaneous changes in central carbon metabolites further indicated reduced TCA flux and overall mitochondrial dysfunction.

This metabolomics workflow provides insight into stress-induced metabolic dysregulation and supports the development of improved in vitro testing strategies. By identifying sensitive metabolic endpoints, it informs the development of potential translational therapies, for improved chemical safety assessment.

1081 B Association between body composition methods and lipid profile by ^{1}H Nuclear Magnetic Resonance spectroscopy in individuals with excess adiposity: A pilot study

PRESENTING AUTHOR: ***Luiz Rodrigo da Silva Rodrigues, Federal University of São Carlos, Brazil***

CO-AUTHORS: *Étore de Favari Signini, Gabriel Henrique Ribeiro, Ana Carolina Aparecida Marcondes Scalli, Jussara Pereira de Oliveira, Andréia Machado Santos, Letícia Figueiredo Scribone, Antônio Gilberto Ferreira, Aparecida Maria Catai*

SUB-THEME: Endocrine and metabolic diseases

Introduction: Body composition assessment allows the characterization of fat distribution patterns and is important in cardiometabolic risk evaluation. ^{1}H NMR spectroscopy enables the analysis of lipid profiles by lipoprotein subclasses. In this context, it becomes relevant to investigate whether different body composition measurements are related to lipid parameters obtained by ^{1}H NMR using the IVDr platform. Aim: To investigate the relationship between body composition assessment using two methods and lipid profiling by ^{1}H NMR. Methods: The sample consisted of 18 individuals (55.6% women), with a mean age 28±5 years and body fat percentage of 40.1±5.9%, with a clinical normal lipid profile. The protocol was performed over 3 days: Day 1: Samples were collected after 10 hours of fasting, stored at -80°C, and processed by ^{1}H NMR. Day 2: Dual-energy X-ray absorptiometry (DXA) after 4 hours of fasting; Day 3: Body mass index, waist circumference, hip circumference, waist-to-hip ratio, and waist-to-height ratio. The normality of the variables was assessed using the Shapiro-Wilk test. Variables with normal distribution were analyzed using Pearson's correlation, and those not normally distributed using Spearman's correlation. Due to the sample size, correlations with a value of $|r| \geq 0.700$ were highlighted and considering the Benjamini-Hochberg method (≤0.05). Results: Visceral adipose tissue in grams and android-gynoid index by DXA, hip circumference, and waist-to-hip ratio showed positive correlations with VLDL (1-3) and IDL (particle number and apolipoprotein-B). The android/gynoid index and waist-to-hip ratio had some correlations in common with VLDL-2 (free cholesterol) and VLDL-3 (triglycerides and phospholipids). Conclusions: Different body composition methods may correlate with different, and even the same, lipoprotein subclasses by ^{1}H NMR. The measurements that were correlated are related to central adiposity. Further studies are needed to understand the impact of these findings on cardiovascular risk.

This research was supported by FAPESP: 2016/22215-7; CNPq: 409799/2023-8; CAPES-001.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1082 A Implementation and Clinical Application of a High-Resolution Orbitrap LC-MS Lipidomics Workflow in Biliary Atresia Patients Before and After Kasai Portoenterostomy

PRESENTING AUTHOR: ***Laura Guerra Cal, Federal University of Rio de Janeiro, Brazil***

CO-AUTHORS: *Yasmin Pestana, Betina Carla Bertrand Simões, Ivonete Siviero, Marina Amaral Alves*

SUB-THEME: Endocrine and metabolic diseases

Lipidomics has emerged as a powerful analytical strategy for investigating metabolic dysregulation, disease-associated biomarkers, and molecular mechanisms underlying complex disorders. In hepatobiliary diseases, alterations in lipid metabolism are closely associated with cholestasis, inflammation, fibrosis, and liver dysfunction, highlighting the need for robust and reproducible analytical platforms capable of comprehensive lipid profiling. This study describes the implementation and clinical application of an untargeted high-resolution lipidomics workflow using liquid chromatography coupled to Orbitrap mass spectrometry (LC-MS Orbitrap).

The method optimization and analytical validation were performed using multiclass lipid standards, including phospholipids (PC, PE, PI), lysophospholipids (LPC), sphingolipids (Cer, SM), and neutral lipids (TG, DG, CE). Individual standard injections were used to evaluate chromatographic retention behavior, ionization efficiency, mass accuracy, and fragmentation profiles. Analyses were conducted under optimized LC-MS acquisition conditions, enabling high-resolution detection and reliable characterization of lipid species across multiple lipid subclasses.

Following analytical validation, the workflow was applied to a longitudinal clinical study involving pediatric patients diagnosed with Biliary Atresia undergoing Kasai portoenterostomy. Plasma samples were collected at four time points: preoperative baseline, 7 days after surgery, and 6 and 12 months postoperatively. Lipid extraction was performed using a standardized protocol followed by untargeted LC-MS Orbitrap analysis.

Preliminary results demonstrated robust chromatographic separation, high reproducibility, and broad lipidome coverage in biological samples. Distinct lipidomic signatures were observed between preoperative and postoperative samples, particularly involving phospholipid, sphingolipid, and neutral lipid metabolism, suggesting dynamic metabolic remodeling after surgical intervention. Longitudinal analyses collected at 6 and 12 months post-surgery further revealed persistent alterations in lipid homeostasis potentially associated with disease progression, biliary drainage recovery and hepatic adaptation.

This implemented workflow establishes a reliable platform for high-resolution clinical lipidomics and demonstrates its applicability for longitudinal metabolic monitoring in biliary atresia. These findings supporting the identification of biomarkers and pathophysiological mechanisms in pediatric cholestatic diseases.

1083 B Plant-Derived Metabolites as Modulators of Amylin Aggregation

PRESENTING AUTHOR: ***Giulia Bennici, King Abdullah University of Science and Technology, Saudi Arabia***

CO-AUTHORS: *Abdul-Hamid Emwas, Mariusz Jaremko*

SUB-THEME: Endocrine and metabolic diseases

Type 2 diabetes mellitus (T2DM) affects millions of people worldwide and remains a major global health challenge. One of the key pathological features of the disease is the aggregation of human islet amyloid polypeptide (hIAPP), also known as amylin, a 37-amino-acid peptide that forms amyloid fibrils in pancreatic islets. This aggregation process contributes to β-cell dysfunction, cellular toxicity, and disease progression. Despite growing interest in targeting protein misfolding and aggregation, effective modulators of hIAPP aggregation and their mechanisms of action remain insufficiently explored.

In this study, we explored medicinal plants as a source of potential modulators of hIAPP aggregation using an integrative strategy combining metabolomics, in vitro biophysical assays, and molecular modeling. Plant extracts were chemically characterized through NMR- and mass spectrometry–based metabolomic profiling, enabling the annotation of bioactive metabolites. Identified compounds were subsequently evaluated using molecular docking to investigate their potential interactions with the hIAPP monomer and to prioritize candidates for further investigation.

Plant extracts and selected metabolites were then tested for their effects on peptide aggregation. Aggregation kinetics and fibril formation were monitored using Thioflavin T fluorescence assays and microscopy techniques. In addition, the cytotoxicity associated with hIAPP aggregation and the potential protective effects of the tested samples were evaluated using MTT cell viability assays.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Our results demonstrate that plant extracts and selected metabolites significantly modulate hIAPP aggregation and reduce aggregation-associated cytotoxicity in vitro. These findings highlight the potential of plant-derived bioactive compounds as promising modulators of amyloid formation and provide valuable insights for the development of novel aggregation-targeted therapeutic strategies for T2DM.

1084 A Chemical Biology Meets Metabolomics: New Insights into Gut Microbiome Metabolism in Primary Sclerosing Cholangitis Disease

PRESENTING AUTHOR: ***Alejandro Torregrosa Chinillach, Uppsala University, Sweden***

CO-AUTHORS: *Peder R. Braadland, Johannes R. Hov, Daniel Globisch*

SUB-THEME: Endocrine and metabolic diseases

Primary Sclerosing Cholangitis (PSC) is a chronic liver disease characterized by inflammation of the bile ducts, leading to impaired bile flow to the gut, and ultimately biliary cirrhosis and liver failure. PSC is considered a rare dysfunction that affects mainly young male individuals in Northern Europe. PSC diagnosis is still challenging as many patients are asymptomatic, and its detection is mostly produced during the evaluation of Inflammatory Bowel Disease (IBD). An effective pharmacotherapy for this disease is still missing, leaving liver transplantation as the only temporary curative therapy with a high risk of relapse. This therapeutic gap highlights the need for reliable diagnostic and prognostic biomarkers for early detection.

The gut microbiome, defined as the community of microorganisms in our gastrointestinal tract, is unique for each individual and is influenced by genetics, lifestyle or diet. Microbiome dysbiosis has been linked to the development of several diseases. Investigation of the co-metabolism represents a huge potential for the discovery of biomarkers. Mass spectrometric metabolomics analysis is the method of choice for analyzing known metabolites. For identification of unknown metabolites, advanced Chemical Biology tools are required but still limited in metabolomics workflows.

Herein, we present a novel Chemical Biology-driven metabolomics workflow for the analysis of human plasma samples to compare the profiles between PSC and healthy controls, as well as PSC patients that underwent liver transplantation. We have applied our sulfatome and glucuronide methodology and we identified more than 140 metabolites from microbiome-host co-metabolism. Furthermore, we utilized our chemoselective probe technologies for carbonyl and carboxylic acid investigations. We have observed crucial metabolic alterations in PSC that are partly reversible by liver transplantation. Finally, depletion of some bacterial metabolite conjugates linked to PSC including indoxyl sulfate and β-hydroxybutyric acid were identified. Our results report on new PSC-relevant metabolites as part of the gut-liver axis.

1085 B STROBE-MetEpi: A Reporting Guideline for Observational Studies in Metabolomic Epidemiology

PRESENTING AUTHOR: ***Tim Ebbels, Imperial College London, United Kingdom***

CO-AUTHORS: *Rachel S Kelly, Stacey N Reinke, Matthias Egger, Royston Goodacre, Julian Little, Craig E. Wheelock, Jessica Lasky-Su, Timothy M D Ebbels*

SUB-THEME: Epidemiology

The rapid growth of metabolomic epidemiology has highlighted substantial variation and incompleteness in the reporting of studies, which has limited the capacity of the field to inform healthcare or public health research. Here, we introduce the STROBE Metabolomic Epidemiology (STROBE-MetEpi) Reporting Checklist, a structured reporting framework, extending the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidance to the metabolomics field, to improve reproducibility, facilitate critical appraisal, and support translation of findings.

The checklist was developed by a core team of 8 researchers and further appraised and tested by 24 reviewers from across the metabolomics and epidemiology fields. We adopted an iterative, evidence-informed development process combining literature reviews, expert input, and internal piloting. Our user-friendly checklist consists of 31 items and is organized into sections that mirror both the STROBE structure and the analytical workflow of a metabolomic epidemiology study.

To evaluate the relevance of our checklist, we assessed how closely current literature adhered to it and found that studies are performing particularly poorly in reporting key areas, including sample handling and pre-analytical processing, instrumentation and data acquisition, data processing, metabolite identification, data quality control / cleaning, and pathway analyses. The poor reporting of these aspects which are crucial for the appropriate contextualization and interpretation of metabolomic epidemiology literature highlights the need for the checklist.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

We believe the STROBE-MetEpi checklist will aid continued development of metabolomic epidemiology, facilitating interdisciplinary research by clarifying the necessary reporting components of metabolomic epidemiology studies. The checklist highlights methodological complexities and challenges specific to metabolomic aspects of epidemiology and provides a shared resource which will aid the community to address them.

1086 A Impact of alcohol, coffee and medications on the metabolome and lipidome

PRESENTING AUTHOR: ***Adetunji Toriola, Washington University School Of Medicine, United States***

CO-AUTHORS: *Saira Khan, Yueshui Lyu, Vaishnavi Mamillapalle, Myung Sik Jeon, Ghazaleh Pourali, Chongliang Luo, Jingqin Luo*

SUB-THEME: Epidemiology

Background: Alcohol, coffee intake as well as certain medications have been associated with various health outcomes, including cancer and cardiovascular diseases. However, few studies have comprehensively investigated within the same study population how these factors are associated with the metabolome and lipidome. We, therefore, leveraged untargeted metabolomics and lipidomics to determine the associations of alcohol and coffee intake, supplement use (calcium, vitamin D, multivitamins), medications (aspirin, acetaminophen, ibuprofen, statins) with metabolites and lipid species in women.

Methods: Our study population comprises 702 premenopausal women. Our final analyses included 857 metabolites and 828 lipid species, after excluding those with a high proportion of missing values. We used multivariable linear regression models, adjusted for confounders to evaluate the associations of these factors with metabolites and lipid species. We corrected for multiple testing separately using Benjamini–Hochberg false discovery rate (FDR adjusted p-value

Results: Alcohol intake was associated with the largest number of metabolites and lipid species. We observed significant associations for 123 metabolites and 89 lipid species across several pathways (ceramides, phospatidylcholines, triacylglycerols, and androgenic steroids). Some of the strongest associations were observed for Ethyl Glucuronide,CE(16:1), PC(16:0/16:1), TAG51:4-FA15:0, 5alpha-Androstan-3alpha,17beta-Diol Monosulfate (2). Coffee intake was associated with 46 metabolites, with the strongest associations observed for quinate, and 3-hydroxypyridine sulfate.Multivitamin use was associated with 18 metabolites, while statin use was associated with 35 lipid species and 4 metabolites. We observed no significant associations for calcium, vitamin D, aspirin, acetaminophen, and ibuprofen after correcting for multiple testings.

Conclusions: Alcohol, coffee, multivitamins, and statins are associated with several metabolites and lipid species across different subpathways. Our findings offer valuable insights into biomarkers of exposure and how potential pathways through which these exposures can impact health outcomes.

1087 B The Metabolome/Exposome-wide Association Atlas of General Health in European Adolescents: lessons from the ATHLETE project

PRESENTING AUTHOR: ***Cristina Balcells Nadal, The Francis Crick Institute, United Kingdom***

CO-AUTHORS: *Panagiotis Vorkas, Alexandros Siskos, Estelle Renard-Dausset, Léa Maitre, Lesley Hoyles, Martine Vrijheid, Hector C Keun*

SUB-THEME: Exposome research

Two decades ago, the HELIX consortium started collecting extensive environmental, molecular and health data across six European birth cohorts to investigate how pregnancy and early-life exposures shape childhood health trajectories. We now report findings from the ATHLETE project, which followed up 780 of these HELIX participants during the critical developmental stage that constitutes adolescence.

In ATHLETE, we comprehensively characterised the serum and urine metabolome and chemical exposome of the participants, measured through liquid chromatography (LC) sequential window acquisition high-resolution SWATH-MS (Sequential Window Acquisition of all Theoretical Mass Spectra). This approach enabled large-scale untargeted profiling of endogenous metabolites, dietary-derived compounds, microbial metabolites, and environmental chemicals within the same analytical framework. Furthermore, we have cross-sectionally associated this comprehensive data resource with general health outcomes, including mental health and wellbeing, cognitive performance, as well as respiratory and cardiovascular health outcomes. Additionally, we have investigated the links between the gut microbiome and gut microbial metabolites, food-derived exposures, and the endogenous host metabolome to delineate diet-microbiome-host interaction signatures during the onset of adolescence.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

The result of this work, a curated Metabolome/Exposome-wide Association Atlas, provides an integrated snapshot of metabolic and environmental determinants of adolescent health. This resource constitutes a powerful tool to advance the understanding of biological pathways and uncover candidate metabolic biomarkers with predictive value in general adolescent health and early disease risk stratification.

1088 A High Sensitivity CCS-Enabled Screening of Pharmaceuticals and Microbial Metabolites in Complex Matrices

PRESENTING AUTHOR: ***Erica Forsberg, Bruker, United States***

CO-AUTHORS: *Beixi Wang, Haoqi Nina Zhao, Birgitta Ryback, Victoria Deleray, Liling Fang*

SUB-THEME: Exposome research

Exposomics research requires high analytical sensitivity and selectivity to detect xenobiotics present at trace levels in complex biological and environmental samples. Many compounds occur at concentrations too low to consistently trigger MS/MS, which limits confident annotation. Ion mobility derived collision cross section values provide an important orthogonal dimension for identification. In this study, trapped ion mobility spectrometry was applied to improve the detection and annotation of pharmaceuticals and microbial metabolites across diverse matrices.

Four sample types were examined including SPE-extracted wastewater, marine water, human plasma and human feces. Analyses used a reversed phase 100% aqueous compatible C18 column with a 19-minute gradient coupled to a timsMetabo. Sodium formate and Agilent ESI-L TuneMix provided mass and mobility calibration at the start of each run. Data was processed in MetaboScape 2026 with compound annotation using GNPS ReFrame Drug CCS and GNPS Ion Mobility libraries along with additional metabolite and natural product libraries.

Across wastewater sample extracts, ~10000 features were detected, with MSMS spectra acquired for >60% of the features. A total of 32 pharmaceuticals were annotated along with >2000 additional unique matches. Major classes included cardiovascular drugs, NSAIDs, antibiotics, stimulants, illicit drug markers, industrial chemicals and endogenous metabolites. Annotations met thresholds of <5 ppm mass accuracy and <3% CCS deviation.

In fecal extracts, spiked antiretroviral drugs spanning 229 to 775 Da were correctly identified. More than 1000 additional compounds were annotated, including amino acids, nucleosides, peptides, sphingolipids, microbial short chain fatty acids, xenobiotics and dietary phenolics.

Marine water extracts yielded ~6800 features with >600 library annotations. Detected compounds included coastal contaminants such as organophosphate esters, plasticizers, surfactants and siloxanes along with microbial osmolytes including betaine, choline, amino acids and fatty acids. These results demonstrate the utility of CCS-supported ion mobility workflows for confident detection of trace-level analytes in environmental and biological samples.

1089 B Taurine as a Modulator of Macrophage Metabolism: Insights from non-targeted GC-MS Profiling

PRESENTING AUTHOR: ***Benedikt Beine, TU Braunschweig, Germany***

CO-AUTHORS: *Hiller, Karsten*

SUB-THEME: Immunometabolism

Taurine is a sulfur-containing β-amino acid primarily derived from cysteine. Unlike canonical amino acids, taurine is not incorporated into proteins, yet it represents one of the most abundant free amino acids in mammalian tissues. Intracellular taurine concentrations reach the millimolar range, particularly in tissues with high metabolic and oxidative demand such as the heart, retina, and immune cells.

Functionally, taurine contributes to calcium homeostasis, osmoregulation, antioxidant defense, and modulation of inflammatory signaling. It has shown protective effects against oxidative and inflammatory stress in animal models and has demonstrated robust beneficial outcomes in clinical studies addressing metabolic syndrome. However, despite its physiological abundance and therapeutic potential, its direct impact on cellular metabolism remains largely unexplored.

Given the high intracellular taurine concentrations in immune cells and its established role in inflammatory regulation, we investigated its influence on macrophage metabolism. Using non-targeted GC-MS–based metabolomics, we observed pronounced alterations in the metabolic profile upon taurine supplementation. Principal component analysis revealed that taurine-induced metabolic changes exceeded those occurring during classical inflammation response, indicating a

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

substantial metabolic reprogramming effect. Our findings suggest that taurine may act as a potent modulator of macrophage metabolism, warranting deeper mechanistic investigations into its role in immunometabolic regulation.

1090 A Immune sensing of cancer status through MR1-small molecule antigen presentation

PRESENTING AUTHOR: ***Mitchell McInerney, Monash University, Australia***

CO-AUTHORS: *Wael Awad, Michael N. T. Souter, Yang Kang, Carl J. H. Wang, Kean Chan Yew Poa, Mohamed R. Abdelaal, Ngoc H. Le, Chloe M. Shepherd, Conor McNeice, Lucy J. Meehan, Adam G. Nelson, Jeremy M. Raynes, Jeffrey Y. W. Mak, James McCluskey, Zhenjun Chen, Ching-Seng Ang, David P. Fairlie, Jérôme Le Nours, Patricia T. Illing, Jamie Rossjohn, and Anthony W. Purcell*

SUB-THEME: Immunometabolism

T cells recognise and react to the presentation of foreign antigens displayed on the surface of all nucleated human cells. Peptide and lipid antigen presentation has dominated immunological research in this domain, but the lesser known MR1-mediated metabolite presentation system is emerging as an intriguing potential therapeutic target. MR1 was originally shown to present microbially derived vitamin B2 precursors to signal a localised infection to specialised T-cells, but evolutionary conservation of the MR1 gene indicates a broader purpose, prompting an expectation of many more ligands that mediate T-cell responses are waiting to be found. Using an unbiased mass spectrometry approach, here we identify pyridoxal (vitamin B6) as a naturally presented endogenous MR1 ligand, capable of activating the 7.G5 T-cell receptor, shown previously to recognise and kill cancer cells with high specificity. Subsequent metabolomic quantitation of pyridoxal and related vitamers in a panel of cancerous cells revealed vastly higher endogenous vitamin B6 levels compared to non-cancerous cell types, providing a potential mechanism for pyridoxal mediated cancer recognition by MR1-reactive T-cells.

1091 B 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*

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 pro-inflammatory M1 polarization in 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 the metabolic changes induced by butyrate treatment in both resting and activated macrophages, which might help uncover the molecular mechanisms underlying its anti-inflammatory effects.

Using three macrophage models, RAW 264.7 cells, THP-1-derived macrophages, and bone marrow-derived macrophages (BMDMs), we treated cells with varying concentrations of butyrate under both basal and LPS-stimulated conditions. Metabolites were extracted and analyzed 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 tricarboxylic acid (TCA) cycle intermediates and amino acid levels. Furthermore, butyrate partially reversed or prevented 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 can influence host immune responses and highlights the potential of SCFA-driven immunometabolic reprogramming in controlling inflammation.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1092 A Improved sensitivity in quantitative profiling of inflammatory and pro-resolving lipid mediators in tissue samples using nanoflow chromatography

PRESENTING AUTHOR: ***Susan Bird, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Rahul Ravi Deshpande1; Vasundhara Kain2; Ciara J. Sallowicz1; Thiago Mattos1; Susan S. Bird1; Ganesh Halade2*

SUB-THEME: Immunometabolism

Introduction: Lipid mediators—including eicosanoids and specialized pro-resolving mediators (SPMs) such as resolvins, maresins, protectins, and lipoxins—are critical biomarkers of inflammation and immune regulation. Their analysis in biological matrices is challenging due to low abundance, structural similarity, and chemical instability, requiring highly sensitive and selective workflows. Here, we evaluate nano-flow liquid chromatography (LC) with different mobile-phase additives to enhance sensitivity compared to conventional high-flow LC. Detection is performed using two high-sensitivity mass spectrometers in targeted and simultaneous quantitation and discovery (SQUAD) workflows. The optimized method is applied to heart and spleen tissue extracts from mice.

Methods: Lipid mediator standards were used for method optimization on the Thermo Scientific Vanquish Neo UHPLC with an OptiSpray PepMap column and OptiSpray ion source. Detection was performed using a Thermo Scientific Stellar mass spectrometer with a dual-pressure linear ion trap and the Orbitrap Astral mass spectrometer with a SQUAD workflow. Mobile-phase additives evaluated included formic acid, ammonium formate, and ammonium fluoride. High-flow separations (300 µL/min) were compared to nano-flow separations (500 nL/min).

Results: Using PRM in negative ion mode, 23 standards were monitored during optimization. Nano-flow LC increased sensitivity for all analytes, with improvements ranging from one to three orders of magnitude compared to high flow. Among additives, 1 mM ammonium fluoride provided up to 1–2 orders of magnitude greater sensitivity than formic acid. Limits of detection and quantitation were established from calibration curves. The final method quantified 84 lipid mediators using 12 internal standards across 32 heart and spleen extracts from healthy and obese mice. Robustness was confirmed through repeated QC injections. Astral SQUAD data were additionally explored using untargeted workflows to expand biological insight.

1093 B Multi-platform metabolomics reveals a distinct preserved biochemical signature in a unknown material found on an Egyptian foetal mummy

PRESENTING AUTHOR: ***Elisabete Pires, University of Oxford, United Kingdom***

CO-AUTHORS: *James Mcullagh, Jose Luis Capelo, Hugo Santos, Luciana Carvalho, Dana Brown, Samantha Harris*

SUB-THEME: Inborn errors of metabolism and neonatal screening

Mummified remains preserve complex mixtures of endogenous biomolecules, embalming substances, and exogenous inputs, making their interpretation challenging. Here, we applied a multi-platform metabolomics approach to characterise an unknown putty-like material (light pink coloured; *c.*1 cm in diameter) found between the wrapped foetus mummy and its cartonnage case and compared it samples from the wrappings and cartonnage.

Polar metabolites were analysed using complementary mass spectrometry-based platforms: anion chromatography–mass spectrometry (AEC–MS), reversed-phase C18 liquid chromatography-mass spectrometry (LC–MS), and an amino acid-targeted assay. Principal Component Analysis of all datasets separated samples into two groups: (1) the unknown material and (2) wrappings and cartonnage.

The unknown material was characterised by enrichment in glycerol, glucose 1-phosphate, and glycerol 3-phosphate, together with amino acids and related compounds including L-proline, β-alanine, seryltheronine, aspartylthreonine, glycylproline, γ-aminobutyric acid (GABA), and glutamic acid. In contrast, C18 profiling also detected flavonoid-, terpenoid-, and azo-like compound-features, consistent with contributions from embalming materials and/or environmental contamination. The selective enrichment of glycerol derivatives and amino acid-related metabolites in the unknown material supports an endogenous biochemical component within this sample.

These results demonstrate the value of multi-platform metabolomics for distinguishing biochemically discrete materials within complex archaeological contexts and for disentangling endogenous and exogenous molecular signals. The unknown material retains a distinct metabolite profile consistent with degraded biological remains, potentially preserved within an embalming matrix. This study highlights the potential of metabolomics to investigate biomolecular preservation and post-mortem molecular transformation in mummified remains.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1094 A Untargeted Metabolomics in Action: Translating Biochemical Insights into Clinical Relevance for Cystinosis

PRESENTING AUTHOR: ***Purva Kulkarni, Radboud University Medical Center, Netherlands***

CO-AUTHORS: *Annemarie de Vreugd, Udo Engelke, Lorena Stoica, Elena Levtchenko, Lambertus van den Heuvel, Koenraad R. P. Veys, Jonathan Martens, Mirian CH Janssen, Dirk J. Lefeber, Purva Kulkarni*

SUB-THEME: Inborn errors of metabolism and neonatal screening

Background: Untargeted metabolomics provides an unbiased, system-wide approach to uncover metabolic alterations, enabling biomarker discovery for diagnosis, disease severity, and pathophysiology. We applied this approach to cystinosis, a lysosomal storage disorder caused by CTNS mutations leading to intracellular cystine accumulation, primarily affecting the kidneys and eyes. Our goal was to identify clinical biomarkers predictive of disease progression and extra-renal complications, illustrating how untargeted metabolomics can connect molecular mechanisms with clinically relevant outcomes.

Methods:Plasma samples from cystinosis patients currently on therapy (n=21; 8 patients including multiple time points) and controls (n=20) were analyzed using untargeted ultra-high-performance liquid chromatography mass spectrometry. Data analysis included: (1) Targeted analysis focused on known cystinosis biomarkers, metabolites associated with the 57-kb CTNS deletion and known chronic kidney disease (CKD)-related metabolites. (2) Untargeted analyses comparing case versus control groups and stratified comparisons among deletion-positive and negative, and control samples. Statistical analyses included PLS-DA and correlation with plasma chitotriosidase activity and white blood cell cystine levels to identify clinically relevant metabolic signatures.

Results: Targeted analysis confirmed that patients with the 57-kb CTNS deletion show elevated sedoheptulose and erythritol, correlating with white blood cell cystine levels, while uric acid and serine associate with plasma chitotriosidase activity. CKD biomarkers (e.g., p-cresol sulfate and creatinine) were increased in advanced disease (stages 4–5). PLS-DA on the untargeted metabolomics data identified four novel sulfur-containing features unique to patients, potentially reflecting cysteamine therapy. Structural elucidation is ongoing via LC-MS/MS and infrared ion spectroscopy, followed by validation with chemical standards.

Conclusion: Our study demonstrates that untargeted metabolomics can uncover both known metabolites and novel metabolic alterations in cystinosis, linking specific biochemical changes to either genetic variants, disease severity or treatment status, all using a single assay. More broadly, these findings highlight the potential of untargeted metabolomics as a powerful clinical tool for rare diseases.

1095 B Non-invasive metabolomics reveals system-wide metabolic rewiring in Inborn Errors of Metabolism: insights from Glycogen Storage Diseases

PRESENTING AUTHOR: ***Yasmin Pestana, Federal University of Rio de Janeiro, Brazil***

CO-AUTHORS: *Yasmin Pestana; Bruno Andrade Paranhos; Fernanda Bertão Scalco; Hélio Fernandes Da Rocha; Rafael Garrett; Marina Amaral Alves*

SUB-THEME: Inborn errors of metabolism and neonatal screening

Glycogen Storage Disease Type Ia (GSDIa) and Type IIIa (GSDIIIa) are inherited metabolic disorders caused by distinct enzymatic defects in glycogen metabolism, glucose-6-phosphatase deficiency in GSD1a, restricted to liver and kidney, and debranching enzyme deficiency in GSD3a, with hepatic and muscular involvement resulting in systemic energetic imbalance. Although primarily associated with defects in carbohydrate metabolism, the broader metabolic consequences and host–microbiome interactions remain incompletely understood. Here, we applied untargeted LC-HRMS metabolomics to non-invasive fecal and urine samples from pediatric patients with GSD1a and GSD3a to investigate systemic metabolic alterations, accounting for disease-specific dietary treatments as potential confounders.

Distinct yet convergent metabolic signatures were identified in both disorders, involving lipid metabolism, bile acid homeostasis, mitochondrial function, oxidative stress and gut microbiota-derived metabolites. GSD1a was characterized by disrupted bile acid metabolism, altered acylcarnitine profiles and increased dicarboxylic acids, suggesting impaired mitochondrial β-oxidation with overflow to microsomal ω-oxidation as a compensatory pathway. . Changes in secondary bile acids and microbiota-derived metabolites further supported gut–liver axis dysregulation. In contrast, GSD3a exhibited metabolic adaptations consistent with enhanced lipid and amino acid utilization, oxidative stress and altered microbial aromatic amino acid metabolism, reflecting its broader hepatic and muscular energetic compromise. Comparative analysis

suggests stronger hepatic metabolic disruption in GSD1a, whereas GSD3a displayed broader systemic energetic remodeling involving liver and muscle metabolism.

Overall, our findings demonstrate that glycogen storage diseases promote extensive metabolic rewiring beyond glycogen metabolism alone. Integrated urinary and fecal metabolomics highlights features consistent with mitochondrial dysfunction and gut–microbiome interactions as central components of disease pathophysiology. In GSD1a, bile acids, acylcarnitines, and dicarboxylic acids emerge as candidate non-invasive biomarkers and potential therapeutic targets; in GSD3a, aromatic amino acid-derived metabolites and markers of oxidative stress represent additional disease-specific readouts warranting further validation.

1096 A Leveraging direct infusion high-resolution mass spectrometry (DI-HRMS) based untargeted metabolomics for biomarker discovery in HMG-CoA synthase deficiency

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

CO-AUTHORS: *Marne Hagemeijer, Susana Gorden, Klaas Koop, Mark Wijnen, Anne Luesink, Judith Jans, Monique de Sain van der Velden*

SUB-THEME: Inborn errors of metabolism and neonatal screening

Background: Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 deficiency (HMGCS2D) is a rare inborn error of ketogenesis caused by pathogenic variants in the *HMGCS2* gene. Patients typically present in infancy during acute illness or prolonged fasting with hypoglycemia, hepatomegaly, metabolic acidosis, and hyperammonemia. However, biochemical findings during acute episodes are non-specific, variable and may normalize during stable periods. Therefore, diagnosis is frequently delayed, with genetic testing pursued only after recurrent metabolic decompensation. As timely diagnosis during acute illness is crucial to initiate appropriate treatment and prevent severe complications, there is an unmet clinical need to improve diagnostics. To address this gap, we performed proof-of-concept untargeted plasma metabolomics to identify HMGCS2D-specific metabolic signatures that may enable earlier diagnosis.

Methods: Plasma samples from HMGCS2D patients (n=2) collected during metabolic crisis (n=3) and stable periods (n=3) were analyzed using non-quantitative direct-infusion high-resolution mass spectrometry (DI-HRMS)-based untargeted metabolomics, as previously described (PMID: 30641898; PMID: 38028537). An R-based peak-calling and annotating pipeline generated Z-scores for over 14,000 metabolite annotations. Z-scores were calculated relative to a healthy reference cohort (n=70) and Z-scores >2.0 or <−1.5 were considered significantly dysregulated. Findings were validated by targeted analysis of acyl-carnitines and amino acids in a subset of samples.

Results: Crisis samples showed extensive metabolic dysregulation, with 95 consistently altered features predominantly involving pathway related to fatty acid synthesis, amino acid metabolism and arachidonic acid metabolism. In contrast, samples obtained during stable periods exhibited fewer metabolic alterations. Notably, arachidonic acid metabolism was disrupted across all samples, irrespective of clinical status, which was not previously reported.

Conclusion: HMGS2D is associated with a distinct metabolic signature, characterized by crisis-associated disruptions in fatty acid metabolism, energy metabolism, and arachidonic acid metabolism. Several candidate biomarkers were identified that may improve diagnostic accuracy, particularly during acute metabolic episodes. Further validation studies are warranted to assess their clinical utility.

1097 B Mass spectrometry-based Lipidomics reveals lipidome shifts Associated with Enterovirus Persistency in an In Vitro Model

PRESENTING AUTHOR: ***Lackson Kashobwe, Vrije Universiteit Amsterdam, Netherlands***

CO-AUTHORS: *Amirbabak Sioofy-khojine, Kirsi-Maarit Lehto*

SUB-THEME: Infectious diseases

Increasing evidence indicates that lipid metabolism plays a critical role in β-cell survival, insulin secretion, immune signaling, and inflammatory stress. Perturbations in lipid pathways have been associated with β-cell dysfunction and may contribute to the development of type 1 diabetes (T1D). Members of Coxsackievirus B (CVB), implicated as environmental triggers of T1D, can establish persistent infections, alter lipid homeostasis, and remodel host lipid membranes to support viral replication.

To investigate virus-induced metabolic reprogramming under chronic stress, we established long-term persistently infected human pancreatic ductal adenocarcinoma cell line (PANC-1) and confirmed stable, non-lytic viral persistence across consecutive passages (once per week) for up to 55 passages. Cells were cultured under sustained chronic inflammatory

stress to model prolonged metabolic pressure. Comprehensive lipid profiling was performed using LC-MS–based lipidomics, enabling quantification of glycerophospholipids, sphingolipids, and neutral lipids, which are crucial components of cellular membranes and metabolic pathways affected by viral infection.

Across thirteen models, seven exhibited increased accumulation of cholesteryl esters, triglycerides, and specific phosphatidylcholine and phosphatidylethanolamine species, consistent with enhanced neutral lipid storage and membrane remodeling. In contrast, six models showed increased lipid degradation, suggesting a shift toward lipid catabolism.

Together, these findings indicate that persistent CVB infection under chronic inflammatory stress drives distinct lipid remodeling states that may disrupt membrane homeostasis and cellular stress pathways. This work provides mechanistic insight into virus-associated metabolic reprogramming and highlights lipid dysregulation as a potential contributor to pathways linked to T1D pathogenesis.

1098 A Untargeted LC-HRMS/MS metabolomics identifies polyamine-driven metabotypes in longitudinal clinical isolates of Pseudomonas aeruginosa

PRESENTING AUTHOR: ***Audrey Le Gouellec, University Grenoble Alpes, France***

CO-AUTHORS: *Victor Mora, Razak Tidjani, Oriane Moyne, Florence Castelli, Benoit Cournoyer, Ina Attree, Eric Faudry, Mylène Robert-Genthon, Dominique Bicout, Max Maurin, Bertrand Toussaint, Karen Moreau, Louis Felix Nothias, Sophie Abby, Syvie Elsen, Audrey Le Gouellec*

SUB-THEME: Infectious diseases

Abstract: Chronic cystic fibrosis (CF) lung infection by Pseudomonas aeruginosa provides a relevant model to study metabolic adaptation during long-term host colonization. Here, we applied an untargeted LC-HRMS/MS metabolomics approach to longitudinal clinical isolates in order to determine whether distinct metabolic trajectories emerge during chronic infection. A total of 32 CF patients were included, and bacterial isolates were analyzed using an untargeted workflow, yielding 3,090 variables from 196 injections performed on biological triplicates. Metabolomics data were examined together with phenotypic traits, antibiotic susceptibility profiles, and clinical lung function parameters.

Multivariate analyses identified three major metabotypes within the isolate collection, indicating that chronic adaptation is accompanied by reproducible changes in the bacterial metabolome. Among the metabolites contributing most strongly to this structuration, polyamines—and particularly spermidine and putrescine—were the main discriminants. These metabotypes were associated with several phenotypic traits related to pathogenic adaptation, including cytotoxicity, growth, mucoidy, and pigmentation, and were also linked to instability in lung function.

To further interpret these metabolic differences, representative isolates with high or low polyamine production were characterized by transcriptomics, whole-genome sequencing, and functional genetics. These analyses supported the involvement of two spermidine biosynthetic routes and showed that impairment of spermidine biosynthesis was associated with reduced cytotoxicity. Altogether, the results indicate that polyamine-related signatures reflect biologically relevant differences between clinical isolates.

This study identifies polyamine metabolism as a major source of metabolic heterogeneity in chronic P. aeruginosa infection and supports the use of untargeted metabolomics to characterize pathoadaptive trajectories in longitudinal clinical collections.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1099 B GC-MS Metabolomics Uncovers Sex-Specific Variations in the Tuberculosis Metabolome

PRESENTING AUTHOR: ***Derylize Beukes, NMP, South Africa***

CO-AUTHORS: *Derylize Beukes, Laneke Luies, Du Toit Loots, Ilse du Preez*

SUB-THEME: Infectious diseases

Despite advances in metabolomics research, significant challenges persist in fully elucidating the metabolic perturbations caused by tuberculosis (TB), particularly due to variability introduced by confounding factors such as sex. Current literature emphasizes the critical oversight of sex as a variable in metabolic studies. With 63.4% of metabolites in the healthy metabolome showing sex-related differences, it is necessary to integrate sex as a critical factor in TB metabolomics research. This study aimed to explore the influence of sex on the sputum metabolome of pulmonary TB patients in a South African cohort (n=228).

The study employed untargeted GCxGC-TOFMS to analyse sputum samples. The cohort consisted of 31 TB-positive patients (7 females, 24 males) and 197 TB-negative patients (32 females, 165 males). Univariate statistical analysis was applied to compare TB-positive and TB-negative groups, segregated by sex, to identify significant metabolite differences. Differentiating compounds were stringently shortlisted by selecting those meeting the statistical selection criteria (absolute fold change >2; BH FDR p-value 1).

The results indicated notable sex-based variations in carbohydrate, lipid, and amino acid metabolites, with females exhibiting a more substantial decrease in carbohydrate levels compared to males. In the TB-positive gender comparison subgroup, 7 annotated differentiating compounds were identified as statistically significant, including 1 carbohydrate and 6 lipids as well as 25 unannotated compounds. In the TB-negative gender comparison subgroup, 19 annotated differentiating compounds were identified, including 13 carbohydrate, 1 lipid, 2 amino acids, 2 exogenous metabolites, and 71 unannotated compounds.

These findings underscore the importance of considering sex as a critical variable in metabolomics studies to identify clear and meaningful disease biomarkers. By integrating sex-based analyses, future research can provide more personalised approaches in the diagnosis and treatment of TB.

1100 A Integrating Machine Learning and LC-MS-based Metabolomics to Validate Ethnopharmacological Anti-plasmodial Medicinal Plants

PRESENTING AUTHOR: ***Pfano Witness Maphari, University of Johannesburg, South Africa***

CO-AUTHORS: *Ntakadzeni Madala, Mthokozisi Simelane, Gerrit Koorsen, Msizi Mhlongo*

SUB-THEME: Infectious diseases

Malaria remains a major global health challenge, with 282 million cases reported in 2024, of which 95% occurred in Africa. The increasing resistance to existing antimalarial drugs highlights the urgent need for novel therapeutic agents. Traditional medicinal plants, rooted in ethnopharmacological knowledge, offer a promising yet underexplored resource for drug discovery. However, limitations in scientific validation of traditional plants as treatment options, accurate identification of specific plant species pose significant challenges. This study aimed to integrate machine learning (ML) and liquid chromatography-mass spectrometry (LC-MS)-based metabolomics to validate the anti-plasmodial potential of selected medicinal plants. Four plant species (Acacia nilotica, Acacia karroo, Gardenia thunbergia and Vangueria infausta) were profiled using LC-MS, analysing both leaf and root extracts obtained with multiple solvents to capture a broad range of metabolites. A total of 112 metabolites were identified, predominantly belonging to phenylpropanoids (e.g., flavonoids and cinnamic acids), and lipid-related compounds (e.g., terpenes and steroids), with methanolic extracts yielding the highest diversity. In parallel, five supervised ML models were developed for active compound prediction and ethnopharmacological validation, with Support Vector Machine and Random Forest demonstrating superior performance (AUC = 0.97). These models predicted 23 potentially active compounds from the 112 compounds LC-MS annotated compounds. Mapping these compounds to their respective plant sources enabled validation of ethnopharmacological claims and identification of species enriched in bioactive compounds, with Acacia nilotica showing the highest enrichment. To further validate these computational predictions, both the plant extracts and the predicted candidate compounds will be subjected to anti-plasmodial assays to confirm their biological activity. Overall, this study demonstrates the effectiveness of integrating metabolomics and machine learning to accelerate bioactive compound discovery and support the scientifics validation of traditional medicinal plants for malaria treatment.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1101 B Machine learning-guided identification and Metabolomics Profiling of Novel SARS-CoV-2 Mpro Inhibitors and Medicinal Plants

PRESENTING AUTHOR: ***Anathi Msobo, University of Johannesburg, South Africa***

CO-AUTHORS: *Gerrit Koorsen, Lizelle Piater, Msizi Mhlongo*

SUB-THEME: Infectious diseases

The continued global burden of COVID-19 underscores the need for novel antiviral agents. Although medicinal plants are a rich source of bioactive compounds, their exploration remains constrained by reliance on ethnobotanical knowledge, limiting the discovery of new therapeutics. Integrating machine learning (ML) with metabolomics offers a data-driven strategy to expand this search space while enabling experimental validation. This study aimed to identify potential SARS-CoV-2 main protease (Mpro) inhibitors and associated medicinal plants using an integrated ML-metabolomics approach. Five supervised ML models were developed, of which Random Forest (RF), Support Vector Machine (SVM), and Logistic Regression (LR) demonstrated the best predictive performance with AUC values of 0.93, 0.91, and 0.91, respectively. These models were applied to compounds from the TCMSP database, predicting 530 active compounds from 6087 candidates. Consensus predictions were subjected to enrichment analysis, identifying five highly enriched medicinal plants, with Licorice emerging as a top candidate. LC-MS-based metabolomics profiling of Licorice enabled the annotation of 38 compounds. Among the annotated compounds, isoliquiritigenin was confirmed as an ML-predicted compound, while the 37 compounds represented newly identified compounds not part of the predicted list. These findings provide partial validation of the ML-driven prioritization pipeline while demonstrating the complementary strength of metabolomics in capturing broader phytochemical diversity. The identification of numerous additional compounds highlights opportunities for discovering novel antiviral candidates. Future work will focus on molecular docking and molecular dynamics simulations to evaluate their inhibitory potential against SARS-CoV-2 Mpro.

1102 A Evaluation of Physiological Metabolites from Kramnik Mice Tissue Samples Following 2 Week Cannabidiol (CBD) Treatment

PRESENTING AUTHOR: ***Maano Valerie Masiphephethu, North-West University, South Africa***

CO-AUTHORS: *Shayne Mason*

SUB-THEME: Infectious diseases

Background: Cannabidiol (CBD) has been shown to have strong anti-inflammatory and antioxidant properties. However, the subtle details of the potential metabolic side effects remain underexplored. This study explores CBD treatment in mice over 2 weeks and the metabolic effects in specific organs.

Methodology: Kramnik mice were administered CBD intrabuccally at 10 mg/kg concentrations over 2 weeks. After treatment, liver, kidney, and brain tissues were collected, and metabolites were extracted using a monophasic Bligh-Dyer method. Metabolite analysis was conducted via proton nuclear magnetic resonance (1 H-NMR) spectroscopy. Principal component analysis (PCA) was used to visualise the data grouping and distribution, and quantitative univariate statistical comparisons were done.

Results: Significant 1H-NMR peaks in brain tissue indicated the presence of critical amino acids and organic acids essential for brain metabolism and immune response. Liver samples showed elevated levels of sugar metabolites, suggesting increased hepatic metabolism with CBD treatment. Kidney samples showed enhanced metabolic balance as indicated by amino acid metabolism.

Conclusion: CBD treatment positively influences metabolism across all examined tissues in Kramnik mice, enhancing essential immune pathways. These results highlight the potential of cannabinoids to complement conventional treatment regimens without negative side effects.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1103 B Guinea Pig GC×GC-TOFMS Metabolomics Reveals Serum Metabolic Alterations Comparable to Human Tuberculosis Studies

PRESENTING AUTHOR: ***Ilse Du Preez, North-west University, South Africa***

CO-AUTHORS: *Rochelle Caudron, Monique Opperman, Yolandy Lemmer, Heena Ranchod, Laneke Luies, Ilse du Preez*

SUB-THEME: Infectious diseases

Tuberculosis (TB) remains the leading infectious disease worldwide, with rising rifampicin (RIF) resistance posing a significant threat to treatment efficacy. Although guinea pigs are widely used as animal models in TB research, their application in metabolomics studies remains limited. This study investigated the translatability of the guinea pig model to the human TB metabolome using untargeted comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) to characterise serum metabolic alterations associated with Mycobacterium tuberculosis (Mtb) infection and RIF treatment.

Comparative analysis between Mtb-infected guinea pigs and healthy controls identified 28 annotated differential metabolites, of which 27 have previously been reported in human blood-based TB metabolomics studies. These metabolites were predominantly associated with lipid and amino acid metabolism, suggesting host immune-mediated metabolic reprogramming and Mtb-driven exploitation of host lipid and energy pathways during infection.

Assessment of treatment-associated metabolic alterations identified 55 annotated differential metabolites across short- and medium-term RIF treatment groups compared with untreated animals. Fifteen of these metabolites overlapped with findings from previous human anti-TB treatment metabolomics studies, indicating conserved metabolic responses to RIF exposure across species. Altered pathways suggested dynamic modulation of host lipid utilisation, immune and energy metabolism, hepatic function, and gut microbiota-associated metabolism during treatment. In addition, 11 treatment-associated metabolites not previously linked to anti-TB therapy were identified, representing potentially novel biomarkers requiring further investigation.

Overall, this study demonstrates that untargeted GC×GC-TOFMS metabolomics can detect biologically relevant metabolic changes in guinea pig serum associated with both Mtb infection and RIF treatment, with substantial overlap observed between guinea pig and human metabolomic profiles. These findings support the translational relevance of the guinea pig TB model for metabolomics-based biomarker discovery and therapeutic monitoring. However, interpretation of treatment-associated findings should consider limitations including small sample sizes, limited guinea pig metabolomics datasets, and methodological variability across existing studies.

1104 A Metabolic fingerprinting for understanding altered pathways in dengue infection

PRESENTING AUTHOR: ***María Monge, CIBION-CONICET, Argentina***

CO-AUTHORS: *Maximilian Rey, Eliana Castro, Genoveva Heliszkowski, Mariela García, Mariela Videla, Nicolás Zabalegui, Diego Álvarez, Mariela Bollini, María Eugenia Monge*

SUB-THEME: Infectious diseases

Dengue fever is the most prevalent mosquito-borne viral disease and a major global public health concern, being endemic in more than 100 countries. In 5–10% of cases, acute infection progresses to dengue hemorrhagic fever or dengue shock syndrome, potentially leading to death if not properly treated. By evaluating metabolic profiles associated with viral infection in model systems, we expect to contribute to the understanding of altered metabolic pathways, identify new predictors of disease progression and targets for treatment.

The model system consisted of infection of the hepatic human cell line Huh-7, representative of dengue virus tropism in humans, with dengue virus type 2 at a multiplicity of infection of 0.1. Control (n = 12) and infected (n = 12) cells were maintained in culture medium supplemented with 2% fetal bovine serum for 48 h. A protocol was optimized for cell harvesting, quenching, extracting and profiling the endometabolome from in vitro models. Samples were analyzed using a reversed-phase UHPLC-QTOF-MS-based metabolic fingerprinting method and data were acquired in positive and negative electrospray ionization modes. Data processing and curation were performed using TidyMS, the Python-based pipeline for preprocessing LC-MS data for untargeted metabolomics workflows. Unsupervised multivariate statistical models were applied for data quality review. Mann–Whitney U tests were applied after correcting for multiple comparisons.

A data matrix of analytically robust features was obtained after data curation. Binary comparisons were conducted to investigate metabolic alterations due to viral infection. Univariate statistical models yielded statistically significant

discriminant metabolic features in the fingerprinting assay. Among them, 7-ketocholesterol and 7-hydroxycholesterol were identified using chemical standards and exhibited higher levels in control samples. Current efforts focus on identifying the remaining discriminant features.

1105 B Integrating Ionizing Radiation and Infection Stressors in a Dual-Hit Model to Identify Biomarkers Supporting Novel Nuclear Emergency Biodosimetry Assays

PRESENTING AUTHOR: ***Evan Pannkuk, Georgetown University, United States***

CO-AUTHORS: *Anika Kot, Jerry Angdisen, Morgan DiGiorgio, Lorreta Yun-Tien Lin, Raymond Song, Eric Wang, Igor Shuryak, Albert J. Fornace Jr. and Heng-Hong Li*

SUB-THEME: Infectious diseases

Effective medical triage following radiological or nuclear incidents requires rapid, high-throughput biodosimetry capable of accurately reconstructing ionizing radiation (IR) dose. Metabolomics of noninvasive biofluids is a leading candidate platform; however, real-world exposures are frequently complicated by infection and supportive care, which can alter endogenous metabolic signatures and confound dose estimation. IR-induced immune suppression and barrier dysfunction increase susceptibility to secondary infections, yet the impact of infection on radiation-responsive metabolite biomarkers remains poorly defined.

Here, we evaluated combined radiation–infection effects in C57BL/6 mice infected with Klebsiella pneumoniae or Listeria monocytogenes and exposed to 0, 2, or 6 Gy X-rays. Urine and serum were collected 1 day post-irradiation and analyzed using global metabolomics, lipidomics, gene expression, and procalcitonin measurements. Multivariate random forest modeling was applied to identify metabolites jointly predictive of radiation dose and infection status.

Principal component analysis of urine demonstrated clear dose-dependent separation in both infected and non-infected mice. L. monocytogenes infection alone modestly perturbed host metabolism, increasing nicotinic acid and reducing tricarboxylic acid cycle intermediates, partially masking canonical radiation responses. Notably, several established radiation-responsive metabolites—including acetylcarnitine, propionylcarnitine, creatine, taurine, acetylspermidine, and hexosamine-valine-isoleucine (Hex-V-I)—exhibited enhanced fold changes in infected irradiated mice, improving high-dose discrimination, although Hex-V-I responses were attenuated relative to non-infected irradiated controls. In serum, infection induced broad lipidomic remodeling, particularly decreases in phosphatidylcholines and lysophosphatidylcholines, masking typical radiation-associated lipid reductions. However, select lipids (LysoPC(14:0), LysoPC(22:5), PC(42:8), PC(42:11)) retained strong dose responsiveness independent of infection.

Receiver operating characteristic analysis demonstrated robust classification performance (AUROC 0.88–1.0). A combined urine–serum random forest model achieved a radiation dose RMSE of 1.31 Gy with 90% infection classification accuracy, outperforming single-biofluid approaches. These findings support development of resilient, multi-omic biodosimetry panels capable of distinguishing radiation injury from sepsis under complex exposure conditions.

1106 A Urine and serum untargeted multiplatform metabolomics in of urinary tract infections and urosepsis patients.

PRESENTING AUTHOR: ***Joanna Raczak-Gutknecht, Medical University Of Gdańsk, Poland***

CO-AUTHORS: *Joanna Raczak-Gutknecht, Paweł Wityk, Margot Biesemans, Beata Krawczyk, Renata Wawrzyniak, Wiktoria Brzezińska, Michał J. Markuszewski*

SUB-THEME: Infectious diseases

Sepsis is a severe condition that carries a high risk of mortality. It is often difficult to stratify patients with common urinary tract infections (UTIs) from those in whom the condition may progress to urosepsis. Therefore, there is a significant need for a deeper understanding of the patomechanisms of the disease and the identification of reliable indicators of progression

In the present study, serum and urine samples were collected from UTI (n=27) and urosepsis patients (n=22) The samples were analyzed using two complementary analytical techniques: gas chromatography coupled with single quadrupole mass spectrometry detection (GC-MS) and liquid chromatography hyphenated with high resolution mass spectrometry detection (LC-Q-ToF/MS). Analyzes were performed in a scan mode. The obtained raw data sets were preprocessed according to QA/QC criteria and then statistically analyzed using advanced multivariate chemometric methods (PCA, OPLS-DA). The results showed increased activity in several metabolic pathways in both UTI and urosepsis patients, including the pentose phosphate pathway, the TCA cycle, the malate–aspartate shuttle, and pyruvaldehyde degradation. The observed alterations in energy production, redox balance and mitochondrial metabolism are associated with the rising metabolic demands of the host

during disease progression and could serve as a basis for identifying host-derived biomarkers to improve early risk stratification and the clinical management of patients progressing from UTI to urosepsis. Although the obtained results are promising, further studies are needed using a targeted approach on larger patient cohorts.

Acknowledgments:

This work was supported by the National Science Center (grant number 2018/29/B/NZ7/02489 and by the NdS-II/SP/0438/2024/01 grant under the Ministry of Education and Science program (Science for Society II).

1108 A Mechanisms of Action and Pathogenicity of Corynebacterium pseudotuberculosis Glutaredoxin: Insights from a Cellular Model

PRESENTING AUTHOR: ***Thyerre Santana da Costa, UNICAMP, Brazil***

CO-AUTHORS: *Thyerre Santana da Costa, David Fernández-Ramos, Fernando Lopitz-Otsoa, Félix Royo, Matheus Luquirini Penteado dos Santos, Beatriz González Valle, Carolina Gismene, Raghuvir Krishnaswamy Arni, Oscar Millet, Ljubica Tasic*

SUB-THEME: Infectious diseases

Corynebacterium pseudotuberculosis, the etiological agent of caseous lymphadenitis, remodels its secretome during infection, including the release of a glutaredoxin-like protein (Grx). Although redox proteins can act as unconventional virulence factors, the impact of secreted Grx on mammalian host cells remains poorly understood. This study investigated whether recombinant C. pseudotuberculosis Grx triggers structural and metabolic reprogramming in murine hepatic progenitor MLP-29 cells. Recombinant Grx was expressed and purified, and its effects were evaluated by MTT assay, confocal microscopy, Annexin V/PI flow cytometry, RT-qPCR, label-free proteomics, Ingenuity Pathway Analysis, and 1H NMR-based metabolomics. Grx induced dose-dependent cytotoxicity, with marked loss of MLP-29 cell viability at concentrations ≥ 50 µmol L-1. At sublethal Grx concentrations, confocal imaging revealed MLP-29 cells with pronounced actin remodeling, stress-fiber formation, nuclear enlargement, mitochondrial redistribution, and intracellular/perinuclear accumulation of His-tagged Grx. Flow cytometry showed a strong increase in early apoptosis of the Grx-exposed MLP-29 cells with limited progression to late apoptosis, consistent with a predominantly caspase-independent mechanism. Proteomics analyses of the Grx-exposed MLP-29 cells indicated disruption of integrin-to-cytoskeleton signaling, repression of epithelial adhesion programs, predicted inhibition of NRF2-mediated oxidative stress response, and activation of pathways associated with mitochondrial dysfunction and rRNA processing. RT-qPCR further supported host-cell reprogramming, with increased Smad4, α-SMA, and NRF2 expression and reduced COX5b in the Grx-exposed MLP-29 cells. 1H-NMR metabolomics on the Grx-exposed MLP-29 cells demonstrated a distinct metabolic signatures, characterized by decreased glycine, alanine, threonine, and acetate, alongside increased leucine, isoleucine, and succinate, indicating perturbation of amino acid metabolism and TCA cycle activity. The observed succinate accumulation and amino-acid imbalance support a mitochondria-centered pathometabolic state, highlighting metabolomics as a key approach to uncover host-cell responses to bacterial redox effectors. These findings identify Grx as a potential unconventional virulence factor that penetrates host cells and promotes coordinated cytoskeletal, mitochondrial, and metabolic remodeling.

1109 B Plasma Metabolome and Lipidome Stability in Hospitalized Patients: Effects of Refrigerated Storage Time and additional Freeze–Thaw Cycle

PRESENTING AUTHOR: ***Sander Guttorm, Oslo Metropolitan University, Norway***

CO-AUTHORS: *Liana Gukasyan, Christian Prebensen, Niclas G. Karlsson, Aleksander Rygh Holten, Dag Kvale, Helge Rootwelt, Katja Benedikte Prestø Elgstøen & Erik Koldberg Amundsen*

SUB-THEME: Laboratory Medicine

Background: Global metabolomics and lipidomics are increasingly used in clinical research, yet pre-analytical factors such as storage time and freeze—thaw cycles can compromise data quality. While stability has been studied in healthy volunteers, evidence from patient-derived samples remains scarce.

Objective: To evaluate the impact of post-centrifugation refrigerated storage duration (0, 24, and 72 h at 4°C) and one additional freeze—thaw cycle on the plasma metabolome and lipidome in acutely hospitalized patients.

Methods: EDTA plasma was collected from 20 patients acutely hospitalized in a medical ward and stored at 4°C for 0, 24, and 72 h post-centrifugation. All samples underwent one additional freeze—thaw cycle and were analyzed using global liquid chromatography–mass spectrometry (LC-MS) metabolomics and lipidomics.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Results: Principal component analysis showed strong clustering by patient rather than by storage condition, indicating that inter-individual biological variation dominated over pre-analytical effects. Over 90% of the global metabolome and lipidome remained stable across conditions. However, 116 annotated compounds exceeded the Cohen's d threshold, including lactate, hypoxanthine, oxoproline, FA(20:4), and LPC(16:0). Metabolomic alterations accumulated progressively over 72 h, while lipidomic changes were most pronounced within the first 24 h. The freeze—thaw cycle preferentially affected PUFA-containing lipids, with 27 lipids and 4 metabolites exceeding the effect size threshold.

Conclusion: EDTA plasma from hospitalized patients is largely robust to common pre-analytical handling conditions. However, specific metabolites and lipids, some serving as established clinical biomarkers, show storage-related changes that may confound interpretation. These findings support feasible, automation-compatible sample workflows for clinical omics studies.

1110 A — Plasma metabolomic signatures reveal association with Haemoglobin B mutational status and distribution of haemoglobin types as well as alterations in microbial metabolism in Sickle Cell Disease (SCD)

PRESENTING AUTHOR: ***Soumen Kanti Manna, Saha Institute of Nuclear Physics, India***

CO-AUTHORS: *Asmita Saha, Debasish Prusty, Chinmay Kumar Sahoo, Blessymol Varghese, Prasanta Purohit, Manoj Kumar Patro, Samira Kumar Behera, Sulakshana P Mukherjee*

SUB-THEME: Laboratory Medicine

Introduction and aim: The clinical manifestations of SCD vary significantly. Determinants beyond haemoglobin(Hb) profile remain poorly understood. These may include non-genetic modifiers like nutrition, metabolism and microbiome, which may be reflected in metabolome. Relationships between plasma metabolome and HBB mutational status or haemoglobin profile also remain unexplored. These were examined here to identify potential implications for disease management.

Methods: Plasma metabolites from healthy (n=10, 23.1±3.0 years), Sickle-cell traits, aka, SCT (n=6, 32.2±7.5 years) and SCD (n=11, 26.3±13.8 years) cases were analysed using GC-MS. Multivariate and univariate analyses (with different normalization methods and corrections for multiple comparisons) of metabolic signature were performed. The association with HBB mutational status and distribution of different haemoglobin types was examined.

Findings and significance: SCD plasma metabolome reflected changes in multiple metabolic pathways associated with central carbon, amino acid, lipid and glutathione as well as microbial metabolism. Lactic acid tended to increase with concomitant derangement of TCA cycle intermediates in SCD as well as SCT cases indicating glycolytic shift. Distinct association between HBB mutation (trait or disease) status and metabolic signatures were observed. Metabolic signatures were found to discriminate healthy, SCT and SCD samples according to mutation (SCT~SCD)-, allele status- and disease with AUCROC > 0.9. Metabolites were found to be oppositely correlated with abundances of normal haemoglobin(HbA0) and SCD(HbS) as well as foetal (HbF) haemoglobin, which is known to attenuate symptoms and complications. Interestingly, few metabolites showed distinct correlation with specific haemoglobin types in healthy and SCD cases. These indicated unique associations between HBB mutation, SCD manifestation, haemoglobin profile and metabolic homeostasis, for the first time. These signatures have potential implications on nutrition, energy metabolism, oxidative stress response, gut and neurological health and immunity. These invite further studies to examine their utility to predict and modulate SCD progression and severity via management of nutrition and gut health.

1111 B — From Sample to Signal: Analytical Validation of a Gut Metabolomics Pipeline for Microbiome-Informed Precision Nutrition — The BIOMATEST PLUS Pilot Study

PRESENTING AUTHOR: ***Laura Sierra, Universidad EAFIT - Astrolab Biotecnología, Colombia***

CO-AUTHORS: *Mariana Lopez-González, Mónica Cala, Maria Clara Arrieta*

SUB-THEME: Microbiome research

The human gut microbiome is a dynamic ecosystem central to host metabolic and immune homeostasis. Leveraging it for precision medicine requires multiomics approaches integrating microbial composition with the intestinal chemical landscape. We aimed to establish an integrated metagenomics and LC-MS/MS metabolomics protocol for gut metabolome characterization, validate a standardized sampling methodology assessing chemical stability across collection devices and preservation conditions, correlate microbial abundances with metabolite profiles, and develop a clinically actionable panel of 40 intestinal metabolites for precision nutrition. Fecal samples from 10 healthy volunteers were collected using three preservation strategies: immediate freezing at −80°C (gold standard), 95% ethanol, and OMNImet•GUT liquid transport device. Samples were analyzed by LC-MS/MS using C18 and HILIC columns, processed in GNPS for molecular networking and

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

MetaboAnalyst for multivariate statistics. A PLS-DA model anchored to the gold standard enabled supervised discrimination between preservation conditions, evaluating solvent and temperature effects and classifying stable versus labile metabolites. Spearman correlations were computed between bacterial genera and metabolite families. Applying the proprietary BIOMATEST framework, 40 intestinal metabolites were curated and assigned evidence-based scores reflecting their functional role in host gut metabolism. LC-MS/MS identified 780 metabolites (C18) and 9 (HILIC) across 90 chemical families, dominated by steroids, bile acids, fatty acids, and terpenoids. While overall metabolome structure was preserved across conditions, a subset of features showed statistically significant abundance changes across storage time points within the same liquid transport device, indicating time-dependent degradation. Inter-individual variation was the primary driver of global variability. Multiomics correlations revealed predominantly negative associations between bacterial genera and chemical families, with one notable positive correlation (ρ = 0.72). The proposed pipeline reliably characterizes the gut metabolome, confirming ethanol and OMNImet•GUT preserve the overall chemical profile, though storage time within liquid devices requires strict control. The scored 40-metabolite panel provides a robust foundation for clinical precision nutrition.

1112 A Routes of cannabis administration differentially shape gut-brain metabolic signatures resolved by metabolomics

PRESENTING AUTHOR: ***Bashar Amer, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Nichole Reisdorph, Katrina Doenges, Michael Armstrong, Rahul R. Deshpande, Susan S. Bird*

SUB-THEME: Microbiome research

Bile acids and related metabolites regulate host metabolism, gut-brain signaling, and microbe-host interactions and are sensitive to xenobiotic exposure and route of administration. Cannabis consumption alters physiology and behavior; however, the metabolic consequences of different delivery routes remain poorly characterized.

We applied a metabolomics workflow to profile matched fecal, brain, and cecum samples from C57BL/6J mice administered cannabis via intraperitoneal injection, oral gavage, or free feeding (n=31, including controls). The platform enabled concurrent targeted bile acid quantitation and untargeted metabolite discovery across biological matrices. Chromatographic separation was performed using reverse-phase LC coupled to high-resolution MS2 acquisition. Calibration curves generated from bile acid standards demonstrated robust linearity across six orders of magnitude. The method achieved a lower limit of quantification of 2.85 femtomoles and a lower limit of detection of 0.71 femtomoles on the column. MS2 fragmentation coverage exceeded 80% across matrices, enabling confident annotation of low-abundance metabolites.

Untargeted analysis revealed route-dependent metabolic signatures across gut and brain tissues. Distinct alterations were observed in neurochemical pathways, including tryptophan, phenylalanine, and dopamine-related metabolites, with profiles varying by administration route. Differences in bile acid composition and metabolite abundance between fecal, cecal, plasma, and brain samples indicate that delivery route influences both systemic exposure and gut-derived metabolic signaling.

These findings demonstrate that integrated quantitative and discovery metabolomics can resolve route-specific metabolic remodeling across the gut-brain axis and provide insight into how cannabis administration modality shapes host metabolic responses.

1113 B Bifidobacterium species show distinct associations to aromatic lactic acid enantiomers in the infant gut

PRESENTING AUTHOR: ★ ***Ahmed Gaafar, University Of Copenhagen: The Faculty Of Science, Denmark***

CO-AUTHORS: *Martin Laursen, Giorgia La Barbera, Henrik Roager*

SUB-THEME: Microbiome research

Bifidobacterium species are key producers of aromatic lactic acids, which may shape early-life immune development via stereospecific activation of the hydroxycarboxylic acid receptor 3 (HCA3). In vitro studies show that Bifidobacterium generates both D- and L-enantiomers of aromatic lactic acids. Yet, the enantiomers of aromatic lactic acids in infants and their relationship to specific Bifidobacterium species remain unexplored. Here, we present the first study to quantify enantiomer-specific aromatic lactic acids in infant feces using a validated chiral LC-MS/MS method. In a longitudinal cohort of 25 infants with fecal samples collected every 2–4 weeks from birth to 6 months of age, fecal concentrations of aromatic lactic acids enantiomers were quantified and associated with absolute abundance of four Bifidobacterium species measured by qPCR. Overall, aromatic lactic acids accumulated in feces during early infancy with D-PLA exhibiting the steepest age-associated increase. Species-level analysis revealed distinct associations with B. longum subsp. longum predominantly linked to D-

enantiomers of aromatic lactic acids, while B. bifidum showed stronger associations with L-enantiomers. B. longum subsp. infantis and B. breve exhibited moderate contributions across both enantiomers. These findings suggest chirality as a potential key dimension of Bifidobacteria-immune interactions and highlight the importance of enantiomer-resolved analyses in infant gut microbiome research.

1114 A Integrating metagenomics and metabolomics to study the gut microbiome and host relationships in sports across different energy systems

PRESENTING AUTHOR: ***Monica Cala, Universidad de Los Andes, Colombia***

CO-AUTHORS: *Vivana Aya, Daniel Pardo-Rodriguez, Laura Vega, Juan David Ramírez*

SUB-THEME: Microbiome research

The gut microbiome plays a critical role in modulating host metabolism, influencing energy production, nutrient utilization, and overall physiological adaptation. In athletes, these microbial functions may be further specialized to meet the unique metabolic demands of different sports disciplines. This study explored the role of the gut microbiome in modulating host metabolism among Colombian athletes by comparing elite weightlifters (n = 16) and cyclists (n = 13) through integrative omics analysis. Fecal and plasma samples collected one month before an international event underwent metagenomic, metabolomic, and lipidomic profiling. Metagenomic analysis revealed significant microbial pathways, including L-arginine biosynthesis III and fatty acid biosynthesis initiation. Key metabolic pathways, such as phenylalanine, tyrosine, and tryptophan biosynthesis; arginine biosynthesis; and folate biosynthesis, were enriched in both athlete groups. Plasma metabolomics and lipidomics revealed distinct metabolic profiles and a separation between athlete types through multivariate models, with lipid-related pathways such as lipid droplet formation and glycolipid synthesis driving the differences. Notably, elevated carnitine, amino acid, and glycerolipid levels in weightlifters suggest energy system-specific metabolic adaptations. These findings underscore the complex relationship between the gut microbiota composition and metabolic responses tailored to athletic demands, laying the groundwork for personalized strategies to optimize performance. This research highlights the potential for targeted modulation of the gut microbiota as a basis for tailored interventions to support specific energy demands in athletic disciplines.

1115 B Mapping Gut Metabolic Recovery After Antibiotics Using Untargeted LC-MS Metabolomics

PRESENTING AUTHOR: ***Priyanka Reddy, University of Melbourne, Australia***

CO-AUTHORS: *Wen Yin, Calum J. Walsh*

SUB-THEME: Microbiome research

The gut microbiome plays a critical role in host health, and faecal microbiota transplantation (FMT) has emerged as a promising strategy to restore microbial and metabolic balance after antibiotic disruption. In this study, we investigated metabolic alterations in faecal samples from antibiotic-treated mice to evaluate the effectiveness of FMT in restoring gut function.

Samples were collected and extracted under controlled conditions, and metabolic profiles were acquired by liquid chromatography-mass spectrometry (LC-MS). The resulting data were subjected to a comprehensive computational workflow, beginning with preprocessing and feature extraction, followed by statistical analysis to compare treatment groups. Data interpretation was supported by multiple software platforms, including MZmine, MetaboAnalyst, and network-based tools such as GNPS and Cytoscape, which enabled clustering of related features and visualisation of global metabolite patterns. Structural annotation of compounds was further guided by spectral library matching and in silico prediction tools such as MS2Query, providing putative chemical identities for discriminant features.

This study outlines a workflow for untargeted faecal metabolomics, integrating LC-MS acquisition with open-source computational tools for preprocessing, statistical analysis, and compound annotation. By combining feature-based methods with network-driven annotation platforms, this framework is designed to enable reproducible characterization of metabolic alterations in microbiome studies. Ongoing work will apply this approach to evaluate the impact of antibiotics and FMT on gut metabolic profiles, with the goal of advancing understanding of microbiome-associated mechanisms and supporting future translational applications.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1116 A SQUAD bile acid profiling reveals cannabis-driven remodeling of the gut microbiome

PRESENTING AUTHOR: ***Scott Peterman, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Nichole Reisdorph, Katrina Doenges, Nathan Anderson, Winona Booher, Bashar Amer, Susan Bird*

SUB-THEME: Microbiome research

Primary bile acids synthesized in the liver are transformed by gut microbes into secondary bile acids that regulate immune and inflammatory pathways. Although cannabis has been proposed to exert anti-inflammatory effects, its impact on gut microbial composition and bile acid metabolism remains unclear. We hypothesized that cannabis-induced microbiome remodeling would be reflected in functional shifts in bile acid composition rather than diversity changes alone.

Male and female C57BL/6J mice received a complex cannabis extract (≤10 mg/kg) or vehicle every other day for two weeks (n=12/group). Fecal samples were analyzed by 16S rRNA sequencing to assess microbial composition. Matched fecal and brain samples were analyzed using a SQUAD (Simultaneous Quantitation and Discovery) metabolomics workflow enabling targeted bile acid quantitation alongside untargeted profiling. Chromatographic separation was performed by UHPLC coupled to a Thermo Scientific modified Tribrid Orbitrap-linear ion trap mass spectrometer, enabling high-resolution MS1/MS2 acquisition with parallel reaction monitoring (PRM)-based quantitation. Calibration curves generated from bile acid standards demonstrated linear responses across 5–6 orders of magnitude, with lower limits of quantification in the low femtomole range. MSn-based quantitation improved selectivity for co-eluting bile acid isomers.

Cannabis exposure significantly reduced alpha diversity in female mice ($q = 0.0192$) and altered overall microbiome composition in both sexes ($p = 0.001$). Taxa associated with bile acid metabolism, including Alistipes, Rikenellaceae, and Oscillospirales, were significantly altered. Metabolomic profiling revealed shifts in primary and secondary bile acids in fecal samples, accompanied by broader metabolic perturbations, including neurotransmitter-related metabolites and cannabinoid-associated compounds.

These findings demonstrate that cannabis exposure remodels gut microbial communities and functionally reshapes bile acid metabolism. Integrated quantitative and discovery metabolomics provides mechanistic insight into host-microbiome interactions and supports bile acids as mediators linking microbial remodeling to systemic metabolic outcomes.

1117 B Multi-OMICS analyses of fecal extracts to explore the microbiome of rats following administration of an agonist for the GPR40 receptor

PRESENTING AUTHOR: ***Adriana Tirado, Waters Corporation, Mexico***

CO-AUTHORS: *Adam King, Robert S. Plumb, Ian D. Wilson*

SUB-THEME: Microbiome research

Fasiglifam (TAK-875) is an orally bioavailable, selective GPR40/FFAR1 agonist developed for Type 2 diabetes, promoting glucose-dependent insulin release. Although early dose-finding studies showed reduced hypoglycemia risk, development stopped after Phase III trials due to liver toxicity concerns. Previous metabolism studies indicated that most TAK-875 metabolites are eliminated in feces, and growing evidence shows bidirectional interactions between drugs and the gut microbiome that can influence drug efficacy. This study applied a multi-omic strategy to explore TAK-875–related microbiome and biological changes in a rat model.

The study used 15 male and 9 female Sprague Dawley rats, initially pair-housed then singly housed for a 96-hour study duration. Animals received fasiglifam intravenously (5 mg/kg) or orally (10 or 50 mg/kg), with vehicle controls for both routes. Fecal and biological fluid samples were collected from predose to 96 hours. Fecal samples were extracted for polar metabolites (Folch extraction) and proteins, which were reduced, alkylated, and digested using FASP.

Metabolite extracts were analyzed using a 5-minute HILIC-UHPLC method coupled to a multi-reflecting Q-TOF MS. Proteomic datasets used the same MS with capillary LC, acquired using DDA and DIA (including stepping quadrupole) with Enhanced Duty Cycle to improve MS2 sensitivity and sequence coverage. Proteomic searches used curated FASTA databases combining rat (Uniprot) and gut-microbial protein sequences, informed by shotgun metagenomics.

Integrated proteomic and metabolomic analyses revealed coordinated dysregulation across the time course, aligning with TAK-875 pharmacometabodynamics. Notably, expression of G proteins such as GPR84 and GPR43 increased, suggesting altered regulation of medium-chain fatty acid signaling and immune-related short-chain fatty acid pathways.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1118 A Untargeted metabolomics for antileishmanial hits discovery: Mechanistic insights of natural product-derived neolignans

PRESENTING AUTHOR: ***Daniela Tristão, UFABC (Universidade Federal do ABC), Brazil***

CO-AUTHORS: *Sara S. L. Melchert, Luiz Felipe D. Passero, Coral Barbas, Ángeles López-Gonzálvez, João H. G. Lago*

SUB-THEME: Neglected tropical and infectious diseases

Cutaneous leishmaniasis is a neglected tropical disease affecting up to one million individuals annually, predominantly in tropical and subtropical regions. Current treatments show limited efficacy, highlighting the need for new molecular prototypes.

Plant-derived natural products are recognized sources for bioactive compounds. Phytochemical investigation of Nectandra paranaensis (Lauraceae) resulted in the isolation of two diastereomeric neolignans: meso-dihydriguaiaretic acid (1) and threo-dihydroguaiaretic acid (2). Both were active in vitro against intracellular amastigotes of Leishmania (L.) amazonensis (EC50 of 42.5 µM and 40.4 µM, respectively), without citotoxicity toward J774 mammalian cells (CC50 > 300 µM, SI = CC50/EC50 > 7.0). Against promastigotes, activity was stereochemistry-dependent - while 1 displayed moderate potency (EC_{50} = 89.3 µM; SI = 3.4), 2 was inactive. These results support their potential as antileishmanial molecular scaffolds.

To elucidate their mechanisms of action, an untargeted metabolomics approach was designed. Promastigotes (MHOM/BR/73/M2269) and infected BALB/c mouse bone marrow-derived macrophages were treated at EC50 concentrations, with amphotericin B as positive control. Metabolites were extracted and analyzed using complementary CE-MS and HILIC LC-MS platforms in both ionization modes, followed by univariate and multivariate statistics analyses.

Over promastigotes, amphotericin B induced broad metabolite depletion. In contrast, compound 1 displayed a more specific effect, consistent with its stereochemistry-driven activity. Increased arginine with reduced ornithine and citrulline suggests impaired arginine conversion and potential limitation of polyamine biosynthesis. However, elevated spermidine indicates activation of alternative polyamines pathways. Decreased proline and increased cystine may further support this metabolic adaptation. In amastigotes, infection upregulated nucleotide and polyamine pathways. Unlike promastigote model, amphotericin B selectively reduced spermidine and putrescine, confirming polyamine pathway inhibition. Conversely, neolignans 1 and 2 increased arginine-related metabolites and broadly affected amino acid and energy metabolism. Additionally, 2 enhanced nucleotide biosynthesis, suggesting that different neolignans stereochemistry may lead to distinct mechanisms, despite similar in vitro activities.

1119 B Analysis of miR-372 and miR-373 in metabolic shift on macrophages infected with Leishmania infantum

PRESENTING AUTHOR: ***Angeles Lopez-Gonzalvez, Universidad CEU San Pablo, Spain***

CO-AUTHORS: *Bruno Prado Eleuterio, Ángeles López-Gonzálvez, Coral Barbas, Sandra Muxel*

SUB-THEME: Neglected tropical and infectious diseases

Metabolomic approaches can clarify how metabolic intermediates are modulated during infection, and whether miR-372/373 family inhibition can block metabolic reprogramming. HIF-1α is a transcription factor related to the expression of genes that participate in glycolytic metabolism and the lactate production pathway. Leishmania can reprogram macrophage carbohydrate and lipid metabolism and induce inflammation. In previous data, we observed increased miR-372/373 in THP-1 macrophages infected with L. amazonensis and functional inhibition of these miRNA reduced infection. HIF1A 3'UTR mRNA is a predicted target of miR-372/373. We hypothesize that the infection with L. infantum increases the expression of human miR-372/miR-373, and these molecules alter the mRNA/protein levels of HIF-1α and consequently a network of target genes involved in glycolysis in macrophages, increasing susceptibility to infection. In this project, we aim to evaluate the function of miR-372/373 in the regulation of glucose metabolism in macrophages infected with Leishmania infantum. In analysis the metabolome using an untarget CE-MS with negative ESI strategy and found the different profile of hexoses and metabolites related to glycolysis and pentose-phosphate and identified a significant number of the metabolites contained in these pathways in infected compared to uninfected macrophages.

Comparing the metabolic fingerprint of miR-372/373-inhibited to negative-control THP-1 macrophages, enrichment was detected in the pentose-phosphate pathway, glycolysis-gluconeogenesis pathway and fructose metabolism, these scored lower than the previous comparison (infected-uninfected). The pentose-phosphate pathway can serve as a diversion from glycolysis, since it is inhibited by the infection with L infantum. When comparing miR-inhibited to negative control groups, glycolysis performed higher in the enrichment analysis than the pentose-phosphate pathway, meaning that it can be more

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

active with the inhibition of miR-372 and miR-373. This is consistent with the idea that the two microRNAs play an important role in the infection of Leishmania infantum in macrophages.

1120 A HILIC-MS-Based Metabolomics Reveals Dose-Associated Responses of Leishmania amazonensis to Antileishmanial Drugs

PRESENTING AUTHOR: ***Emanuel Nunes, Universidade Estadual Palista, Brazil***

CO-AUTHORS: *Márcia Aparecida Graminha, Maria Eugenia Monge, Ian Castro-Gamboa*

SUB-THEME: Neglected tropical and infectious diseases

Drug-induced metabolic perturbations can reveal biochemical signatures associated with parasite stress and pharmacological response. In this study, an untargeted LC–MS-based metabolomics workflow was applied to investigate the metabolic response of Leishmania amazonensis promastigotes exposed to two chemically and mechanistically distinct antileishmanial drugs: miltefosine and pentamidine. Parasite viability was evaluated by MTT assay to define biologically relevant dose ranges. Intracellular and extracellular fractions were then extracted and analyzed by hydrophilic interaction liquid chromatography coupled to high-resolution tandem mass spectrometry (HILIC-HRMS/MS). Data were acquired in full-scan and data-dependent MS/MS modes, with quality assurance and quality control based on system suitability standards, pooled QC samples, and blank filtering. Feature extraction and alignment were performed in MZmine, followed by exploratory statistical analyses, including PCA, hierarchical clustering, volcano plots, and Spearman correlation with drug concentration.

Both drugs induced dose-associated metabolic changes, with distinguishable profiles between low- and high-dose groups. Miltefosine exposure was associated with alterations in choline-containing species, phospholipids, carnitines, and nucleobases, suggesting metabolic remodeling related to membrane perturbation, lipid metabolism, energy metabolism, and purine salvage. Pentamidine treatment showed a predominant depletion of small peptides, diketopiperazines, and nucleobases, including xanthine and cytosine, suggesting disturbances in nitrogen metabolism, protein synthesis, and nucleic acid-related processes. Although structural annotations remain putative and require further validation, these preliminary results demonstrate the applicability of HILIC-HRMS/MS metabolomics to describe drug-induced metabolic remodeling in L. amazonensis. This workflow provides a basis for identifying metabolic markers of antileishmanial drug response and for guiding future targeted validation of pathways involved in parasite stress.

1121 B Untargeted metabolomics reveals cardiac metabolic alterations caused by Trypanosoma cruzi infection

PRESENTING AUTHOR: ★ ***Hanna de Sa, UFBA, Brazil***

CO-AUTHORS: *Breno C. Barreto, Maria Vitoria G. das Neves, Maria Gabriela S. Santos, Carine M. A. Cardoso, Juliana F. Vasconcelos, Milena B. P. Soares, Gisele A. B. Canuto*

SUB-THEME: Neglected tropical and infectious diseases

Chagas disease (CD), caused by Trypanosoma cruzi, has been one of the leading causes of cardiac death in Latin America. Its pathogenesis and progression are still poorly understood. Additionally, there are no efficient drugs available for the treatment of this disease, especially at advanced stage. Thus, there is a need to elucidate the metabolism involved in this disorder. This work aimed to evaluate metabolic changes in cardiac tissues during the acute phase of Chagas Disease using untargeted metabolomics. Mice were infected with T. cruzi, and 60 days after infection, hearts were collected to compose the infected group (IFC, n = 5). The control group (CTR, n = 5) was composed of hearts from healthy mice. Metabolomics analyses were performed by RPLC-MS [ESI(+) and ESI(-)] and GC-MS. The results showed 228 significant annotated metabolites (level 3 according to MSI) by RPLC-MS and 23 by GC-MS (levels 2 or 3), based on multivariate (PLS-DA) and univariate analysis (t-test/Mann-Whitney test, FDR corrected). Metabolic pathway analysis highlights alterations in multiple pathways, including those correlated with arachidonic acid (AA), sphingolipid, and energy metabolism. These alterations are related to fatty acid dysregulation, which may contribute to the observed decrease in ATP levels in the IFC group and may be associated with mitochondrial dysfunction that could affect the heart's metabolic flexibility, especially leading to heart failure. Furthermore, dysregulations of sphingolipid and AA metabolism are linked to increased inflammatory activity, correlated with elevated eicosanoid levels, and is associated with increased ceramide and sphingomyelin in the IFC group. Our results demonstrated interesting metabolic changes in the cardiac tissue of mice infected with T. cruzi, which could contribute to development of myocarditis. These findings confirm speculations already reported in the literature and provide new insights into the consequences of infection on cardiac tissue and cardiac failure.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1122 A Metabolomic profiling of 6-OHDA-induced neurotoxic effects in an optimized SH-SY5Y model of Parkinson's disease

PRESENTING AUTHOR: ***Wanderleya Faulhaber, Federal University of Juiz de Fora, Brazil***

CO-AUTHORS: *Wanderleya Toledo dos Santos Faulhaber, Matheus Fernandes Alves, Fernanda Brito Leite Dornelas, Albert Katchborian-Neto, Fernanda Maria Pinto Vilela, Daniela A. Chagas-Paula, Ana Claudia C. Paula*

SUB-THEME: Neurological disorders

Parkinson's disease (PD) is a neurodegenerative condition marked by the gradual degeneration of dopaminergic neurons. Metabolomics has become a powerful approach for identifying metabolic disturbances linked to neurodegenerative processes. This study investigated metabolomic alterations related to oxidative stress using an optimized cellular model of PD based on 6-hydroxydopamine (6-OHDA) exposure in SH-SY5Y cells, designed to require a minimal number of cells. Cellular and extracellular extracts from both treated and untreated cells were examined by ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). Multivariate statistical methods were applied to identify metabolites that were differentially regulated between the experimental and control groups. The analysis revealed pronounced modifications in metabolites associated with oxylipin biosynthesis. Specifically, a decrease was detected in compounds derived from docosahexaenoic acid (DHA), including putative hydroxydocosahexaenoic acid (HDoHE) and epoxydocosapentaenoic acid (EpDPE) isomers, suggesting disruption of metabolic routes related to neuroprotection and anti-inflammatory activity. Conversely, an elevation in oxidation products of 18-carbon polyunsaturated fatty acids, mainly linoleic acid, was observed, indicating enhanced lipid peroxidation in the induced model. These metabolic shifts illustrate the effect of 6-OHDA-triggered oxidative stress on lipid metabolic pathways. Overall, the results demonstrate that 6-OHDA exposure leads to substantial remodeling of bioactive lipid metabolism, particularly within the oxylipin class, highlighting the relevance of lipid peroxidation in PD pathogenesis. Furthermore, these metabolites may serve as potential biochemical indicators of oxidative stress in cellular models of Parkinson's disease and contribute to a deeper understanding of mechanisms underlying dopaminergic neuron degeneration.

1123 B Association of a five-metabolite and early-symptom profile with Parkinson's disease and its clinical progression

PRESENTING AUTHOR: ***Juan José Oropeza Valdez, UNAM, Mexico***

CO-AUTHORS: *José Pedro Elizalde-Díaz, Osbaldo Resendis Antonio, Jaquelin Leyva -Hernández, Laura Adalid-Peralta, Mayela Rodríguez-Violante, Rupasri Mandal, David S Wishart, Yamilé López-Hernández, Eduardo Martínez -Martínez*

SUB-THEME: Neurological disorders

Parkinson's disease (PD) urgently requires blood-based markers that flag pathology before disabling motor decline. This study measured absolute concentrations of 144 plasma metabolites in 20 neurologically healthy adults and in 40 PD patients clinically classified as intermediate (PD-I) or progressive (PD-II). A multinomial logistic regression model coupled with a genetic algorithm was built to examine how changes in metabolite concentrations relate to disease stage and to assess their exploratory discriminative performance in this cohort. Five metabolites: glutamine, butyric acid, indoleacetic acid, phosphatidylcholine aa C40:2, and acylcarnitine C12:1 emerged as the smallest biomarker set that consistently separated controls, PD-I, and PD-II. When three non-motor manifestations often present in the prodromal phase (drooling, REM behavior disorder and depression) were added, the combined profile clearly distinguished controls from early-stage patients and improved classification of intermediate versus progressive disease. The selected metabolites play roles in gut-derived signaling, mitochondrial beta-oxidation, and membrane lipid homeostasis, while the clinical variables mirror the recognized early spread of alpha-synuclein pathology, together offering a coherent snapshot of systemic changes across PD progression. Because the panel can be quantified from a single small plasma aliquot and a brief clinical interview, it represents a promising exploratory finding that requires validation in larger, independent cohorts before any consideration for clinical application or pre-symptomatic screening.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1124 A The Biochemistry of Dominance and Submission: Metabolomic Insights into Social Hierarchies in Mice

PRESENTING AUTHOR: ***Maxim Itkin, Weizmann Institute of Science, Israel***

CO-AUTHORS: *Albert Pinhasov, Natalya M Kagan, Sergey Malitsky*

SUB-THEME: Neurological disorders

The endocannabinoid system (ECS) plays a central role in regulating emotional, cognitive, and social behaviors. However, its contribution to personality traits such as dominance and submissiveness remains poorly understood. In this study, we applied a combined lipidomics and metabolomics approach to characterize biochemical differences in the brains of selectively bred dominant (Dom) and submissive (Sub) mice.

Targeted and untargeted LC–MS analyses revealed elevated levels of long-chain polyunsaturated ethanolamides, including anandamide (AEA) and docosatetraenoyl ethanolamide (DEA), in Sub mice. These changes were accompanied by increased levels of their precursor fatty acids and upregulation of key biosynthetic enzymes (FADS2, ELOVL5), suggesting altered PUFA metabolism.

Functional studies demonstrated phenotype-dependent effects of DEA: acute administration reduced nociception and increased locomotion in Sub mice, while subchronic treatment enhanced dominance behavior in Dom and wild-type mice.

Additionally, polar metabolite profiling revealed distinct metabolic signatures between groups: Sub mice showed stress-associated metabolites, while Dom mice were enriched in energy metabolism and neurotransmitter precursors.

Together, these findings highlight the ECS as a key modulator of social hierarchy and demonstrate that metabolomic profiling can capture biochemical correlates of behavioral phenotypes, providing new insights into the neurobiology of personality and psychiatric disorders.

1125 B Phase 1 pilot clinical study of the psychedelic 5-MeO-DMT reveals acute autonomic and metabolomic signatures in healthy volunteers relevant to mental health

PRESENTING AUTHOR: ***Albert Katchborian-Neto, Federal University of São Carlos, Brazil***

CO-AUTHORS: *Matheus Fernandes Alves, Lucas Vendrami Rimi, Luiz Paulo Melchior de Oliveira Leão, Miller Santos Ferreira, Gabriel Henrique Ribeiro, Mauricio Augusto Pinto Moreno da Silva Alves, Daniele Ferreira Dias, Marisi Gomes Soares, João Henrique Ghilardi Lago, Daniela Aparecida Chagas-Paula and Tiago Arruda Sanchez*

SUB-THEME: Neurological disorders

5-Methoxy-N,N-dimethyltryptamine (5-MeO-DMT) is a rapid-acting psychedelic with growing interest as a potential intervention for difficult-to-treat mental disorders, yet its acute physiological and biochemical effects in humans remain poorly defined. Here, we report a Phase 1 pilot study in healthy volunteers with prior psychedelic experience integrating psychometric assessment, cardiovascular monitoring, and untargeted metabolomics of blood and saliva samples collected before, during, and after inhaled 5-MeO-DMT exposure under controlled conditions. To support data reproducibility and confident analysis, samples were orthogonally characterized by UPLC-HRMS and 1H-NMR. Because blood sampling during peak effects of short-acting psychedelics is operationally challenging, a standardized venous-access protocol was also implemented to enable safe serial collection during the acute phase. Healthy volunteers (n=18) received a single 7.5 mg vaporized dose, with comparison to a non-placebo control group. Subjective effects were assessed using MEQ30 and EDI. Participants showed complete mystical-type experiences, high ego dissolution, and improved well-being, with no relevant adverse effects. Blood pressure and heart rate increased transiently and returned to baseline, while heart rate variability decreased after dosing, consistent with acute autonomic modulation. Untargeted metabolomics using multivariate statistical analyses of UHPLC-HRMS data revealed acute changes in metabolites associated with redox homeostasis, energy and lipid metabolism remodeling. For example, in all volunteers, an NMR signal at 4.11 ppm (quartet, J = 6.9 Hz), supported the annotation of lactate, which increased markedly in blood during the acute phase and declined post-experience. This consistent lactate response indicates an acute systemic bioenergetic shift during 5-MeO-DMT exposure and identifies a candidate translational biomarker linking metabolic demand, autonomic activation, and biological pathways potentially relevant to therapeutic response. These findings support the safety, tolerability, and biomarker-mapping potential of untargeted metabolomics in early-phase clinical studies involving short-acting psychedelics for mental health research.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1126 A Serum lipidomics identifies severity and outcome biomarkers in mild traumatic brain injury

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

CO-AUTHORS: *Henrique C. Ribeiro, Ilias Thomas, Alex M. Dickens, Virginia F. J. Newcombe, Jussi P. Posti, Endre Czeiter, Daniel Duberg, Tim Sinioja, Matilda Kråkström, Isabel R. A. Retel Helmrich, Kevin K. W. Wang, Olli Tenovuo, Ewout W. Steyerberg, Andrew I. R. Maas, András Büki, Tuulia Hyötyläinen, David K. Menon*

SUB-THEME: Neurological disorders

Traumatic brain injury (TBI) causes long-term disability with significant societal burden. It is commonly classified as mild, moderate, or severe using the Glasgow Coma Scale. However, better phenotypic characterization is needed for improved management and outcome prediction. Lipid profile alterations in serum have emerged as candidate prognostic markers reflecting TBI pathophysiology. We analyzed serum lipid profiles from 929 mild TBI patients in CENTER-TBI, a large European multicenter cohort, with 6-month outcomes assessed by the Glasgow Outcome Scale–Extended (GOSe). Serum was collected within 24 hours of admission and profiled by UHPLC-MS using class-specific standards. Lipid and protein data were integrated using ordinal elastic-net and validated by proportional odds logistic regression. Models were adjusted with variables included in the CRASH clinical outcome prediction model and validated in an independent test set. Compared with a clinical baseline model, combined models achieved AUCs of 0.702–0.802, highlighting key contributions from lipids LPC(18:2), LPC(20:5), PC(O-34:3) and proteins GFAP, NFL, and S100B. In a subset of 103 CENTER-TBI patients, MRI diffusion metrics (Fractional Anisotropy - FA, Mean Diffusivity - MD) were linked with serum lipidomics. Increasing injury severity was associated with higher MD, lower FA, and distinct lipid patterns, indicating that serum lipid profiles reflect white matter microstructural damage. In summary, serum lipidomics, integrated with protein biomarkers, shows strong potential for outcome prediction in mild TBI, addressing an important clinical unmet need.

1127 B LC-MS/MS Metabolomics of Cerebrospinal Fluid in Pediatric Tuberculous Meningitis

PRESENTING AUTHOR: ***Ontefetse Neo Plaatjie, North West Univeristy, South Africa***

CO-AUTHORS: *A. Marceline Tutu van Furth, Martijn van der Kuip, Shayne Mason*

SUB-THEME: Neurological disorders

Introduction: Tuberculous meningitis (TBM) has high mortality, partly due to delayed diagnosis and profound metabolic disruption. Acylcarnitines and amino acids are key regulators of brain energy metabolism and are often altered in TBM, while disturbances in tryptophan metabolism have been linked to disease severity. We profiled these pathways in cerebrospinal fluid (CSF) to characterise metabolic changes and assess their ability to distinguish TBM from viral meningitis (VM) and non-meningitis controls (NMC).

Method: We performed targeted LC-MS/MS metabolite profiling of acylcarnitines (n = 144), tryptophan metabolites (n = 130) and amino acids (n = 137). Significant differential metabolites from each pathway were evaluated for their diagnostic potential, and their relationship with clinical metadata, including CSF features and neuroimaging findings (CT and MRI), were assessed.

Results: Free carnitine emerged as the strongest marker for TBM, followed by quinolinic acid from the tryptophan-kynurenine pathway. Both metabolites showed moderate to strong correlations with basal enhancement and hydrocephalus, key radiological features of TBM. Most amino acids, including but not limited to proline, glycine, alanine, lysine and valine were significantly elevated in TBM compared to controls and VM, whereas tryptophan was significantly reduced in both TBM and VM and showed limited value in distinguishing between them. Tryptophan also demonstrated a negative correlation with CSF lymphocyte counts.

Conclusion: Free carnitine and quinolinic acid could contribute as potential markers for the diagnosis of TBM. Altered carnitine and amino acid metabolism suggest major disruptions in fatty acid and energy metabolism in TBM. CSF tryptophan depletion in both TBM and VM, together with its negative correlation with lymphocytes, reflects an activation of indoleamine-2,3-dioxygenase as a shared immune response in meningitis. At the same time, the elevation in kynurenine metabolites highlights enhanced activation of the tryptophan-kynurenine pathway in TBM.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1128 A Metabolomics and Transcriptomics Reveal Hippocampal Lipid Remodeling and Reduced Energy Demand Following Chronic Cannabidiol Treatment

PRESENTING AUTHOR: ***Sacaia Alvim Santos Romani, University of Campinas - UNICAMP, Brazil***

CO-AUTHORS: *João Paulo Dorta Machado, André Schwambach Vieira, Alessandra Sussulini*

SUB-THEME: Neurological disorders

Cannabidiol (CBD) is FDA-approved for the treatment of severe epilepsy. However, molecular mechanisms underlying its therapeutic effects remain unclear. Given the hippocampus role in seizure generation and neuroplasticity, understanding how chronic CBD exposure reshapes molecular pathways in this region is essential to elucidate its mechanism of action. Therefore, we hypothesized that sustained CBD treatment induces coordinated metabolic and transcriptional remodeling, thereby modulating pathways relevant to neuronal function and plasticity. To test this hypothesis, we investigated hippocampal molecular alterations in C57BL/6 mice following 7 days of CBD treatment using a multi-omics approach.

Untargeted metabolomics was performed by LC-HRMS after biphasic extraction, enabling analysis of polar metabolites by HILIC and less polar compounds by RPLC with acquisition in both ionization modes. Data processing and metabolite annotation were conducted using MZmine, GNPS, and SIRIUS, with quality-control filtering to ensure robust metabolite detection and annotation. Statistical analyses were performed in MetaboAnalyst and R. For transcriptomics, RNA from the hippocampal CA1 subregion was sequenced on an Illumina HiSeq 2500 platform, aligned using the STAR, and differential expression assessed with DESeq2. Multi-omics integration was performed through joint pathway analysis.

Metabolomics revealed increased membrane phospholipids, including phosphatidylcholines, phosphatidylethanolamines, and phosphatidylserines, suggesting reinforcement of neuronal membrane composition and synaptic stability. Neuroprotective lysophosphatidylethanolamines (LPE 18:1 and LPE 22:6) and the mitochondrial antioxidant coenzyme Q10 were also elevated, indicating enhanced antioxidant capacity and mitochondrial support. Transcriptomics showed downregulation of genes involved in energy metabolism, including components of the electron transport chain and ribosomal biogenesis.

Integrated multi-omics analysis revealed concerted molecular adaptation characterized by membrane lipid remodeling and enhanced antioxidant defenses alongside reduced neuronal energy demand. These findings suggest a previously unreported metabolic mechanism underlying the antiepileptic effects of chronic CBD treatment and demonstrate the power of multi-omics integration for elucidating biochemical mechanisms in neuropharmacology.

1129 B Circulating metabolomic changes in Lennox-Gastaut syndrome and their correlation with clinico-radiological severity

PRESENTING AUTHOR: ***Aditi Pandey, Centre of BioMedical Research, India***

CO-AUTHORS: *Jayantee Kalita, Firoz M.Nizami, Ashish K.Dubey*

SUB-THEME: Neurological disorders

Lennox-Gastaut syndrome (LGS) is an epileptic encephalopathy characterized by multiple types of seizures typically occurring between 1 and 7 years of age, cognitive impairment and characteristic electroencephalographic abnormalities. There is no definite cure for this condition; the seizures can be managed to some extent through medical, dietary and sometimes surgical interventions. LGS is also frequently refractory to anti-seizure medication (ASM).

Circulating metabolomic profile may give insight into the ongoing metabolic pathway abnormalities in these patients, but there is no such study. We report NMR-based metabolomic profile in LGS and its association with clinical severity, MRI changes and EEG findings. LGS children between 2-18 years were included based on clinical and EEG diagnostic criteria. Detailed neurological examinations, frequency and type of seizures, EEG changes, cranial MRI and NMR-based serum metabolomic profile were measured. The Clinical Global Impairment Severity Scale (CGI-S) was used to rate the severity of LGS. Twenty-six LGS patients and 11 healthy matched controls were included. The median age of the patients was 6 (range 2-17) years, and 19 were males. Their median CGI-S score was 6, and all had more than one type of seizure.

Spectra were recorded on 800 MHz NMR spectrometer and eight metabolites namely lactate, glucose, glutamate, pyruvate, glutamine, glycine, citrate and creatinine were crucial for discrimination of LGS from the controls, among which glutamate was upregulated and citrate, pyruvate, and glutamine were downregulated in LGS. Glutamate was associated with developmental quotient ($r = -0.48$) and pyruvate with focal seizures ($r = 0.47$) and cystic encephalomalacia on cranial MRI ($p = 0.02$). NMR metabolomic profile including glutamate, glutamine, glycine, glucose, pyruvate, lactate, citrate and creatinine can

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

discriminate LGS from the controls. In view of significant perturbations in glutamate, the effect of anti-glutamatergic ASM may be explored in controlling seizures and brain damage.

1130 A UHPLC-QTOF-based metabolomics reveals changes in the plasma and cardiac metabolome of mice fed Aristotelia chilensis (maqui)

PRESENTING AUTHOR: ***Lia Olivares, Universidad de Cocepción, Chile***

CO-AUTHORS: *Lia Olivares-Caro, Daniela Nova-Baza, Oscar Ramírez, Danilo Escobar-Avello, Jorge Fuentealba*, Claudia Mardones**

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Cardiovascular disease (CVD) is one of the leading causes of death in Chile and worldwide, representing a major public health problem. Polyphenols are compounds commonly found in vegetables, fruits, and other foods. These bioactive substances have demonstrated antioxidant properties, playing a crucial role in the prevention of chronic diseases by reducing oxidative stress in the body. It has been reported that Maqui (*Aristotelia chilensis*) possesses a high polyphenolic content and is capable of providing beneficial effects on human health. Its main polyphenols include anthocyanins, alkaloids, flavonoids, coumarins, ferulic acid, and caffeic acid. The aim of this study was to evaluate whether chronic consumption of maqui-enriched murine food produces changes in the plasma and cardiac metabolome of C57BL/6J mice that could potentially be associated with CVD prevention. Using a metabolomics strategy based on UHPLC-QTOF, changes in the plasma and cardiac metabolome of mice that consumed maqui for a period of 8 months were evaluated (C: control; A: maqui). Methanolic extracts from murine plasma and heart tissue were analyzed randomly by UHPLC-DAD-QTOF in negative ionization mode. Quality control (QC) samples were included. Subsequently, the obtained data were processed using MetaboScape 3.0 and MetaboAnalyst 6.0 software. Significant features were selected using Volcano-plot analysis (FC: 1.5, $p < 0.05$).The results showed a spontaneous separation between both groups (C-A) using exploratory methods (PCA). Quality control samples clustered in the center of the PCA, validating the instrumental and technical reproducibility of the analysis. Principal components (PC) 1 and 2 in the plasma and heart analyses explained 66.3% and 65.4% of the variance, respectively. Volcano-plot analysis identified 42 and 57 significant features in heart and plasma samples, respectively. In conclusion, maqui-supplemented feeding induces changes in the plasma and cardiac metabolome that could be associated with cardiovascular protection, making the identification of significant metabolites essential. FONDEQUIPEQM170023,FONDECYT1230625,FONDECYT3240171

1131 B Characterising marathon-induced metabolic perturbations and subsequent recovery thereof using untargeted 1H-NMR metabolomics

PRESENTING AUTHOR: ***Du Toit Loots, BioMMet, North-West University, South Africa***

CO-AUTHORS: *Rachelle Bester, Zinandré Stander, Shayne Mason, Karen Keane, Glyn Howatson, Tom Clifford, Emma Stevenson*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Introduction: Although physical activity is a health-promoting, popular global pastime, participation in extreme endurance events results in numerous adverse metabolic, immunologic and physiological perturbations, that diminish athletic performance and adversely affect the overall health of an athlete, especially in the absence of sufficient recovery. A comprehensive understanding of the metabolic changes occurring during and following extreme endurance events can inform the development of improved training and recovery strategies.

Methods: This investigation employed an untargeted proton nuclear magnetic resonance (^{1}H-NMR) metabolomics approach to characterise the changing serum metabolite profiles of 15 athletes at 4 time points (pre-, immediately post-, 24 h post-, and 48 h post-marathon).

RESULTS: The results indicate 26 metabolites to significantly fluctuate, indicating dramatic changes to ATP and glycogen use and synthesis, fat catabolism, aerobic and anaerobic glycolysis, ketogenesis, amino acid catabolism (in particular the BCAAs) and the TCA cycle, in response to the high energy demands of a marathon. Metabolites associated with muscle and kidney damage, changes to the gut microbiota, energy drink consumption, and delayed-onset muscle soreness during the recovery period, were also significantly altered. Although a complete recovery of those metabolites associated with the energy-producing pathways and fuel substrate stores occurred within a 48-hour recovery period, several other metabolites remained perturbed beyond this period (phenylalanine, tyrosine, BCAAs and proline), indicating incomplete recovery of other physiological processes.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

CONCLUSION: This study provides a comprehensive description of the marathon-induced metabolic changes and post marathon recovery and additionally suggests pre- and post-marathon nutrition and supplementation strategies (involving fats/ketones, the type of carbohydrate, phenylalanine, tyrosine, BCAAs and proline) for improving the health and performance of athletes who participate in such extreme endurance events. Furthermore, the "late-stage recovery" metabolite markers identified may also later be investigated for personalized monitoring of athletic health/recovery/performance.

1132 A Evaluating metabolic adaptations to high- versus low-nutrient diets in dairy cows through NMR-based metabolomics

PRESENTING AUTHOR: ***Victoria Gomez, CENUR Litoral Norte, Universidad de la República, Uruguay***

CO-AUTHORS: *Alberto Casal, Andrés López-Radcenco, Guillermo Moyna*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Diets based in total mixed ration (TMR) enhance the performance of dairy cows, resulting in increased milk yield, improved body condition scores, and better metabolic status and reproductive performance. In contrast, pasture-based diets provide cost-effective, high-nutritional-value feeding strategies that positively impact animal welfare and environmental sustainability. When dairy cows transition from 100% TMR to pasture-based feeding regimes, their metabolism rapidly adapts to changes in the quantity and type of nutrients absorbed. Metabolomics offers a comprehensive method to examine these alterations in metabolism and nutrient mobilization across various biofluids. This study applies NMR-based metabolomics to investigate metabolic changes, shifts in energy balance, and nutrient dynamics. We analyzed the serum, urine, and milk metabolic profiles of twelve cows initially fed a high-nutrient TMR diet in a controlled environment before transitioning to a pasture-based system. Samples were collected during the TMR phase, and two and ten days after the transition. PCA of ^{1}H NMR biofluid profiles revealed a clear differentiation between the two feeding systems, highlighting metabolic trends associated with the dietary transition and subsequent adaptation. Although most urinary nitrogen is excreted as urea, OPLS-DA identified significant differences in minor nitrogen species, including hippurate, creatine, N-methylamines, and TMAO. OPLS-DA of serum ^{1}H NMR profiles also revealed alterations in nitrogen metabolites such as choline, betaine, dimethylglycine, and methylamines. Additionally, we observed alterations in metabolites linked to energy metabolism, including lactate, b-hydroxybutyrate, alanine, acetate, and isobutyrate. These findings confirm that nitrogen metabolism is strongly influenced by diet, particularly in grass-rich systems with high crude protein content. Initially, the milk metabolite profile reflects feeding system changes, but metabolic shifts quickly compensate them to maintain milk nutritional composition. Our results highlight the importance of metabolomics-based approaches in the development of sustainable nutrition and management systems that focus on increasing production levels while ensuring animal welfare.

1133 B Whole-Grain Rye Feeding Drives Tissue-Specific Host–Microbial Metabolic Responses Beyond Conventional Clinical Endpoints in a Mouse Model

PRESENTING AUTHOR: ***Ambrin Farizah Babu, University Of Turku, Finland***

CO-AUTHORS: *Marjo Tuomainen, Artur Sannikov, Tomi Tuomainen, Ville Koistinen, Topi Meuronen, Marko Lehtonen, Olli Kärkkäinen, Iman Zarei, Retu Haikonen, Anna Kårlund, Pasi Tavi, Kati Hanhineva*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Dietary interventions for metabolic diseases are evaluated using circulating clinical risk markers; however, they provide limited insight into tissue-specific mechanisms and may overlook functional differences between diets with similar clinical phenotypes. This limitation is particularly relevant for complex whole foods such as whole-grain rye (WGR), a Nordic staple commonly consumed as sourdough-fermented bread, where fermentation may alter nutrient and phytochemical availability, fiber structure, and gut-microbial utilisation.

To investigate whether fermentation shapes host metabolic responses to rye bread (RB) that are not captured by conventional clinical endpoints, mice were fed low-fat (LF) diets supplemented with fermented (LF-FRB) or unfermented rye bread (LF-URB). Systemic and tissue-specific responses were assessed by integrating untargeted metabolomics across plasma, heart, muscle, spatially resolved intestinal tissues, and feces with fecal metagenomics.

The LF-background enabled detection of functional metabolic responses to WGR without confounding diet-induced metabolic disturbances. Despite indistinguishable clinical phenotypes (body weight, plasma glucose, insulin, liver, and lipid parameters) across diets, both RBs showed higher betaines and tryptophan-derived microbial metabolites along the gut–plasma axis. Fermentation produced distinct tissue metabolic signatures: LF-FRB uniquely promoted lipid remodeling in muscle and intestinal tissues, characterized by increased acylcarnitines, glycerophospholipids, sphingolipids, and monoacylglycerols, whereas LF-URB showed higher antioxidant compounds (e.g., ergothionine) and lowered potentially

harmful microbial metabolites, including p-cresol sulphate. Metagenomic analysis revealed diet-associated shifts in microbial taxa, including lower Adlercreutzia mucosicola and higher Lachnospiraceae taxa in both RBs, and lower Bacteroides acidifaciens in LF-URB; all previously linked to mucosal and metabolic functions in perturbed contexts.

These findings provide the first systems-level multi-organ characterization of WGR, revealing fermentation-driven metabolic and microbial divergence despite indistinguishable clinical phenotypes. Integrated multi-compartment metabolomics and metagenomics indicate that tissue-level metabolic responses to food-processing may precede measurable clinical changes, highlight early metabolic adaptations, and provide mechanistic insight into diet–microbiome interactions relevant to metabolic health.

1134 A Metabolomics identifies the impact of a One Health approach applied to the rapeseed food chain on animal and human health

PRESENTING AUTHOR: ★ ***Katia Mekbel, AMU, France***

CO-AUTHORS: *Sophie Lemosquet, Florence Gondret, Têko Gouyou, Alain Bouchereau, Jean-Christophe Avice, Jacques Trouverie, Catherine Tardivel, Ljubica Svilar, Claire Vigor, Jean-Marie Galano, Thierry Durand, Jean-Charles Martin*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Oilseed rape is one of the major oilseed crops cultivated in France and Europe, and a source of antioxidant and anti-inflammatory bioactive compounds, including polyphenols and glucosinolates. However, the effects of genotype and processing methods on the transfer of these bioactives along the food chain and their health impact on animal and human consumers remain poorly understood.

This study investigated whether rapeseed genotype and processing technology affect the metabolic systems of pre-calving heifers, their post-delivery milk quality and those of their milk-consuming calves and ob/ob mice. Two contrasting oilseed rape genotypes, Mambo and Bonanza (high and low antioxidant potential, respectively), were processed to cake using either a conventional degradative method or an optimized bioactive-preserving method, resulting in 4 experimental diets. Forty-five cows were fed these diets for 9 weeks, including 4 weeks during gestation and 5 weeks during lactation. The milk produced was then administered to their calves and to ob/ob mice.

Primary metabolomics analyses were performed in cow plasma, in milk, and in the plasma of milk-consuming animals using LC-HRMS-based suspect screening combined with multivariate statistical analyses. Targeted LC-HRMS quantification of 17 isoprostanoids and prostaglandins (PGE2, PGF2α) was used to assess oxidative stress and inflammatory status.

The optimized Mambo diet induced a distinct metabolic signature associated with modulation of oxidative stress, inflammatory pathways, and muscle metabolism in all animal models. This was accompanied by specific changes in the milk metabolome of dairy cows. Targeted analyses confirmed significant reductions in isoprostanoids and inflammatory prostaglandins under this dietary condition.

Overall, our findings demonstrate that rapeseed genotype and processing technology influence meal quality, as well as animal and consumer health. Importantly, this study illustrates how metabolomics can integrate the effects of rapeseed genotype and processing technology on metabolism and health across the food chain, providing a mechanistic link between farm practices and biological outcomes.

1135 B Unraveling Convergent Phenolic Metabolites: Human Evidence that Distinct Plant Extracts Generate Shared Bioactive Metabolites

PRESENTING AUTHOR: ***Álvaro Fernández-Ochoa, University of Granada, Spain***

CO-AUTHORS: *Patricia Fernández-Moreno, María del Carmen Villegas-Aguilar, María de la Luz Cádiz-Gurrea, Noelia Sánchez-Marzo, María Herranz-López, Enrique Barrajón-Catalán, Vicente Micol, Martina Mantini, María Cristina Barbalace, Cristina Angeloni, David Arráez-Román, Antonio Segura-Carretero, Álvaro Fernández-Ochoa*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Introduction and Aim: Research on dietary bioactive compounds often focuses on native phytochemicals, overlooking that the molecules reaching human circulation are metabolites generated through host and microbial biotransformation, which can be the true bioactive effectors involved in the modulation of non-communicable chronic diseases. Within this framework, we propose the convergent metabolites hypothesis, whereby structurally distinct phenolic precursors from different botanical sources generate identical bioavailable metabolites. As a proof of concept, this study aimed to identify convergent metabolites derived from Olea europaea (OE) and Lippia citriodora (LC) leaf extracts and evaluate their biological relevance.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Methodology: An acute double-blind human intervention trial was conducted in 25 healthy volunteers receiving a single 500 mg dose of OE or LC leaf extracts. Multiple plasma and urine samples were collected over 24 h and analysed using an untargeted HPLC-ESI-QTOF-MS metabolomics workflow with QC-based analytical quality control and Feature-Based Molecular Networking. The bioactivity of selected metabolites was evaluated in vitro in SH-SY5Y neuronal and BV-2 microglial cell models.

Findings and Significance: Untargeted profiling revealed 66 circulating metabolites in plasma (9 convergent) and 808 altered metabolites in urine (92 convergent), highlighting urine as a key biofluid for capturing phenolic-derived metabolites. Hydroxytyrosol and homovanillic acid conjugates emerged as major convergent metabolic nodes. In vitro assays confirmed antioxidant activity of hydroxytyrosol-related compounds in neuronal cells. Shared metabolites showed source-dependent nutrikinetic profiles, with OE-derived metabolites reaching Tmax earlier than those derived from LC, suggesting that combining botanical sources may extend systemic exposure to bioactive metabolites.

Contribution to the Discipline: This study demonstrates metabolic convergence across different plant matrices and highlights circulating metabolites as functional effectors of dietary phenolics. These findings provide insight into how distinct plant sources generate shared bioactive metabolites in humans and support future strategies for designing functional foods or nutraceutical with enhanced biological impact.

1136 A Validating urinary metabolites as non-invasive biomarkers of the omega-3 index: A nutrikinetics study

PRESENTING AUTHOR: ***Mariana Buitrago, McMaster University, Canada***

CO-AUTHORS: *Brittany C. MacIntyre, David M. Mutch, Philip Britz-McKibbin*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

The omega-3 index (O3I) is a clinically relevant biomarker of cardiovascular health that reflects the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content of erythrocyte membrane phospholipids. Despite its value, routine O3I screening is limited by the need for blood collection and the short-term stability of erythrocytes in biorepositories. To address this gap, we previously reported 17 urinary metabolites as potential non-invasive biomarkers of the O3I in a secondary randomized, placebo-controlled trial of high-dose EPA and DHA only supplementation relative to olive oil. Herein, we outline results from the Biomonitoring of the Omega-3 Index (BIO-OMIX) validation study, designed to further investigate dynamic temporal changes in urinary metabolites associated with O3I measured in serum and red blood cells. Twenty-four healthy adults (n = 12 F, n = 12 M; 19–30 y) consumed ~3 g/day of an omega-3 fish oil supplement (OMEGOR® Vitality 1000) for 8 weeks, followed by an 8-week washout. First-void morning urine samples were collected daily for three days and then every two weeks following the supplementation and washout periods. Urine samples were analyzed using multisegment injection–capillary electrophoresis–mass spectrometry (MSI-CE-MS) under positive and negative ionization with full-scan acquisition. Both targeted and untargeted data analysis approaches independently validated time-dependent urinary metabolites associated with O3I changes. A previously unidentified divalent anion showed a robust, reversible temporal response and moderate correlations with plasma and erythrocyte O3I (p

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1137 B Postprandial cholic acid exposure modulates lipid handling and hepatic inflammatory signaling in a rat model

PRESENTING AUTHOR: ***Larissa Rodrigues, University of São Paulo, Brazil***

CO-AUTHORS: *Lucas Xavier Martins de Oliveira, Tiago Augusto Cavalcanti de Albuquerque Oliveira Abib, Ana Luiza Oliveira Souza, Wermerson Assunção Barroso, Carlos Mario Donado-Pestana, Karsten Hiller, Lorraine Brennan, Jarlei Fiamoncini*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

During the postprandial period, changes in circulating nutrients and signaling molecules trigger coordinated physiological responses affecting energy metabolism and inducing a transient inflammatory reaction. Among these metabolites, bile acids (BA) show one of the largest increases after meal ingestion. Despite growing in vitro evidence supporting their role as modulators of metabolism and inflammation, their in vivo postprandial effects remain poorly characterized. This study aimed to characterize BA-mediated regulation of lipid handling and inflammation during the postprandial phase using integrated metabolomics and molecular approach in a rat model. Following a 10-h fast, seventy Sprague–Dawley rats were allocated to seven groups. Experiments began at the onset of the dark cycle (18:00 h) to align with natural feeding behavior. A baseline group was euthanized under fasting conditions; remaining animals consumed a mixed energy-dense meal (60% lipid) and received either vehicle (control) or cholic acid (CA, 360 mg/kg) by gavage immediately post-feeding. Animals of each group were euthanized at 60, 120, or 240 min post-intervention. Meal ingestion produced marked postprandial metabolic responses. Plasma triglycerides and cholesterol peaked at 120 min (p p = 0.0135, respectively), and changes in lipoprotein composition were observed across the postprandial time course. CA attenuated the plasma triglyceride response versus control (p = 0.0159), driven primarily by lower VLDL-triglyceride content, and reduced non-esterified fatty acid levels at 120 min (p = 0.0318). Hepatic triglycerides and cholesterol peaked at 120 min (~50% and ~40%); broader shifts in the hepatic metabolite profile were also detected. Postprandial NF-κB pathway activation was evidenced by elevated hepatic TLR4 and phosphorylated NF-κB, and was more pronounced in controls than in CA-treated animals. These findings demonstrate that acute postprandial CA exposure attenuates triglyceride-rich lipoprotein accumulation and might affect hepatic NF-κB inflammatory signaling, revealing a dual metabolic and anti-inflammatory role for BA in vivo.

1138 A Sensitive, high-coverage plasma metabolomics in diet-induced obesity enabled by parallel acquisition method

PRESENTING AUTHOR: ***Rafael Melani, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Rafael Melani, Bashar Amer, Brandon Bills, Sunandini Yedla, Rahul Ravi Deshpande, Daniel Hermanson, Thomas Moehring, Susan S. Bird*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Advances in plasma metabolomics demand mass spectrometers (MS) that combine high sensitivity, rapid acquisition, and comprehensive structural characterization. Here, a Tribrid MS was evaluated for enhanced sensitivity, faster polarity switching, and improved parallelization between the 2 mass analyzers. These advances enable concurrent acquisition of high-resolution MS^1 and rapid MS^n within a single experiment, increasing data density and throughput while minimizing duty-cycle constraints.

Analytical performance was benchmarked using NIST SRM 1950 plasma with a metabolomics strategy that integrates targeted quantitation and untargeted metabolomics into a single workflow. Plasma samples spiked with isotope-labeled amino acids were extracted with methanol and analyzed by reversed-phase LC-MS. Acquisition combined sensitive tMS^2 quantitation in parallel with high resolution-based MS^1 and accelerated MS^n scans for confident metabolite annotation.

The tMS^2 quantitation demonstrated high quantitative performance, achieving limits of quantification of ~0.5 fmol on column and limits of detection of ~0.25 fmol for multiple amino acids, with linear dynamic ranges spanning up to six orders of magnitude. Parallel high-resolution acquisition delivered sub-ppm mass accuracy, stable retention times, and consistent signal response across replicates. Faster polarity switching and enhanced parallelization improved feature fragmentation rates and metabolite annotation coverage, particularly when combined with intelligent data-dependent acquisition.

The optimized workflow was applied to plasma samples from diet-induced obesity mouse models (male and female mice fed high- or low-fat diets). The enhanced capabilities of the method enabled deep metabolome coverage and reproducible profiling, supporting robust comparative analysis of diet- and sex-associated metabolic differences in complex biological samples.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1139 B Acute and Short-Term Calafate-derivated products Consumption Modulates Plasma and Urinary Metabolomic Profiles in Healthy Men

PRESENTING AUTHOR: ***Mariana Opazo Torres, Universidad de Concepción, Chile***

CO-AUTHORS: *Daniela Nova, Lía Olivares, Francisca Vidal, Claudia Radjokovic, Andrea Sánchez, Luis Bustamante, Claudia Mardones*

SUB-THEME: Nutrimetabolomics & dietary biomarkers

Calafate is a native Patagonian berry rich in anthocyanins and other phenolic compounds with reported antioxidant and metabolic regulatory properties, positioning it as a promising candidate within the field of functional foods and nutraceuticals. However, evidence regarding its metabolic effects in humans remains limited. This research evaluated the impact of acute and short-term calafate consumption on plasma and urinary metabolomic profiles in healthy young men. A commercially available juice fortified with dehydrated calafate was chemically characterized using UHPLC-DAD-QTOF-MS/MS. Antioxidant capacity was assessed using CUPRAC and ORAC assays. In a longitudinal pilot intervention, healthy young men consumed the beverage once daily for five days. On day 1, blood samples were collected during the postprandial phase (0,30,60,120,240 min) following overnight fasting, while urine samples were obtained at baseline and day 5. Metabolomic changes were evaluated using UHPLC-QTOF-MS/MS-based untargeted metabolomics with reverse-phase C18 and HILIC separations. Data processing was carried out using MSDial and MetaboAnalyst 6.0 software. Multivariate and univariate analyses, including volcano plots ($p<0.05$; FC ≥ 2) and one-way ANOVA with Tukey's post hoc test, were performed to identify discriminant metabolites associated with calafate intake. Databases (KEGG, HMDB, etc.) were used for annotation. The beverage exhibited high antioxidant capacity and anthocyanin content. Consumption significantly increased plasma antioxidant capacity and total phenolic content 4 h post-intake. Metabolomic analysis identified 10 discriminant plasma metabolites ($p<0.05$), mainly associated with fatty acid metabolism. Glycochenodeoxycholic acid showed a dynamic postprandial response, with a significant increase ($p<0.05$) at 1 h followed by a decline at 4 h. Urinary metabolomic profiles also showed distinct clustering between day 1 and day 5 samples, while metabolite annotation remains ongoing. These findings suggest that calafate consumption in humans modulates antioxidant status, lipid, and bile acid metabolism, representing the first metabolomics study characterizing metabolic responses to calafate intake.

1140 A Selective NMR Signal Suppression to Enhance Sensitivity in Metabolomics and Lipidomics

PRESENTING AUTHOR: ***Abdul-Hamid Emwas, KAUST, Saudi Arabia***

CO-AUTHORS: *Upendra Singh, Ruba Al-Nemi, Fatimah Alahmari, Mariusz Jaremko*

SUB-THEME: Other

Abstract: NMR spectroscopy is a central tool in metabolomics and lipidomics. Yet, its sensitivity in complex biological samples is often limited by dominant high-abundance signals, such as sugars in plant-derived materials and methyl/methylene groups in lipid-rich systems. These intense resonances mask low-concentration metabolites of biological importance.

We present optimized NMR acquisition strategies based on selective signal suppression and band-selective excitation, integrating water presaturation with excitation sculpting (1D presat-^{1}H-ES) and targeted suppression approaches applied to 1D and 2D experiments (J-resolved, TOCSY, HSQC). Validation across sugar- and lipid-rich samples, including dates, honey, serum, dairy products, and plant oils, demonstrated up to 2–4 fold enhancement of weak signals, improved cross-peak visibility, and better multivariate discrimination [1-2].

This approach expands metabolite coverage, improves comparative quantification, and reduces acquisition time, supporting more robust metabolomics and lipidomics analyses in complex biological matrices.

References: Singh U, Emwas AH, Jaremko M. Enhancement of weak signals by applying a suppression method to high-intense methyl and methylene signals of lipids in NMR spectroscopy. Rsc Advances. 2024;14(37):26873-83.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1141 B Association of Human Retinal and Choroidal Tissue Metabolic Profiling with Age-related Macular Degeneration

PRESENTING AUTHOR: ***Deeba Husain, Harvard Medical School, United States***

CO-AUTHORS: *Ines Lains, Kevin Mendez, Roshni Bhat, Haemin Kang, David M. Wu, Ivana K. Kim, John B. Miller, Demetrios G. Vavvas, Jessica Lasky-Su, Joan W. Miller*

SUB-THEME: Other

Purpose: We and others have shown that plasma metabolomics can provide insights into the pathophysiology of age-related macular degeneration (AMD). However, human retinal and choroidal tissue metabolomics remains largely unexplored, and it remains unclear how circulating metabolomic profiles relate to in situ changes in AMD. This work aimed to evaluate human tissue metabolomic profiles in AMD and to compare them to our previously reported results. Methods: Prospectively designed cross-sectional study including 38 human donor eyes (24 AMD, 14 controls) over age 50, enucleated within 6 hours post-mortem. Color fundus photographs were taken to assess AMD. Both a 6 mm macular and peripheral punch were obtained. The neurosensory retina was then separated from retinal pigment epithelium (RPE)/choroid from these samples. All samples were snap frozen, stored at -80C in sterile vials and were shipped frozen. Non-targeted mass spectrometry analysis was conducted by Metabolon Inc. Multivariate logistic regression models, accounting for age and gender, were used for analysis with $p < 0.05$ determined as statistically significant. Results: In macula neurosensory retinal tissue, 45 metabolites (out of 559 measured) were significantly associated with AMD. Most of them were lipids (n=35, 77.7%), including lysophospholipids (7), phosphatidylcholines (11), phosphatidylethanolamines (3) and phosphatidylinositols (3). In the macular RPE/choroidal tissue there were 6 metabolites (among 552) significantly associated with presence of AMD, mostly (n=4, 66.67%) lipids. In the peripheral neurosensory retina, 5 (among 554) metabolites were significantly associated with AMD, with the majority (n=3) being amino acids. The peripheral RPE/Choroid showed 8 (among 565) metabolites significantly associated with AMD. When comparing these results to our previous reports in human plasma, the majority of the significant differences observed overlapped, with the strongest signals being seen with phosphatidylcholine and phosphatidylethanolamine metabolites. Conclusions: To our knowledge, this is the first study investigating metabolomic profiles of human retinal/choroidal tissue in AMD.

1142 A Linking indices of high-intensity interval exercise to changes in the human plasma metabolome

PRESENTING AUTHOR: ***Glykeria Avgerinou, Aristotle University of Thessaloniki, Greece***

CO-AUTHORS: *Daniel Marques de Sa e Silva, Anatoli Petridou, Helen Gika, Georgios Theodoridis, Vassilis Mougios*

SUB-THEME: Other

High-intensity interval exercise (HIIE) induces profound and rapid shifts in energy metabolism, yet the metabolic underpinnings of these responses and the degree to which they vary between individuals remain incompletely characterized. Standard physiological indices (such as respiratory gases) provide valuable but limited insight into the complexity of exercise metabolism. Plasma metabolomics can capture the broad biochemical landscape of responses to HIIE. However, associations between physiological indices and metabolic shifts remain unexplored.

This study aimed to examine associations between exercise variables (specifically, indices of aerobic metabolism) and plasma metabolite profiles after a HIIE intervention.

Twenty-five recreationally active adults (13 men, 12 women) completed two identical HIIE sessions separated by at least one week, after standardized nutrition. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER) were continuously monitored via ergospirometry. Carbohydrate oxidation, fat oxidation, and energy expenditure were calculated from these variables. Venous blood was collected at baseline, immediately post-, and one hour post-exercise for the preparation of plasma, which was analyzed using two complementary metabolomics platforms, hydrophilic interaction liquid chromatography and gas chromatography, coupled to mass spectrometry. Changes in metabolite levels were correlated with physiological indices using Pearson's and Spearman's correlations, as appropriate.

Most indices of aerobic metabolism during HIIE were highly replicable between sessions. Energy derived primarily from carbohydrate oxidation (by 87% on average), with a trend (decrease in the 2nd session) reminiscent of adaptations to high-intensity interval training. Fourteen annotated metabolites (alanine, arachidonate, ethanolamine, glutamine, ketoleucine, acetylcarnitine, glycine, hypoxanthine, lactate, phosphate, propanoate, pyruvate, 2-hydroxyisobutyrate, glutamate) exhibited consistent shifts in both exercise sessions. Correlation analysis showed novel metabolite-specific associations between these shifts and physiological indices, thus contributing to the biochemical interpretation of metabolic changes with HIIE.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

These findings highlight the value of integrating dual-platform metabolomics with standard physiological monitoring to better characterize exercise metabolism.

1143 B Longitudinal assessment of gut microbiome alterations associated with psychedelic-assisted psychotherapy for PTSD

PRESENTING AUTHOR: ***Savannah Rivera, Southampton University, United Kingdom***

CO-AUTHORS: *Giorgia Caspani, Veronica Santiago, Madhuriben Panchal, Yi Wu, Franklin Nobrega, Jonathan Swann*

SUB-THEME: Other

First-line treatments of PTSD generally involve a combination of psychotherapy and medical treatments; however, these often have limited efficacy, with more than a third of people not fully achieving complete remission. The bidirectional relationship between the gut-brain axis has led to findings implicating gut dysregulation in individuals with PTSD as one of the key factors driving its pathogenesis. Psychedelics, in turn, have emerged as a promising adjunct to psychotherapy for neuropsychiatric disorders, potentially via modulation of the gut microbiome.

This study utilises a combination of metabolomic (liquid chromatography mass spectrometry [LC-MS]) and metagenomic (full shotgun sequencing) techniques to capture the longitudinal dysregulation of gut profiles in stool and blood samples of military veterans with PTSD (N=18) involved in a psychedelic ceremonial retreat in the Peruvian Amazon forest. Samples were collected at timepoint T1 (baseline), T2 (post-ayahuasca) and T3 (3-month follow-up) along with psychological assessments for PTSD (PCL-5), depression (BDI-II) and anxiety (STAI). Uni- and multivariate comparison analysis were conducted in R and significant differences (p<0.05) evaluated by t-tests.

Significant and persistent metabolite alterations were observed particularly in sex-specific models using blood samples from participants at T1 versus T3 including lipids such as PS 34:2 and L-specific amino acids including tyrosine and phenylalanine, precursors to dopamine. Further, notable alterations in fatty acids such as DiCA(12:0) were observed in faecal samples between subclinical and clinical PTSD.

While previous studies have already implicated the potential for dietary lipids to be related to the pathogenesis of PTSD, our next study phase will aim to identify the pattern of dysregulation in the gut microbiota throughout the timepoints and establish their association with our findings. In doing so, expand our understanding of the mechanisms employed in the gut-brain axis.

1144 A Glycaemia-residualised plasma metabolomics identifies progression-associated markers in prediabetes

PRESENTING AUTHOR: ***Joanna Godzien, Medical University of Bialystok, Poland***

CO-AUTHORS: *Joanna Godzien, Krzysztof Solowiej, Patrycja Mojsak, Julia Zelkowska, Katarzyna Miniewska, Magdalena Bossowska, Filip Bossowski, Katarzyna Maliszewska, Anna Cito-Rojewska, Paulina Konopka, Lukasz Szczerbinski, Edyta Adamska-Patruno, Maria Górska, Adam Kretowski, Michal Ciborowski*

SUB-THEME: Other

Prediabetes is metabolically heterogeneous: some individuals remain stable, whereas others progress to type 2 diabetes despite comparable baseline glycaemia. This study used glucose-adjusted plasma metabolomics to distinguish metabolites reflecting current glycaemic status from those associated with future progression.

Baseline plasma samples from 42 participants with prediabetes from the 1000PLUS cohort were analysed using complementary LC-MS and GC-MS workflows. Over five years, 24 participants developed type 2 diabetes, and 18 remained prediabetic. Previously detected discriminatory LC-MS features and GC-MS metabolites were re-evaluated after residualising metabolite intensities against baseline 120-min glucose and HbA1c. Raw and adjusted datasets were compared using cross-validated ROC analysis, recursive feature elimination, and bootstrapped elastic net modelling.

Adjustment for glycaemic measures substantially changed the discriminatory behaviour of many metabolites, indicating that part of the original metabolomic signal reflected baseline disease severity. In contrast, a limited set of metabolites remained stable after residualisation. Lysine, LPC 18:2;O, LPC 18:1, glutamic acid, and one unannotated LC-MS feature retained similar cross-validated AUCs before and after adjustment, suggesting a relationship with progression risk beyond baseline glucose status. Lysine showed the most reproducible behaviour and was repeatedly selected in bootstrapped elastic net models. Glucose-adjusted feature selection also improved multivariate performance, with mean AUC increasing from 0.682 to 0.833 and confidence intervals becoming narrower.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

These results show that residualisation against clinically relevant glycaemic variables can refine metabolomic biomarker discovery in longitudinal prediabetes studies. The persistence of lysine and selected lysophosphatidylcholines after glucose adjustment suggests that they may represent progression-associated metabolic markers rather than markers of current glycaemia. This approach may support the development of metabolomics-based tools for identifying individuals with prediabetes at increased risk of progression to type 2 diabetes.

1145 B Metabolomics approach to physiological adaptive mechanisms in repetitive confined-water scuba diving under mild hyperoxia using ^{1}H-NMR

PRESENTING AUTHOR: ***Kyu-Bong Kim, Dankook University, South Korea***

CO-AUTHORS: *Jung Dae Lee, Hyang Yeon Kim, Suhkmann Kim, Gi-Wook Hwang, Ho-Seong Lee*

SUB-THEME: Other

Background: This study aims to elucidate the multifaceted effects of scuba diving, particularly focusing on the physiological adaptive mechanisms and metabolic aspects involved in confined-water scuba diving.

Methods: Selected 10 healthy male college students in their 20s and conducted confined-water scuba diving sessions once a day, five times a week, for 80 minutes each se ssion over a period of 8 weeks. Measurements were taken on changes in body composition, cardiorespiratory fitness, and hormonal levels. Additionally, alterations in metabolites of urine samples were observed using ^{1}H-NMR.

Results: Body fat decreased significantly at 4 and 8 weeks ($p < 0.05$). Air consumption decreased at both times ($p < 0.001$), while forced vital capacity increasedat 4 w ($p < 0.05$) and 8 w ($p < 0.01$). Physical fitness index improved at 4 w ($p < 0.01$) and 8 w ($p < 0.001$). Dopamine increased progressively at IA ($p < 0.05$), 4 w ($p < 0.01$), and 8 w ($p < 0.001$). Norepinephrine increased at 4 and 8 w ($p < 0.001$). Cortisol fell immediately and at 4 w ($p < 0.001$) but rebounded at 8 w ($p < 0.01$). In urine, 89 metabolites were detected; 9 (urea, ethanol, hippurate, 2-octenoate, creatine phosphate, taurine, glucose, serine, threonine) differed significantly after diving.

Conclusion: Based on the metabolic findings regarding the adaptive mechanisms of confined-water scuba diving, these identified metabolites could serve as key factors and significant biomarkers for understanding the adaptation mechanisms of scuba diving.

1146 A Systemic disturbances in the plasma and PBMC metabolome assessed in a mouse model of Hutchinson–Gilford progeria syndrome

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: Other

Hutchinson-Gilford progeria syndrome (HGPS) is an ultrarare genetic disorder (prevalence 1 in 18-20 million) caused by the expression of progerin, a mutant form of lamin A protein, leading to accelerated aging and premature death at an average age of 14.5 years. Currently, reliable molecular biomarkers for HGPS detection are lacking, limiting early diagnosis and disease monitoring. Thus, this study aimed to characterize the metabolomic profiles of plasma and PBMCs in a mouse model of HGPS to identify metabolic alterations linked to the disease phenotype. Plasma and PBMC samples collected from six HGPS mice and six wild-type controls were analysed using a targeted metabolomic assay, allowing profiling of ca. 500 metabolites. Orthogonal partial least squares discriminant analysis (OPLS-DA) revealed a clear separation between the HGPS and control groups in both sample types (plasma: R2=0.993, Q2=0.967, p-value=$5.765\times10^{-}18$; PBMC: R2=0.999, Q2=0.958, p-value=$4.542\times10^{-}4$). Metabolites that contributed significantly to group discrimination were selected using the OPLS-DA model (based on the p-corr and VIP values). A total of 224 metabolites in plasma and 112 in PBMCs differed significantly between groups. The most prominently affected metabolite classes included triacylglycerols (115 altered in plasma and 22 in PBMCs), phosphatidylcholines (36 and 37), lysophosphatidylcholines (11 and 3), and cholesteryl esters (11 and 3), highlighting a profound perturbation of lipid metabolism. These findings demonstrate that HGPS is associated with marked systemic metabolic alterations, particularly within lipid pathways. The identified metabolomic signatures enhance understanding of HGPS pathophysiology and may pave the way for the development of novel biomarkers to improve disease detection and potentially guide therapeutic interventions. Founding: 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.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1147 B ^{1}H NMR Based Metabolic Profiling of Endurance Horses

PRESENTING AUTHOR: ***Gabriel Anderson, Universidad de la República, Uruguay***

CO-AUTHORS: *Gabriel Anderson, Fernanda Mutay, Victoria de Brun, Gonzalo Marichal, Guillermo Moyna, Andrés López-Radcenco*

SUB-THEME: Other

Raid is an endurance discipline of major cultural, social, and economic importance in Uruguay that has grown considerably in recent years. These submaximal endurance competitions cover 60–115 km and place high physiological demands on horses. More than 60% of the animals fail to complete the events, with metabolic disorders accounting for about 70% of eliminations. However, knowledge of metabolic stressors during competition remains limited, challenging the prevention and management of these disorders. This study evaluated metabolic changes during 90 km competitions using serum samples from 50 horses participating in 8 events. Control samples were collected at admission, and post-competition samples were obtained at the end of each horse's participation, distinguishing animals eliminated at 60 km for metabolic or locomotory reasons from those that completed the race. Serum 1H NMR spectra were acquired at 500 MHz, and the processed data were analyzed by principal component analysis (PCA), orthogonal projections to latent structures discriminant analysis (OPLS-DA), and univariate metabolite analysis. Results showed that increased lactate, formate, and citrate levels, together with glucose consumption, were associated with exercise intensity, indicating marked changes in energy metabolism and higher physical effort. Higher concentrations of amino acids, creatine, allantoin, and methylated amines suggested muscle catabolism related to the use of alternative energy substrates. Horses eliminated from competition accumulated acetoacetate, a ketone body associated with an inability to meet energy demands. Overall, these findings characterize the metabolic response of horses exposed to the sustained cardiorespiratory, endocrine, and neuromuscular demands of raid competition. Integrating biochemical data with these findings may help identify biomarkers linked to stress and performance, providing useful tools to improve the health, management, and welfare of endurance horses.

1148 A High-Throughput 4D LC-TIMS-MS Reveals Drivers of Untargeted Metabolomics Coverage

PRESENTING AUTHOR: ***Tommaso Stefani, Institute Of Microbiology VAT CZ61388971, Czech Republic***

CO-AUTHORS: *Anna Michl, Michaela Plechata, Rufin Toghueo, Zdenek Kamenik*

SUB-THEME: Other

Untargeted metabolomics workflows are strongly influenced by analytical and computational choices that affect metabolome coverage and data quality. Using 4D LC-TIMS-MS (timsTOF HT), we examined these effects across more than 1000 samples including SPE-purified microbial extracts, agar-based cultures, and mammalian tissues.

Our results show that sample matrix and extraction strategy strongly shape the detectable metabolomic space. SPE-purified extracts produced relatively clean profiles, whereas solid–liquid extraction of agar cultures introduced substantial medium-derived background signals that required extensive blank subtraction to recover biological features. Collision cross section (CCS) values provided an additional orthogonal parameter for feature validation and helped maintain consistent feature annotation during multi-week analytical sequences where retention time drift occurred. Quality control samples confirmed that biological variability remained distinguishable across large cohorts when appropriate system suitability monitoring and dilution strategies were applied.

To address fragmentation across metabolomics software tools, we implemented a modular workflow integrating results from MetaboScape, SIRIUS, and GNPS into unified molecular networks visualized in Cytoscape. This integration enabled systematic clustering and annotation of large feature sets, illustrating how computational integration complements experimental design in untargeted metabolomics.

Together, these observations highlight practical factors that strongly influence metabolomics datasets and provide guidance for laboratories implementing large-scale 4D LC-TIMS-MS workflows.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1149 B Clinical Translation needs Commercialisation - but how?

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

SUB-THEME: Other

Researchers in clinical lipidomics and metabolomics often want to bring their findings into real-world use, but many are unsure how to protect and share their ideas. In many cases, the value is not a patentable invention, but the practical know-how in workflows, protocols, and data analysis. This raises questions about how to safeguard such knowledge and how to license it responsibly to interested partners.

I invite the community to openly discuss experiences, questions, and concerns around translating and commercializing such non-patent-based expertise. Together with the audience, I aim to map common challenges, collect practical examples, and co-create simple ideas for how we, as a field, can better protect, share, and license our workflows in ways that support both collaboration and clinical impact.

1150 A Analysis of Smoke's and Non-Smoker's urine using GCXGC TOF MS

PRESENTING AUTHOR: ***Maribel Hernandez, LECO MEXICO, Mexico***

CO-AUTHORS: *Tiago Schena, Joe Binkley, John Heim*

SUB-THEME: Other

Metabolomic analysis of urine samples has expanded considerably in recent years, as urine represents an ideal biological medium for the discovery of new biomarkers and the elucidation of metabolic pathways. Such insights can enhance diagnostic accuracy, refine prognostic assessments, and inform therapeutic strategies. Urine samples are easily obtained, allowing serial collection to monitor disease progression and therapeutic response [1].

Currently, metabolomic analyses are mainly performed using GC or HPLC techniques. However, more recently, comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (TOFMS) has been explored due to its ability to expand the peak capacity of chromatographic separation. This increased resolution and improved analyte characterization are essential for a deeper understanding of complex biological matrices.

This study demonstrates the capabilities of GC×GC-TOFMS systems to identify metabolomic profiles and reveal significant differences between diabetic and non-diabetic groups. On average, over 1,000 peaks were detected per sample, with a signal-to-noise ratio of 100. Approximately 25 compounds were identified as those showing the greatest variation between the two groups, including 2,2,4,4-tetramethyl octane, linolenic acid trimethylsilyl ester, cholesterol trimethylsilyl ether, and 2,4,4-trimethyl-1-pentanol, among others. Notably, 1H-purine-2,6-dione, 3,7-dihydro-3,7-dimethyl was found exclusively in the diabetic group and absent in the non-diabetic controls.

In conclusion, this research confirms that GC×GC-TOFMS technology is an excellent tool for characterizing small-molecule metabolites in urine samples. The sample grouping and Fisher ratio features within ChromaTOF enable analysts to efficiently mine data for key chemical differences and metabolites, thereby advancing the search for disease biomarkers.

Lin, C., Tian, Q., Guo, S., Xie, D., Cai, Y., Wang, Z., Chu, H., Qiu, S., Tang, S., & Zhang, A. (2024). Metabolomics for clinical biomarker discovery and therapeutic target identification. *Molecules, 29*(10), 2198.

1151 B Determining site of NAT2 mediated acetylation of vorinostat

PRESENTING AUTHOR: ***Wawrzyniec Haberek, Uppsala University, Sweden***

CO-AUTHORS: *Ioanna Tsiara, Natalia Rameika, Tobias Sjöblom, Daniel Globisch*

SUB-THEME: Pharmacometabolomics

Arylamine N-acetyltransferase 2 (NAT2) is a key metabolic enzyme responsible for the acetylation of aromatic amines and is primarily involved in xenobiotic metabolism. NAT2 activity significantly influences the pharmacokinetics of several commonly used drugs, including isoniazid, clonazepam, sulfasalazine, and various aromatic amines, hydroxylamines, and hydrazines. While NAT2 is known to acetylate amines, its role in modifying other functional groups remains underexplored.

Our recent investigation explored the involvement of NAT2 in the metabolism of histone deacetylase inhibitors (HDAC) specifically vorinostat, which contains a hydroxamic acid moiety. To identify the site of acetylation, vorinostat was subjected

to both chemical and enzymatic acetylation. The resulting compounds were analyzed using high-resolution mass spectrometry (HRMS) to confirm their identity, and 15N NMR spectroscopy was employed to determine the acetylation site.

The data demonstrated that NAT2 is capable of acetylating vorinostat at the oxygen atom of its hydroxamic acid group contrary to the enzyme's traditional classification as a nitrogen acetylating enzyme. This modification significantly enhanced the cytotoxicity of the HDAC inhibitor, indicating a potential role for NAT2 in modulating the therapeutic activity of this drug class.

These findings have implications for pharmacometabolomics and cancer precision medicine using conventional chemotherapeutic drugs, as improving their efficacy and safety may affect over 4 million cancer patients worldwide that receive these drugs as standard of care.

1152 A Multi-omics analysis reveals biochemical and immune dysregulations of HIV exposed infants by in utero exposure to antiviral drug

PRESENTING AUTHOR: ***Shuzhao Li, Jackson Laboratory, United States***

CO-AUTHORS: *Jasmine Chong, Shujian Zheng, Ashlynn Bennett, Joshua M. Mitchell, Jennifer Canniff, Michael J Johnson, Elizabeth Aiken, Shabir Madhi, Daniel Frank, Adriana Weinberg*

SUB-THEME: Pharmacometabolomics

Pregnant women with HIV control viral replication with antiretrovirals and give birth to HIV-exposed uninfected infants (HEU). The children, however, exhibit increased morbidity and mortality due to severe infections, as well as cognitive and growth abnormalities. In this study, we performed high-resolution, untargeted metabolomics and lipidomics on 123 HIV-exposed mother-baby pairs and 117 control pairs without HIV. Detailed immune cell analysis was performed on cohort blood samples, and gut microbiomes were profiled on the mothers.

High concentrations of the antiretroviral drug efavirenz and its metabolites were detected in maternal blood and cord blood. The metabolomic differences between HEU participants and controls reflect perturbed pathways of steroids, tryptophan and bile acids, and they largely consisted of metabolites that were correlated with efavirenz concentrations within the HEU group. The results suggest a major contribution of the drug to the abnormal biochemical profile of HEU infants born to mothers treated with efavirenz.

Integrative analyses of metabolomics, lipidomics, immune cell populations and gut microbiomics were performed at the levels of feature pairs, multiomics factor analysis and hierarchical community networks. The results revealed significant associations among the differentially displayed small molecules, microbiota and immune profiles between HEU and HUU maternal-fetal dyads that may contribute to the immunological defects of HEU infants.

1153 B Longitudinal Metabolomics Reveals Diet-Specific Risk Profiles: A Reference-Based Framework for Precision Nutrition

PRESENTING AUTHOR: ***Dorsa Yahya Rayat, The Metabolomics Innovation Center, Canada***

CO-AUTHORS: *David S. Wishart*

SUB-THEME: Precision health

Precision nutrition requires scalable approaches capable of dynamically monitoring individual metabolic responses to diet and interpreting these changes within a clinically meaningful framework. Although metabolomics provides direct insight into systemic biochemistry and disease risk, comprehensive longitudinal profiling combined with reference-based clinical interpretation remains limited.

This study aimed to develop a reference-based framework for longitudinal metabolomic profiling to support personalized health assessment and metabolism-guided dietary recommendations aligned with individual metabolic risk profiles.

A longitudinal N-of-1 dietary intervention study was conducted integrating plasma metabolomics with continuous physiological and behavioural monitoring across three controlled dietary phases: Fast Food, Mediterranean, and Ketogenic diets. Plasma samples were analyzed using targeted LC-MS, quantifying more than 780 metabolites and over 250 metabolite-derived sums and ratios. Metabolite concentrations were compared against established healthy reference ranges and disease-associated thresholds using a custom risk assessment framework to generate individualized metabolic signatures and diet-specific risk profiles.

Distinct diet-specific metabolomic signatures were observed across amino acid metabolism, lipid metabolism, energy pathways, and gut microbiome-derived metabolites. The Mediterranean diet produced the most favourable metabolic profile,

demonstrating 31% lower cardiovascular-related risk scores compared to the Fast Food diet and 18% lower scores compared to the Ketogenic diet. Favourable metabolic shifts included improved lipid-related ratios and reductions in inflammation-associated metabolites, while metabolites associated with elevated cardiometabolic risk were more prominent during other dietary phases. Longitudinal analysis further demonstrated recovery toward healthy reference ranges within four weeks following dietary transitions.

These findings demonstrate that reference-based longitudinal metabolomic profiling can differentiate metabolic risk patterns across dietary interventions while identifying individualized metabolic vulnerabilities. This framework represents a scalable approach for precision nutrition, supporting early detection of subclinical metabolic dysfunction and enabling data-driven dietary recommendations tailored to individual physiology.

1154 A Adapting and Expanding Quantitative Targeted metabolomic methods for studies in Pregnancy

PRESENTING AUTHOR: ***Natalie Homer, University of Edinburgh, United Kingdom***

CO-AUTHORS: *Scott Denham, Joanna Simpson, Ellie Phelan, Margaux Billen, Rebecca Reynolds, Marius Lahti Pulkkinen, Katri Raikkonen*

SUB-THEME: Pregnancy, maternal and neonatal health

Steroids, including mineralocorticoids, glucocorticoids, and sex hormones play a critical role in pregnancy. LC-MS/MS analysis offers an opportunity to conduct broad steroid profiling by simultaneous multiplex analysis within a given sample. We sought to expand steroid profiling and develop a method that could also measure bile acids and kynurenine metabolites in plasma and in amniotic fluid, to generate rich data for pregnancy biomarker investigation.

For targeted methods that result in quantitative values the challenge in the analytical laboratory is to develop methods that are sensitive enough for reliable data, have an appropriate calibration range, include a system for quality control, a robust workflow for sample preparation, instrumental analysis, data analysis and evaluation.

We developed a workflow for a study in pregnancy for targeted analysis of three metabolic pathways; steroids, bile acids and kynurenine metabolites. A semi-automated sample preparation using phospholipid depletion plate, combined with analysis on an Acquity I-Class UPLC and a Sciex QTrap6500+ mass spectrometer of the three metabolic pathways we developed a workflow that measures 24 steroids, 14 bile acids and 7 kynurenine metabolites from anextract of 100 uL sample. The calibration standards for the three methods were combined, but the analytical measurement involved three injections – and the three datasets were evaluated in turn. Following measurement in plasma we adapted the workflow and validated for measurement of these metabolites in amniotic fluid, adapting the calibration ranges and quality controls to reflect the different sample type.

On application to plasma samples of a pregnancy study we detected 20 steroids, 9 bile acids and 7 kynurenine metabolites, while adapting the method for amniotic fluid samples we detected 18 steroids, 9 bile acids and 4 kynurenine metabolites in 50% or more of the samples. This study revealed concentration range differences between sample types and highlights the challenges that exist when developing workflows for quantitative targeted metabolomic-style methods increasingly required for pregnancy studies.

1155 B Unraveling stage-specific metabolites in human milk and their links to maternal physiology: insights from a Mexican population

PRESENTING AUTHOR: ***Imelda Cecilia Zarzoza Mendoza, Universidad nacional Autonoma de Mexico, Mexico***

CO-AUTHORS: *Lourdes Barbosa-Cortés, Maricela Rodríguez-Cruz, María Cristina Carmona-Isunza, Hilda Sánchez-Vidal, José Carlos Páez-Franco, Cristian Emmanuel Luna-Guzmán, Maricela Morales-Marzana, Juan Manuel Domínguez-Salgado, Judith Villa-Morales*.

SUB-THEME: Pregnancy, maternal and neonatal health

Background/Objective: Human milk is an irreplaceable source of nutrition and is essential for the infant's growth and development right after birth and for early life stages survival. This study aims to characterize and compare the metabolite profiles of colostrum, transitional, and mature milk using an untargeted approach. Additionally, it explores potential correlations between the identified metabolites and maternal nutritional factors.

Methods: This was a longitudinal, prospective, and observational study. We included human milk samples from 113 Mexican women who practiced exclusive breastfeeding. Samples were analized by GC-MS. Metabolites showing significant variation

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

across lactation stages were further analyzed using Friedman tests with post hoc Wilcoxon tests and Bonferroni correction. Correlations with maternal anthropometric were evaluated.

Results: Twenty-three metabolites were identified, including amino acids and derivatives, sugars, fatty acids, and energetic metabolites. Alanine and creatinine decreased during lactation, while aspartate, serine, and valine increased. Rhamnose was higher in colostrum, whereas decanoic, dodecanoic, and tetradecanoic acids increased over time, and 11,14-eicosadienoic acid decreased. Lactic acid declined across stages. Positive correlations were found between several amino acids and maternal anthropometric variables, while lactic acid correlated negatively.

Conclusions: Human milk metabolomic profiles display distinct, stage-specific variations shaped by maternal characteristics, reflecting the dynamic physiological and nutritional demands of the developing infant.

1156 A Longitudinal Urinary Metabolomics in Preterm Infants Associated with Gestational Age and BPD Severity from 6 Months to 2 Years of Age

PRESENTING AUTHOR: ***Meng Han Chiang, Chang Gung Memorial Hospital at Linkou, Taiwan***

CO-AUTHORS: *Chih-Yung Chiu*

SUB-THEME: Pregnancy, maternal and neonatal health

Background: Bronchopulmonary dysplasia (BPD) is a chronic lung disease that primarily affects preterm infants who require mechanical ventilation or oxygen therapy. Although urinary metabolomics has identified metabolic signatures of BPD at 6 months of corrected age, whether these changes persist through 2 years remains unknown.

Methods: A total of 254 urine samples were analyzed at corrected age 6 months (6M, n=139) and 2 years (2Y, n=115). Infants were stratified by gestational age (GA): ≥37 weeks, 28–32 weeks, and <28 weeks; and by BPD severity based on 2019 NICHD criteria: healthy controls (HC), no or mild BPD, and moderate or severe BPD (M+S BPD). Urinary metabolomic profiling was performed using 1H-Nuclear magnetic resonance (NMR) spectroscopy coupled with partial least squares discriminant analysis (PLS-DA).

Results: Nineteen and twenty urinary metabolites were significantly associated with GA, and twelve and thirty-four with BPD severity, at 6M and 2Y respectively ($P < 0.05$). At 6M, acetylsalicylate, hippurate, 3-methyl-2-oxovalerate (3-MOV), N-phenylacetylglycine (N-PAG), indoxyl sulfate, and 4-hydroxyphenylacetate were significantly higher in infants with GA <28 weeks and M+S BPD compared with full-term infants and HC ($P < 0.001$). At 2Y, N-PAG, indoxyl sulfate, and succinate remained elevated in extremely preterm infants with higher fold changes than at 6M. In the M+S BPD group, N-PAG, indoxyl sulfate, acetylsalicylate, and succinate remained elevated, while glutamine, citrate, and 3-MOV further differentiated M+S from no/mild BPD. N-PAG, indoxyl sulfate, acetylsalicylate, and pantothenic acid were significantly differently expressed across all comparisons at both timepoints.

Conclusion: Urinary metabolomic analysis demonstrates that metabolic signatures associated with prematurity and BPD severity, particularly gut microbiome-derived metabolites including N-PAG, indoxyl sulfate, and hippurate, persist from 6 months to 2 years and may become more pronounced over time, highlighting potential targets for early identification and intervention.

1157 B Untargeted Metabolomic and Lipidomic Profiling to Identify Disease-Specific Biomarkers and Evaluate Treatment Response in Cystic Fibrosis

PRESENTING AUTHOR: ***Asma Farjallah, Université de Sherbrooke, Canada***

CO-AUTHORS: *Christelle Bergeron, Dominic Cliche, Simon Couillard, André M. Cantin, Christiane Auray-Blais*

SUB-THEME: Respiratory diseases

Cystic fibrosis (CF) is a rare autosomal recessive genetic disease. It is caused by various mutations in the CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) gene, which disrupts the normal function of the chloride ion channel. Clinical manifestations of CF typically include recurrent respiratory infections, chronic airway inflammation, progressive decline in lung function, and intermittent pulmonary exacerbations. Primary objective of this study: to identify plasma biomarkers in CF patients using untargeted metabolomic/lipidomic approaches, with the goal of improving early detection, diagnostic accuracy, and disease monitoring. Liquid chromatography coupled with time-of-flight mass spectrometry was employed to distinguish untreated cystic fibrosis patients (n=26) from age- and sex-matched healthy controls (n=24) for biomarker discovery. Secondary objective: to evaluate the biochemical impact of three CFTR modulator therapies: elexacaftor/tezacaftor/ivacaftor (ETI; Trikafta®) (n=21) and tezacaftor/ivacaftor (TI; Symdeko®) and lumacaftor/ivacaftor (LI;

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Orkambi®) (n=10), through plasma metabolomic and lipidomic profiling of treated CF patients. Multivariate statistical analysis and pathway enrichment analyses were used. Results revealed dysregulation in the galactose metabolism, glycolysis/gluconeogenesis, bile acid, fatty acid metabolism, steroid hormone biosynthesis, and amino acid catabolism. Moreover, among the CFTR modulator therapies, ETI induced the most extensive metabolic remodeling, with a higher proportion of significantly altered metabolites and lipids across multiple chemical classes. ETI treatment was characterized by consistent downregulation of oxidized fatty acids, eicosanoid-related species, sphingolipids, and membrane-remodeling phospholipids, reflecting reduced inflammation, oxidative and endoplasmic reticulum stress, enhanced β-oxidation, normalization of arachidonic acid signaling, and partial restoration of steroid hormone metabolism. In contrast, LI and TI produced more limited metabolic changes, with persistent lipid and amino acid pathway alterations, suggesting incomplete correction of inflammatory and lipid disturbances. Overall, these metabolomic/lipidomic approaches provide comprehensive insight into biochemical disruptions in CF and support the potential for metabolic profiling to optimize current therapies and guide the development of targeted treatment strategies.

1158 A Short- and long-chain PFAS induce hepatic metabolic alterations revealed by in vitro metabolomics

PRESENTING AUTHOR: ***Filipa Amaro, UCIBIO Faculty of Pharmacy of Porto, University of Porto, Portugal***

CO-AUTHORS: *Filipa Amaro, Joana Pinto, Ester Siniscalchi, Giorgia Maria Varalda, Eleonora Azzinnari, Egidio Iorio, Francesca Marcon, Paula Guedes de Pinho*

SUB-THEME: Toxicology

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals widely used in industry and consumer products due to their high exceptional stability and resistance to degradation. Their environment persistence and potential adverse health effects have raised increasing concerns. Short-chain PFAS have been introduced as safer alternatives, yet their biological impact remains insufficiently characterized. Hence, this study aimed to investigate in vitro metabolic alterations induced in human hepatic cells by the legacy perfluorooctanoic acid (PFOA) and its short-chain substitute, perfluorobutanoic acid (PFBA), using an untargeted multiplatform metabolomics approach. HepaRG cells were exposed for 24h to increasing concentrations of PFAS, and concentrations inducing up to 40% cytotoxicity were selected based on MTT assays (PFOA: 37.5, 75 and 150 µg/mL; PFBA: 75, 150 and 300 µg/mL). Metabolites from the intracellular (endometabolome) and culture medium (exometabolome) of both non-exposed and exposed cells were analysed by GC-MS and 1H NMR spectroscopy. The data were processed and statistically analysed. Multivariate analysis showed that the HepaRG cell endometabolome was significantly altered after exposure to high concentration of PFOA, and to intermediate and high concentrations of PFBA. These changes were characterised by significantly decreased levels of metabolites associated with the oxidative stress response (pyroglutamate, glutamate, threonate and taurine) and energy metabolism (malate). PFOA further disrupted amino acid homeostasis and phospholipid metabolism, indicating impacts on cell proliferation, and signalling pathways. Exometabolome analysis revealed discrimination across all PFAS concentrations, with alterations in metabolites involved in NAD+ biosynthesis (tryptophan and niacin), and fatty acid oxidation (3-hydroxybutyrate). Changes in extracellular amino acid levels were also evident following PFOA exposure. Overall, these results highlight early metabolic markers of PFAS-induced hepatotoxicity and demonstrate the value of metabolomics in the detection of in vitro chemical toxicity. Although the effects were more pronounced for PFOA, PFBA induced similar alterations of potential toxicological relevance.

1159 B Hepatic Untargeted Metabolomics of Sevoflurane Exposure

PRESENTING AUTHOR: ***Mariane Silva, UNESP- Botucatu, Brazil***

CO-AUTHORS: *Maria Vitória Destro, Tony F. Grassi, Cintia Oliveira, Nur Aimi A. Zainurin, Luis A.J. Mur, Leandro G. Braz, Mariana G. Braz*

SUB-THEME: Toxicology

Sevoflurane is a volatile anesthetic widely used in surgical practice due to its rapid induction and recovery, and its recognized clinical safety. Despite its extensive clinical use, the hepatic metabolic effects associated with sevoflurane exposure remain incompletely understood. Approximately 5% of absorbed sevoflurane undergoes hepatic biotransformation, generating hexafluoroisopropanol and inorganic fluoride. In recent years, metabolomics has emerged as a powerful approach to investigate anesthetic-induced biochemical alterations; however, most studies have focused primarily on neurological outcomes in experimental models. Therefore, this study aimed to characterize hepatic metabolomics and oxidative stress following exposure to clinically relevant sevoflurane concentrations in adult rodents. Male Wistar rats were allocated into three groups: control (without anesthetic exposure) and sevoflurane-exposed groups (3.5% and 4.8%) for 2 h under

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

continuous monitoring of vital signs, followed by liver collection. Untargeted metabolomics analysis was performed using flow infusion electrospray high-resolution mass spectrometry (FIE-HRMS). Oxidative stress was evaluated through ferric reducing antioxidant power (FRAP), thiobarbituric acid reactive substances (TBARS), and protein carbonylation assays. Pairwise metabolomic analysis identified 140 differentially abundant metabolites (DAMs) in the 3.5% group and 145 DAMs in the 4.8% group, with 82 shared metabolites between exposure groups. Pathway enrichment analysis revealed significant alterations in lysine degradation, cysteine and methionine metabolism, arginine biosynthesis, and alanine, aspartate, and glutamate metabolism. Shared metabolic alterations included reduced gamma-glutamylcysteine abundance and increased taurine and uric acid levels. Although TBARS and FRAP analyses showed no significant differences between groups ($p>0.05$), protein carbonylation was significantly increased in the 3.5% group compared with control ($p=0.04$). Additionally, decreased levels of tryptophan, lysine, proline, serine, citrulline, ornithine, and aspartate suggest disturbances in amino acid and nitrogen metabolism, including possible modulation of urea cycle-related pathways. Collectively, these findings demonstrate that sevoflurane exposure induces hepatic metabolic disturbances associated with adaptive redox and nitrogen metabolism responses.

1160 A Fast 2D NMR-based metabolomics: an innovative tool to improve contaminant exposure biomarker discovery

PRESENTING AUTHOR: ★ ***Carla Remy, INRAe, France***

CO-AUTHORS: *Marie Tremblay-Franco, Roselyne Gautier, Elisabeth Baeza, Jérémy Ratel, Laurent Debrauwer, Erwan Engel, Cécile Canlet*

SUB-THEME: Toxicology

Persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), are persistent, bioaccumulative, toxic and mobile. Today, despite having been banned for many years, humans are still mainly exposed to POPs through animal-derived foods. To enhance food safety surveillance, 2D nuclear magnetic resonance (NMR) spectroscopy has been evaluated as a non-targeted metabolomics approach to identify liver markers of dietary exposure of chickens to PCBs. To overcome the limitations of 1D NMR for biomarker detection and identification, the performance of 2D NMR was investigated using several fast 2D NMR sequences. Both aqueous and lipidic liver extracts from chickens exposed for 42 days to low dietary doses of dioxin-like PCBs (0.15 and 0.75 ng/kg fresh weight) were analyzed on a 600 MHz NMR spectrometer using 1H 1D NMR and different 2D NMR sequences, namely ultrafast (UF) correlation spectroscopy (COSY), fast COSY, non-uniform sampling (NUS) total correlation spectroscopy (TOCSY) and NUS heteronuclear single quantum coherence spectroscopy (HSQC). Multivariate and univariate statistical analyses were performed on NMR data to discriminate between control and exposed animals and to identify metabolic alterations induced by PCB exposure. Contrary to 1D NMR, 2D NMR provided valid statistical models, demonstrating its added value. Overall, exposed animals showed an increase in fatty acid and triglyceride levels, a decreased in mono- and diglyceride levels and also an alteration in cholesterol levels, suggesting liver metabolic stress. Furthermore, fast 2D NMR sequences proved to be complementary in terms of their informational content: NUS HSQC was less sensitive but allowed better separation of signals from abundant compounds, while fast COSY better highlighted statistically discriminant changes in lower abundance metabolites. Together, these findings demonstrate the potential of 2D NMR-based metabolomics for assessing biological effects associated with contaminant exposure, while remaining compatible with the analysis of a large number of samples in nutritional and toxicological studies.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1162 A METABOLIC ALTERATIONS ASSOCIATED WITH CANNABIDIOL USE IN NEUROPATHIC PAIN: A FECAL METABOLOMICS APPROACH VIA LC-HRMS

PRESENTING AUTHOR: ***Júlia Nunes, Federal University of Rio de Janeiro, Brazil***

CO-AUTHORS: *Vitória Fernandes, Tadeu Montagnoli, Rodrigo De Souza, Gisele Sudo, Marina Amaral*

SUB-THEME: Pharmacometabolomics

Neuropathic pain is a complex chronic condition that is often refractory to conventional therapies, with diabetic neuropathy being one of its most prevalent etiologies. Cannabidiol (CBD) has emerged as a promising therapeutic alternative; however, its mechanisms of action, particularly those involving interactions with the gut microbiota and the gut–brain axis, remain incompletely understood. This study aimed to investigate the metabolic alterations associated with CBD treatment in an animal model of streptozotocin (STZ)-induced diabetic neuropathic pain using an untargeted fecal metabolomics approach. Analyses were performed using high-performance liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Multivariate analysis (PCA) demonstrated that neuropathic pain significantly alters the fecal metabolomic profile compared to control animals. CBD treatment attenuated this pathological metabolic signature, resulting in a profile distinct from both the vehicle-treated and control groups. The identification of differentially abundant metabolites suggested modulation of pain-associated functional dysbiosis, highlighted by the normalization of secondary bile acids, metabolites predominantly of microbial origin such as 3,12-dioxo-5-β-cholan-24-oic acid. Additionally, a significant reduction in pro-inflammatory lipid mediators, including oxylipins and prostaglandin derivatives, was observed. Targeted analysis confirmed the presence of CBD and its major Phase I metabolites (7-OH-CBD and 7-COOH-CBD) in fecal samples, supporting biliary excretion and direct exposure of the intestinal environment to the active compound. Taken together, these findings suggest that CBD modulates host metabolism through interactions with the gut microbiota and attenuation of inflammatory processes, reinforcing the gut-brain axis as a relevant target for the therapeutic effects of cannabinoids in neuropathic pain.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1211 B Discovery and Metabolomic Approach for Stereochemical Profiling of Korean Milk Thistle

PRESENTING AUTHOR: ***Dongyup Hahn, Kyungpook National University, South Korea***

CO-AUTHORS: *Seunguk Yu*

SUB-THEME: Agro-industrial crops and traditional foods

Silymarin, a standardized extract from milk thistle (Silybum marianum), is a complex mixture of flavonolignans renowned for its hepatoprotective effects. Despite its widespread use, the therapeutic efficacy of silymarin often varies due to the presence of numerous diastereomers and unidentified minor isomers. Since the biological affinity and metabolic activity of these compounds are highly dependent on their specific stereochemical structures, a precise stereospecific separation and quantitative analysis are essential. Furthermore, the compositional profile of these isomers fluctuates based on cultivation environments, necessitating an optimized extraction strategy to maximize functional yields. Structural elucidation of the EA layer fractions resulted in the identification of nine major components, including Taxifolin and Silybin A & B, along with three minor isomers, such as Epitaxifolin. This isolation of minor isomers directly from raw milk thistle—rather than via chemical synthesis—represents a significant finding in the phytochemical profiling of S. marianum. These findings provide a robust platform for the standardized production of high-purity silymarin isomers, facilitating further investigation into their stereospecific biological activities.

1212 A Untargeted metabolomics for Geographical Indication support unveils the unique chemical signature of the aromatic rice landrace

PRESENTING AUTHOR: ***Esther de Mello Antonio, UNIRIO - Federal Univ. the State of Rio de Janeiro, Brazil***

CO-AUTHORS: *Luciana Ribeiro da Silva Lima, Adriana Santos da Silva, Carolina Thomaz dos Santos d'Almeida, Hygor Marcos Ribeiro de Souza, José Manoel Colombari Filho, Alexandre Guedes Torres, Cristina Yoshie Takeiti, Mariana Simões Larraz Ferreira*

SUB-THEME: Agro-industrial crops and traditional foods

Rice (Oryza sativa), a global staple food, includes traditional varieties that possess unique phenotypic, chemical, and sensory characteristics shaped by their specific cultivation microenvironment (terroir). Metabolomics has emerged as a robust analytical tool to assess the authenticity and to support geographical indication (GI) certification. This study aimed to establish the metabolic fingerprint of six aromatic rice landraces of Arroz Anã de Porto Marinho (Cantagalo, Rio de Janeiro, Brazil) and compare them with fourteen cultivars from the Active Germplasm Bank (BAG) (reference group) of Embrapa. A sequential extraction was performed to obtain free (soluble) and bound (insoluble) metabolites. An untargeted metabolomics analysis was performed using a UHPLC-HRMS system (Orbitrap Q-Exactive Plus) in negative ionization mode. Data preprocessing and annotation were conducted using Progenesis QI and the GNPS and PubChem databases. Univariate and multivariate statistical analyses (MVA) were performed on MetaboAnalyst 6.0. The initial detection of 2,502 features was refined using a quality-control filter (CVtrans-ferulic acid, p-coumaric acid, caffeic acid, and vanillic acid) and flavonoids (apigenin, rutin, quercetin, myricetin, and catechin). MVA revealed a clear spatial segregation with the absence of overlap between Arroz Anã clusters and BAG cultivars (PC1 51.9%). Phenotypically recognized as aromatic grain, Arroz Anã shared structural similarities with reference aromatic rice regarding the bound fraction and bioactive connections with red-pigmented rice concerning the free fraction. However, it exhibited a unique chemical architecture: specific differential markers, such as oxidized fatty acids (FA 18:1+2O, FA 18:3+2O), citric acid, and LPE 18:2, defined its identity and distinguished it from the control group. The metabolomic approach provides scientific support for the authentication of the aromatic Arroz Anã variety, providing the necessary technical basis for its GI certification and economic valorization.

1213 B Untargeted metabolomic analysis of Biquinho pepper cultivars (Capsicum chinense Jacq.) by UHPLC-HRMS

PRESENTING AUTHOR: ***Guilherme Perez Pinheiro, State University of Campinas, Brazil***

CO-AUTHORS: *Fernando Cesar Sala, Marcia Ortiz Mayo Marques, Emanuel Carrilho, Alexandra Christine Helena Frankland Sawaya*

SUB-THEME: Agro-industrial crops and traditional foods

Low-pungency peppers, including the Biquinho variety of Capsicum chinense, are widely consumed in Brazil for their strong aroma and mild flavor. Besides their traditional use, such peppers possess broad therapeutic potential (e.g., antihyperlipidemic, anti-inflammatory, and antioxidant) as they display several bioactive compounds in their composition, such as flavonoids, carotenoids and capsaicinoids, which may be of interest not only to the food industry as a natural

antioxidant but also to the pharmaceutical industry. Considering the vast potential of these peppers, we compared several Biquinho pepper cultivars to map their potential chemical variability. Seeds of eight Biquinho pepper cultivars were obtained from the Federal University of São Carlos (UFSCar), which were simultaneously cultivated at UFSCar and at the Federal Rural University of Rio de Janeiro; sample processing and analytical parameters were optimized for this study. Untargeted UHPLC-HRMS analyses were performed on a Vanquish Chromatographic System coupled to an Exploris 120 Orbitrap (Thermo) using pepper extracts. The raw data were processed using Progenesis QI software (Waters, Nonlinear Dynamics), while MetaboAnalyst Online was used for statistical analysis and chemometrics. The overall composition from both negative and positive ion modes was closely related among cultivars and between cultivation sites. Several compounds detected in the extracts were annotated, including flavonoids (e.g., luteolin and apigenin derivatives), amino acids, lipids, and capsaicinoids. Capsaicin, as an example, was less intense in the cultivars 2024-1, 2033-4, and Biquinho, followed by 2026-1 and 2028-1, respectively, while dihydrocapsaicin did not vary between cultivars. From the annotated flavonoids, highest intensities were detected in the cultivars 2033-5, 2028-1, and 2026-1. Altogether, we assessed the composition of Biquinho pepper cultivars and selected varieties with lower capsaicinoid content and higher bioactive compound content, plants that may be used for future commercialization and/or product development.

1214 A Geographic modulation of ten cacao metabolomes in Colombian genotypes revealed by ^{1}H NMR profiling

PRESENTING AUTHOR: ***Elkin Galeano, Universidad De Antioquia, Colombia***

CO-AUTHORS: *Juan M. Lopez, Catalina Agudelo, Edison Osorio, Helena Maruenda*

SUB-THEME: Crop quality research

Introduction: The chemical composition of Theobroma cacao beans is a critical determinant of the nutritional and sensory quality of chocolate products, yet it is significantly influenced by the complex interplay between genotype and growing environment.

Aim: This study aimed to characterize the metabolic profiles of ten cacao genotypes—including universal commercial clones and regional varieties—cultivated in two distinct regions of Antioquia, Colombia (Urabá and Northeast), to elucidate the impact of geographic origin on key quality-related metabolites.

Methods: Untargeted and targeted metabolomic profiling was performed using proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy on aqueous–methanolic extracts of cacao beans (n = 98). Spectral data was normalized and analyzed using multivariate statistics, including PCA and PLS-DA, to identify discriminant metabolic patterns. Quantification of 21 metabolites was achieved via qNMR. Nonparametric tests (Kruskal–Wallis and Mann–Whitney) assessed regional and genotypic differences.

Results: Distinct metabolic signatures were associated with cultivation site, with clear clustering by geographic origin in multivariate models (PLS-DA classification accuracy ≈69%). Beans from the Urabá region exhibited significantly higher concentrations of free amino acids, methylxanthines (notably theobromine), and antioxidant-related metabolites such as epicatechin. These profiles correlate with the warm and humid climate of the region and the alluvial soils, suggesting enhanced nitrogen assimilation and secondary metabolite biosynthesis. In contrast, Northeast-grown samples showed comparatively lower metabolite abundance, consistent with more acidic, nutrient-limited soils. Genotype–environment interactions were evident, with clones TSH565, FEAR5, ICS1, and CCN51 displaying particularly strong site responsiveness.

Conclusions: This study demonstrates that local environmental conditions significantly modulate the cacao metabolome beyond genetic effects alone. NMR-based metabolomics provides a robust platform for origin discrimination and for identifying cultivation environments that enhance nutritionally valuable metabolites.

Bibliography: Metabolomic profiling of ten cacao genotypes from Antioquia, Colombia reveals local effects on nutritional composition. J. Food Comp. Anal. 2025, 148. 10.1016/j.jfca.2025.108607

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1215 B Development of an LC-HRMS Metabolomic Workflow for the Characterization of Bioactive Metabolites in Tomato

PRESENTING AUTHOR: ***Bruno Silva Leyes, Universidad Tecnológica del Uruguay, Uruguay***

CO-AUTHORS: *Lucia Pareja, Andrés Pérez*

SUB-THEME: Crop quality research

Tomato (Solanum lycopersicum L.) is one of the most widely consumed vegetables worldwide and represents an important source of bioactive metabolites, including carotenoids, flavonoids, phenolic acids, and volatile compounds. These metabolites contribute to the sensory and nutritional quality of the fruit and have attracted increasing scientific interest due to their potential health benefits. Consequently, comprehensive chemical characterization of tomato metabolites has become an important research focus. In this context, metabolomics based on high-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS) has emerged as a powerful tool for the analysis of complex plant matrices.

The aim of this work is to develop and apply both targeted and untargeted metabolomic strategies for the characterization of metabolites of interest in tomato fruits using LC-HRMS. In an initial stage of the study, analytical and instrumental conditions were optimized using available reference standards in order to improve spectral quality, sensitivity, and data reproducibility. Different ionization, fragmentation, and acquisition parameters were evaluated, along with alternative acquisition modes, to maximize metabolite detection and obtain relevant structural information.

This preliminary work allowed the generation of high-resolution mass spectra and characteristic fragmentation patterns for several metabolites, providing a solid basis for subsequent analyses. In addition, progress was made in the development of a spectral library adapted to the local instrumental conditions, which will support compound identification and annotation in future analyses of tomato extracts. The next stages of the project will focus on applying these metabolomic strategies to real tomato samples in order to further explore their chemical composition and metabolic variability.

1216 A AMDIS-Based Volatileomics Platform for Strawberry Quality Innovation: From Flavor Analysis to Freshness Prediction

PRESENTING AUTHOR: ***Kang Mo Ku, Korea University, South Korea***

CO-AUTHORS: *Eungu Jee, Eunsu Do, Tae-Yeong Ko*

SUB-THEME: Crop quality research

Strawberry aroma quality is a key determinant of consumer preference and market competitiveness. However, volatile organic compounds (VOCs) are chemically diverse and frequently affected by peak co-elution, making accurate identification and interpretation challenging in GC-MS-based aroma analysis. In particular, the use of general commercial mass spectral libraries can generate excessive false hits, complex output files, and prolonged data-processing time. Therefore, crop-specific and user-friendly analytical resources are required to improve the accessibility, reliability, and efficiency of strawberry volatileomics research. In this study, we established an AMDIS-based strawberry VOC user library and applied it to strawberry flavor evaluation and freshness prediction. The library was constructed from TD-GC-MS profiling data obtained from 61 strawberry germplasms from Korea and other countries. It was designed to include practical information for compound interpretation, including compound name, synonym, CAS number, chemical formula, chemical class, retention index, odor description, and odor threshold. The library was prepared in .msl and .msp formats to support AMDIS deconvolution and facilitate the identification of trace compounds in complex chromatograms. Compared with the NIST library, the custom library reduced the average number of matches from 281 to 81, decreased report file size from approximately 686 KB to 22 KB, and shortened processing time per chromatogram from approximately 31 s to 9 s. This platform was further applied to cultivar-dependent VOC profiling, seasonal variation analysis, and the exploration of flavor-improvement target compounds, including gamma-decalactone. In addition, color, soluble solids, titratable acidity, soluble metabolites, amino acids, and VOC data were compared for predictive modeling of strawberry freshness during storage. Among these variables, the VOC-based multilayer perceptron model showed the strongest predictive potential. These results demonstrate that a user-friendly AMDIS VOC library can serve as a practical platform connecting strawberry aroma research, targeted metabolomics, cultivar evaluation, and postharvest quality prediction.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1217 B GWAS of Chickpea Grain Lipidomic Profiles From a Global Diversity Panel

PRESENTING AUTHOR: ***Sally Buck, CSIRO, Australia***

CO-AUTHORS: *Jakob Butler, Raul Ortega, Matthew Taylor, Jeni Pritchard, JP Ral*

SUB-THEME: Crop quality research

Chickpea is an important agricultural crop with high nutritional value and potential for functional ingredient applications. Chickpeas are categorised into two market classes, desi and kabuli, according to grain size and colour, but variation in grain composition including the lipidome within and between classes remains largely unexplored., even though it has significant implications for flavour and storability of grains. Lipidomic analysis was performed in a global Chickpea Diversity panel to identify drivers of grain composition and relationships between nutritional traits. Lipidomic analysis revealed triacylglycerols to be the most abundant lipid class in all chickpeas, regardless of market class, and C18:2 was the most abundant class of fatty acids. The analysis also identified low abundance fatty acids not previously classified in chickpeas (C20:1, C20:2, C18:4, C16:2 and C16:3). A genome-wide association study (GWAS) identified several loci of interest for grain composition, providing promising avenues for breeding and future work. These results highlight important opportunities for developing chickpea varieties with enhanced nutritional profiles and better suitability for emerging markets.

1218 A Characterization of First-Generation Interspecific Strawberry Hybrids: In search of the next commercial Nordic garden strawberry cultivar

PRESENTING AUTHOR: ★ ***Carla Vecenâncio da Silva, University of Turku, Finland***

CO-AUTHORS: *Attiq Rehman, Niina Kelanne, Oskar Laaksonen, Tuuli Haikonen, Baoru Yang, Maaria Kortesniemi*

SUB-THEME: Crop quality research

Garden strawberry (Fragaria × ananassa) is the most produced berry in Finland and loved world-wide. In the Nordic countries, climate change-driven milder winters and increased precipitation create new cultivation challenges. The increasing need for resilient plants that still exhibit desirable sensory attributes, such as red colour and sweet flavour, calls for the development of climate adapted cultivars. New resilience traits and desirable properties can be achieved through crossbreeding. The Natural Resources Institute Finland (Luke) reconstructed F. × ananassa by crossing F. chiloensis and F. virginiana to introduce traits from wild parents and increase genetic variability. In total, 136 hybrid genotypes representing 12 progeny groups and six reference cultivars (five commercial and one white-fruiting) were analysed for volatile profiles using HS-SPME-GC-MS. Yearly difference in the volatile compound contents are evaluated from samples harvested from 2021, 2023, and 2025 show the most stable compounds despite weather differences. Samples from 2023 spanning the widest volatile diversity were selected for trained panel descriptive analysis. Genome-wide association studies found the gene regions associated with the expression of desirable properties found in hybrids. Machine learning algorithms were trained to predict sensory attributes based on volatile composition and identified important contributors to these properties. Both volatile and sensory profiles of hybrids differed from commercial cultivars and varied across progeny groups. Volatile compounds found specifically in wild cultivars, such as methyl anthranilate, were identified in progeny individuals. Hybrids' flavour profiles generally exhibited higher sourness, astringency, and bitterness, and more intense flavour and odour. Many genotypes displayed pale pink or orange hues as opposed to the vibrant darker red colour from cultivars. The successful introduction of new traits from wild parents and identification of corresponding gene regions contributes to future breeding programs and development of resilient and delicious cultivars.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1219 B Brazilian Mining Dam Collapse: Molecular Networking–Guided Metabolomics Reveals Species-Specific Plant Detox

PRESENTING AUTHOR: ***Alan Cesar Pilon, Instituto de Química, Brazil***

CO-AUTHORS: *Marília Elias Gallon, Eduardo Afonso Silva-Junior, Amanda Roberta Corrado, Maria das Graças Lins Brandão, Maria Cristina Teixeira Braga Messias, Alberto José Cavalheiro, Norberto Peporine Lopes*

SUB-THEME: Environmental exposures

In November 2015, a catastrophic environmental disaster struck the state of Minas Gerais, Brazil, which was caused by the collapse of the Fundão dam. Over 40 million m3 of metal-rich mining waste, containing iron, arsenic, mercury, cadmium, and manganese, contaminated more than 650 km of the Doce River basin, causing severe degradation of terrestrial and aquatic ecosystems. While immediate effects included widespread destruction of local habitats, including indigenous settlements, some endemic plant species exhibited remarkable resilience, adapting their metabolism to tolerate extreme exposure to toxic mining waste. Using an untargeted liquid chromatography-tandem mass spectrometry (LC-MSn) metabolomics workflow integrated with multivariate analysis and molecular networking, we profiled metabolic changes in two medicinal plants, Vernonanthura polyanthes (Asteraceae) and Piper aduncum (Piperaceae). Our results showed that exposure to toxic mining waste elicited species-specific responses, with plants triggering biosynthetic pathways that enhanced production of peptides, especially glutathione and lysine-acetylated derivatives, for V. polyanthes, and phenylpropanoids, especially O-methylated C-glycosylated flavonoids, for P. aduncum. These shifts are consistent with defense mechanisms involving metal chelation and redox buffering, whereby glutathione-based peptides and flavonoids mitigate metal toxicity and oxidative stress in plant tissues. Overall, these findings shed light on the mechanisms of plant response under extreme environmental disturbance, providing insights into how metabolic adaptations contribute to ecological stabilization and plant recovery.

1220 A Untargeted GC–MS metabolomics reveals stronger effects of urbanization than seasonality in native Atlantic Forest trees

PRESENTING AUTHOR: ***Silvia Ribeiro de Souza, Environmental Research Institute of the State of São Paulo, Brazil***

CO-AUTHORS: *Fernanda Anselmo-Moreira, Bruno Ruiz Brandão da Costa, Agnès, Borbon, Adalgiza Fornaro, Cláudia Maria Furlan*

SUB-THEME: Environmental exposures

Urban forests are increasingly exposed to environmental stressors, yet the metabolic responses of tropical tree species to urbanization and seasonality remain poorly understood. In this study, we applied untargeted GC–MS metabolomics to characterize the biochemical profiles of 16 native Atlantic Forest species collected from two sites with contrasting levels of urbanization and across dry (Aug-Sep, 2023) and rainy seasons (Jan-Feb, 2024). A total of 298 and 274 features were detected in the polar and nonpolar phases, respectively, with 140 and 135 compounds annotated. Metabolites spanned diverse chemical classes, including sugars, organic acids, amino acids, glycerolipids, and terpenoids, reflecting the complexity of plant metabolic responses. Multivariate analyses revealed significant differences between study areas for both polar and nonpolar datasets, whereas seasonal effects were not statistically significant. These findings indicate that urbanization exerts a stronger influence on metabolomic profiles than seasonal variation. Univariate analyses further identified key metabolites contributing to site differentiation, including erythritol and 5-oxoproline in the polar phase and multiple lipid-related compounds in the nonpolar phase. At the class level, shifts in sugars, organic acids, amino acids, and lipid-related compounds suggest coordinated metabolic adjustments associated with environmental conditions. In contrast, seasonal stability in metabolomic profiles highlights the resilience of these species to short-term climatic variation. These results provide new insights into the biochemical plasticity of tropical tree species and highlight the importance of integrating metabolomic data with physiological and atmospheric measurements to better understand plant–atmosphere interactions in urban environments.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1221 B Microbial Solid-State Fermentation as a Strategy to Enhance Flavor and Bioactive Profiles in Low-Quality Arabica and Canephora Coffee

PRESENTING AUTHOR: ★ ***Juan Dereix, Universidad de los Andes, Colombia***

CO-AUTHORS: *Monica Cala, Andres Fernando Gonzalez, Tatiana Sepulveda, Camila Morales*

SUB-THEME: Fermented foods

Coffee is one of the most widely consumed beverages worldwide, and its sensory quality and functional properties are strongly influenced by post-harvest processing. Controlled fermentation has emerged as a promising strategy to enhance flavor complexity and modulate bioactive compounds, particularly in low-quality beans. This study applied integrated metabolomics and flavoromics approaches to monitor solid-state fermentation (SSF) in low-quality Coffea arabica and Coffea canephora, aiming to improve sensory attributes through microbial-driven biochemical transformations.

Prior to fermentation, green coffee beans were subjected to steam treatment to reduce native microbial load and standardize initial conditions. Subsequently, beans were inoculated with industrial strains of Saccharomyces cerevisiae and Lactobacillus spp., applied individually and as a microbial consortium, and fermented for 48 h under controlled solid-state conditions. Green coffee samples were collected at 0, 24, and 48 h to evaluate time-dependent metabolic changes.

Untargeted metabolomic profiling of green coffee extracts and brewed coffee was performed using LC-qTOF-MS, while volatile compounds in the final beverage were analyzed by HS-SPME-GC-qTOF-MS to assess aroma-related modifications. Multivariate and differential metabolite analyses revealed distinct metabolic trajectories depending on the microbial starter and coffee species. Significant changes were observed in organic acids, amino acids, sugars, phenolic compounds, and alkaloids, indicating active microbial biotransformation and substrate utilization during SSF.

Volatile profiling demonstrated shifts in alcohols, esters, acids, and other aroma-active compounds associated with flavor enhancement. The results suggest that controlled SSF can modulate both metabolic and volatile profiles, highlighting the potential of selected microbial starters to improve flavor complexity and add value to low-quality Arabica and Canephora coffees.

1222 A Effect of fermentation conditions on lactic acid formation in specialty coffee using decision tree machine learning

PRESENTING AUTHOR: ***Pedro Luiz Lima Bertarini, Federal University of Uberlândia, Brazil***

CO-AUTHORS: *Marcela Vieira Caixeta Machado, Parafino Alberto Luís, Fabiana Cristina Lara Peghim, Laurence Rodrigues do Amaral, Matheus de Souza Gomes, Líbia Diniz Santos*

SUB-THEME: Fermented foods

The consumption of specialty coffee has increased worldwide in recent years, driven by consumers seeking beverages of higher quality and differentiated sensory characteristics. Fermentation processes applied to specialty coffees have gained attention because they can enhance and modulate sensory properties, contributing to more complex aroma and flavor profiles. Among the compounds associated with these changes, lactic acid has an important role in specialty coffees, especially after controlled fermentation processes. In specialty coffee, fermentation that promotes lactic acid production typically leads to higher but pleasant acidity, enhanced sweetness and body, and sensory descriptors such as creamy or milky notes, fruity characteristics (berry and citrus), as well as nutty, cocoa, caramel, honey, and brown sugar notes, especially when lactic acid bacteria are combined with selected yeasts. In this study, fermentations of specialty coffees from two different farms were evaluated to investigate the influence of processing conditions on lactic acid formation, considering different fermentation methods (dry or submerged), fermentation times, farm location, and fruit condition (peeled cherry, cherry, or floaters). The lactic acid concentrations were quantified using high-performance liquid chromatography (HPLC). To interpret the results, a machine learning approach based on a decision tree algorithm was applied using a full-training strategy, in which all records were used for both training and testing to obtain the best possible accuracy. The median value of lactic acid content (5.06) was used as the threshold to classify the samples into HIGH and LOW groups. The model reached an accuracy of 84.85%. The decision tree indicated that the most relevant factors for lactic acid levels were fermentation time and fruit condition, while the type of fermentation and farm location showed lower influence. In general, longer fermentation times and the use of higher-quality fruits, such as peeled cherry coffees, were associated with higher lactic acid content.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1223 B Caffeine Dynamics in Immature Coffee Fruits under Post-Harvest Interventions

PRESENTING AUTHOR: ***Líbia Santos, Federal University of Uberlândia, Brazil***

CO-AUTHORS: *Adriana Nogueira Alves, Sandra Lúcia Nagueira, Laurence Rodrigues do Amaral, Matheus de Souza Gomes, Pedro Luiz Lima Bertarini, Líbia Diniz Santos*

SUB-THEME: Fermented foods

Immature coffees (green fruits) are traditionally associated with sensory defects and value loss; however, they represent an underexplored biological matrix for prospecting bioactive metabolites. Applying metabolomics to immature fruits enables the mapping of chemical variability linked to ripening stage and post-harvest practices, supporting segregation, valorization, and standardization strategies. Among key markers, caffeine is a high-impact alkaloid of physiological and technological relevance, acting both as a bioactive compound and as an indicator of processing-driven chemical modulation. Fruits of Coffea arabica L. (cv. Bourbon Amarelo) were evaluated at distinct maturity stages (immature, ripe, and CD) and subjected to combinations of post-harvest steps including: (i) application of the Versatians product, (ii) presence/absence of washing after Versatians treatment, and (iii) controlled fermentation. The experimental design comprised multiple replicated treatments, fruit sample preparation, and targeted quantification of key metabolites, with emphasis on caffeine, which ranged from 3.66 to 6.99 mg g^{-1}. Caffeine quantification revealed a maturity-stage effect, with lower concentrations in ripe fruits than in immature fruits, reaching up to 33% variation between stages. Moreover, no consistent increase in caffeine was associated with the Syngenta product when compared with the respective controls within the same maturity stage. In contrast, fermentation increased caffeine in immature fruits, as fermented samples showed, on average, 34% higher levels than non-fermented immature fruits, suggesting that fermentation can intensify this alkaloid in matrices initially displaying higher baseline variability. Overall, the data indicate that immature coffees exhibit a chemically relevant profile that is responsive to processing, reinforcing their potential for the identification and valorization of bioactive metabolites. Caffeine emerged as a discriminant marker for ripening stage and for fermentation effects, supporting metabolomics as a strategic tool to optimize post-harvest routes and add value to lots with a higher proportion of green fruits.

1224 A Extraction method-dependent recovery of trigonelline and caffeine from coffee husk of two coffee arabica cultivarssXTRACTION METHOD–DEPENDENT RECOVERY OF TRIGONELLINE AND CAFFEINE FROM COFFEE HUSK OF TWO COFFEA ARABICA CULTIVARS

PRESENTING AUTHOR: ***Laurence Rodrigues do Amaral, Federal University of Uberlandia, Brazil***

CO-AUTHORS: *Fernanda Antunes Domingues, Lívia Carneiro Fidélis Silva, Marcelo Antônio Duarte Cruz, Luiza Manuela Alves Braga Cardoso, Matheus de Souza Silva Mateus, Ordarlei José Aparecido Silva, Pedro Luiz Lima Bertarini, Líbia Diniz Santos, Matheus de Souza Gomes*

SUB-THEME: Fermented foods

Coffee husk, a major by-product of coffee processing, is a rich source of bioactive compounds with potential applications in the food, cosmetic, and pharmaceutical industries. Among these compounds, the alkaloids trigonelline and caffeine are recognized for their antioxidant properties and potential functional benefits. This study evaluated the recovery of trigonelline and caffeine from the husk of two Coffea arabica cultivars (Arara and Bourbon) obtained during pulped coffee processing. In addition, different extraction methods were compared to investigate their influence on the recovery of these metabolites, namely infusion, decoction, and cold extraction, using water as the solvent. Quantification of trigonelline and caffeine was performed by high-performance liquid chromatography (HPLC) using a C18 column. All analyses were carried out in triplicate, and statistical differences were assessed by analysis of variance (ANOVA) followed by Tukey's test at a 95% confidence level. Both compounds were detected in the husk of the two cultivars analyzed. Trigonelline concentrations ranged from 6.26 to 7.17 mg g^{-1} in Arara and from 7.58 to 9.43 mg g^{-1} in Bourbon. Caffeine concentrations ranged from 3.97 to 4.87 mg g^{-1} in Arara and from 4.53 to 6.47 mg g^{-1} in Bourbon. Overall, the Bourbon cultivar exhibited higher levels of both metabolites. Extraction method significantly influenced compound recovery, with decoction and cold extraction showing greater efficiency than infusion. The highest concentrations of trigonelline (9.43 mg g^{-1}) and caffeine (6.47 mg g^{-1}) were obtained by cold extraction in the Bourbon cultivar. These results demonstrate that the recovery of these antioxidant alkaloids from coffee husk depends on both the extraction method and the cultivar. The findings highlight the potential of Coffea arabica husk as a source of bioactive compounds and support the valorization of coffee-processing residues for sustainable and functional applications.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1225 B Comparative Metabolic and Chemical Profiling of Apple Vinegars Derived from Juice- and Pomace-Based Fermentation

PRESENTING AUTHOR: ***Qizai Wang, University of Turku, Finland***

CO-AUTHORS: *Niina Kelanne, Oskar Laaksonen, Maarit Heinonen, Baoru Yang, Maaria Kortesniemi*

SUB-THEME: Fermented foods

Apple cider vinegar (ACV) is produced through sequential alcoholic and acetic fermentations, and its quality is strongly influenced by substrate composition. Apple juice, obtained during industrial juice processing, is commonly used as the fermentation substrate. This processing step generates large quantities of apple pomace as a by-product. Pomace differs from juice in its chemical composition and is rich in structural polysaccharides and phenolic compounds. However, comparative studies on fermentation behavior between juice- and pomace-based substrates remain limited.

The aim of this study was to evaluate the effect of fermentation substrate composition on metabolic dynamics during ACV production. The feasibility of apple pomace as a fermentation substrate was also examined.

Three substrates were established from a Finnish apple cultivar: pure apple juice, juice–pomace mixture, and water–pomace mixture. Alcoholic fermentation was conducted with *Saccharomyces cerevisiae*, followed by acetic acid fermentation with unpasteurized commercial vinegar. Ethanol and acetic acid were analyzed by GC–FID, sugars and organic acids were analyzed by ^{1}H NMR spectroscopy, and volatile compounds were characterized by HS-SPME-GC–MS. Multivariate analyses, including PLS-DA and PCA were conducted to discriminate samples and identify metabolites contributing to differences among substrates.

Distinct fermentation kinetics and metabolite evolution patterns were observed among the three substrates. The water–pomace fermentation system exhibited altered sugar utilization, acidification behavior, and volatile compound profiles compared to juice-based systems, indicating that substrate composition influences metabolic transformation during ACV production.

This study provides insight into fermentation metabolism influenced by substrate composition during ACV production. The valorization of apple pomace as an agricultural by-product through fermentation represents a promising and sustainable approach.

1226 A Untargeted UHPLC-HRMS-based metabolomics for fingerprinting sugar-based adulterated honey

PRESENTING AUTHOR: ★ ***Nicolás Zabalegui, CONICET, Argentina***

CO-AUTHORS: *María Eugenia Monge*

SUB-THEME: Foodomics and food security

Honey is a nutritious complex mixture produced by honey bees that contains glucose and fructose in very high concentration, disaccharides, and other minor components such as proteins, enzymes, organic acids, phenols, minerals, and amino acids. It is frequently adulterated by adding inexpensive syrups1. Other less common adulteration practices include mislabeling, the addition of pollen to ultrafiltered honey and feeding bee colonies after the onset of nectar flow. These practices have an impact on human nutrition and health, and affect local and international markets1. A recent study1 showed that 46% of samples imported into the European Union between January 2021 and June 2022 were flagged as potentially fraudulent. Novel and sophisticated methods of adulteration challenge established detection methods.

The present work aimed at identifying novel adulteration markers using a UHPLC-HRMS-based untargeted metabolomics strategy with hydrophilic interaction chromatography and negative electrospray ionization. Honey samples (n=38) collected from different geographical Argentine regions were analyzed to search for known fraud markers as well as novel adulteration compounds. Paired samples collected from the same collection centers were labeled as adulterated or non-adulterated based on Stable Carbon Isotope Ratio Analysis. Sample preparation involved an 80-fold dilution with acetonitrile-water (50:50 v/v). Five different types of quality control samples were used for analysis. Volcano plots, Spearman correlation analysis, and frequency of new feature appearance after adulteration in paired samples were used for data analysis.

The LC-HRMS-based untargeted metabolomics strategy enabled the detection of endogenous honey compounds, several oligosaccharides known as fraud markers, as well as new potential adulteration features. One of the most promising candidates was putatively annotated as hydroxyquinoline. The identification of these novel markers needs to be further confirmed with ongoing tandem MS experiments and retention time matching with chemical standards.

[1] Paiano, V.; Breidbach, A.; Lörchner, C.; et al. Separations 2025, 12 (2), 47. https://doi.org/10.3390/separations12020047

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1227 B Beyond the Root: A Foodomics Workflow Turning Carrot Agrobiodiversity into Circular Bioeconomic Value

PRESENTING AUTHOR: ***Juan Camilo Henao Rojas, AGROSAVIA, Colombia***

CO-AUTHORS: *Monica Cala; Luis Carlos Chivita; Jaison Martínez; Yeraldin Bedoya; Edison Osorio*

SUB-THEME: Foodomics and food security

Antioquia Zana is a multi-institutional research and innovation program supported by Colombia's General Royalties System (SGR) and the Ministry of Agriculture, designed to strengthen the carrot value chain through four coordinated pillars: (i) characterization and strategic use of local carrot agrobiodiversity, (ii) development of differentiated raw materials for functional foods, (iii) development of bio-ingredients for the pharmaceutical and cosmetic sectors, and (iv) market intelligence, technology transfer, and value-chain consolidation. Within this platform, we implemented a "differential quality" workflow that positions metabolomics as a decision backbone for circular, agrobiodiversity-based bioeconomy chains in developing-country contexts, using carrot (Daucus carota L.) as a model crop. The workflow starts with a demand-driven diagnostic that combines bibliometrics, digital trend mining, consumer insights, and market analytics to define target product attributes and to narrow the gap between primary production, R&D, and downstream market requirements for value-added applications. Metabolomics is then applied at two complementary scales. First, untargeted LC–MS fingerprinting of locally cultivated varieties is used to resolve phytochemical diversity, define chemotype-specific markers for traceability, and prioritize metabolite families associated with functional potential. Second, metabolomic profiling of surplus and cosmetically/industrially rejected biomass across distinct microclimates identifies location- and defect-linked chemical signatures that guide bioprospecting and upcycling, directly supporting postharvest loss reduction. These chemical data inform evidence-based selection and optimization of transformation technologies. In Antioquia Zana, processes such as hydrodynamic cavitation and spray drying are deployed to improve preservation, concentration, and stability of bioactive compounds relevant to food and cosmeceutical applications, enabling the conversion of differentiated and surplus carrots into functional ingredient bases. Finally, metabolomic evidence is translated into scalable product concepts through integrated formulation development, physicochemical stability and sensory assessment, and functional/nutritional validation of food and cosmeceutical prototypes. Collectively, this workflow provides an operational roadmap for building circular, science-validated value chains grounded in agrobiodiversity within the Colombian bioeconomy.

1228 A Unraveling Hidden Health Benefits in Our Food : an LC-MS-based framework for rapid screening of functional component in food

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

CO-AUTHORS: *Hiroki Naito*

SUB-THEME: Foodomics and food security

Japan has pioneered systems for evaluating the health functions of food, such as the Foods for Specified Health Uses (FOSHU) system. These functional components include both water-soluble and fat-soluble substances, making it difficult to efficiently identify them from a wide variety of foods. Because technologies for simple and rapid simultaneous analysis of these components are not yet widespread, there is a possibility that useful components in local foods are being overlooked.

Our team has developed an LC-MS-based method for quantitatively analyzing key functional components, including those found in FOSHU products, from a wide variety of food samples using a single procedure. We have conducted experiments using over 50 commercially available FOSHU products (including beverages, gum, and solid foods such as sausages), confirming that functional analysis is possible regardless of the food's form. While general "non-targeted analysis (comprehensive analysis)" can detect more components, it is difficult to identify substances and accurately determine their quantities. Furthermore, even if new components are found, a lack of data on their functionality and safety means that social implementation will take a long time. In contrast, our technology targets components with known functionality and safety, allowing for comparison of component amounts between candidate foods and calculation of quantitative values. In addition, integration with existing pharmacological databases and epidemiological data, we can demonstrate how the detected functional components contribute to human health and longevity. This research can contribute to revealing hidden health benefits in underexplored food and assisting rapid development of new functional food to benefit human health.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1229 B Foodomics insights into bioaccessible antioxidant peptides generated during bovine milk digestion

PRESENTING AUTHOR: ***Diana Vargas, Alpina Productos Alimenticios S.A.S Bic, Colombia***

CO-AUTHORS: *Julián Quintero, Erika Obando, Paola Zapata, Bernadette Klotz*

SUB-THEME: Foodomics and food security

Bovine milk is a food matrix with recognized nutritional value and a role as a source of digestion-derived bioactive compounds. However, peptide signatures generated after standardized in vitro gastrointestinal digestion, and their contribution to functional properties in dairy foodomics, remain insufficiently characterized in UHT matrices. This study used an in vitro digestion and peptidomics workflow to evaluate how foodomics technologies can characterize bovine milk as a bioaccessible peptide-generating matrix. One raw milk reference and three UHT bovine milks from the Colombian market were subjected to oral, gastric, and intestinal phases of the INFOGEST 2.0 protocol. Native samples and final intestinal digests were assessed using $ABTS^{+\cdot}$ and ORAC antioxidant assays. Peptidomic analysis was performed on final intestinal digests, fractionated by molecular weight, analyzed by high-resolution LC-MS/MS, and annotated against bioactive peptide databases.

Antioxidant capacity showed an assay-dependent response: $ABTS^{+\cdot}$ antioxidant activity increased significantly after digestion in all matrices (p 0.05). These results suggest that hydrolysis-derived peptide fractions were more responsive to the $ABTS^{+\cdot}$ radical system than to peroxyl radical-mediated oxidation evaluated by ORAC. Peptidomic profiling identified 23,320 peptide sequences, with 1,500 retained for interpretation. Short peptides, mainly 7–13 amino acids, and the

These findings indicate that simulated gastrointestinal digestion drives bioaccessible peptide generation in bovine milk. In UHT matrices evaluated, matrix-specific peptide profiles were observed after digestion, with peptide pools associated with increased ABTS antioxidant activity. Overall, this work supports foodomics approaches to characterize bovine milk as a complex functional food matrix and understand its relevance for functional nutrition.

1230 A Integrating non-targeted metabolomics and chemoinformatics to map the chemical diversity of Coffea arabica leaf extracts

PRESENTING AUTHOR: ***Davide Rovelli, Univeristy Of Parma, Italy***

CO-AUTHORS: *Davide Rovelli, Ada Nucci, Tito Damiani, Chiara Dall'Asta*

SUB-THEME: Foodomics and food security

Despite their long-standing integration into the ethnomedicine of coffee-growing regions, the recent classification of Coffea leaf infusions as a traditional novel food by the European Union has catalyzed a shift from a mere by-product toward their broader application as botanical extracts. However, the inherent complexity for these products remains a significant industrial hurdle. The plasticity of the botanical matrix, driven by both ecological variability and manufacturing protocols, often results in heterogeneous phytochemical profiles. Such inconsistency is the main limitation that complicates their assessment in terms of efficacy and safety, with the risk of misinterpreting the product's bioactivity, either by inflating the importance of isolated compounds at the expense of metabolic synergies or by failing to detect subtle deleterious effects. To deepen the understanding of coffee leaf composition and their effects in terms of potential bioactivity of the final extract, an in-depth non-targeted mass spectrometry-based metabolomic analysis was performed on coffee leaves extracts (n=27) collected form three different countries (Nicaragua, Mexico, and Laos). The results showed that the metabolome of coffee leaves exhibited significant origin-dependent differences, primarily driven by variations in the relative abundance of specialized metabolite classes (e.g., alkaloids, polyketides, and terpenoids). This highlights a unique metabolome for each origin, indicating a strong influence of local biotic and abiotic factors. Moreover, the resulting data served as foundation for a chemoinformatic pipeline used to map the structural diversity of unique metabolites identified. Overall, this study establishes a robust framework that integrates metabolomic diversity with bioactivity and toxicity scoring. By prioritizing metabolites based on their predicted functional potential, this approach facilitates the valorization of coffee leaves as an high-value matrix and the investigation of the inherent complexity of botanical products, bridging the gap between raw analytical data and targeted biological validation.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1231 B Comprehensive Phenolic Profiling of Traditional Aromatic Rice by Integrated Metabolomics to Support Geographical Indication

PRESENTING AUTHOR: ★ ***Ana Koifman, UNIRIO, Brazil***

CO-AUTHORS: *Ana Bari Koifman, Carolina Thomaz Dos Santos D'Almeida, Hygor Marcos Ribeiro de Souza, Mariella Camardelli Uzeda, Alexandre Guedes Torres, Cristina Yoshie Takeiti, Mariana Simões Larraz Ferreira*

SUB-THEME: Foodomics and food security

Arroz Anã de Porto Marinho, a traditional aromatic rice from Cantagalo (Rio de Janeiro, Brazil), is under evaluation for geographical indication certification. Metabolomics provides molecular fingerprinting to discriminate *terroir*. This study evaluated the effects of pollination system and crop year (C1: 2024; C2: 2025) on its phenolic profile using an integrated untargeted–targeted metabolomics approach. Grains were polished and ground to obtain bran and white rice. Metabolites were extracted with aqueous ethanol followed by alkaline and acid hydrolysis. Untargeted profiling was performed by UHPLC-HRMS (Orbitrap Q-Exactive Plus, ESI+ and ESI-; *m/z* 100–1000), with pooled QC samples and analytical standards analyzed by MS/MS to support annotation in Progenesis QI according to MSI guidelines. Targeted analysis was conducted by UHPLC-QqQ-MS (MRM mode) (Xevo TQ-S) using external calibration and manual validation (TargetLynx, R^2>0.995). Untargeted profiling detected 16,965 molecular features, refined to 86 high-confidence phenolic features after database and QC filtering. PCA explained 82% of total variance and separated white rice and bran (PC1), with tightly clustered white rice samples indicating high compositional stability, while bran samples clustered by crop year, indicating a strong climatic effect. HCA showed that the phenolic profile was primarily driven by grain fraction, followed by crop year and pollination system. Pollination promoted consistent grouping, including clustering between self- and external pollination. Targeted analysis indicated a predominance of hydroxycinnamic acids, with *trans*-ferulic acid as the major compound, followed by *p*-coumaric and caffeic acids. Phenolic levels were 15–25-fold higher in the outer grain layers (bran) than in polished rice, as expected. *Trans*-ferulic and *p*-coumaric acids were the most concentrated phenolic compounds, whereas caffeic acid was detected in white rice only in C2. These findings reinforce the robustness of metabolomics as a powerful approach to support the geographical indication of this traditional rice based on its distinctive phenolic signatures.

1232 A Integrated 1H NMR Metabolomics and Melissopalynology for Geographic Traceability of Argentine Export Honey.

PRESENTING AUTHOR: ★ ***Paula Burdisso, IBR-CONICET, Argentina***

CO-AUTHORS: *Guillermina Andrea Fagúndez, María Gabriela Tamaño, Rodolfo Maximiliano Rasia, Andrea Valeria Coscia, Paula Burdisso*

SUB-THEME: Foodomics and food security

Introduction and Aim: Argentina is a leading global honey producer, exporting 95% of its production. Establishing geographic authenticity is vital for market differentiation but remains challenging due to overlapping chemical profiles. This study established an analytical framework integrating 1H NMR metabolomics and melissopalynology to characterize and predict the geographic origin of honey from central Argentina.

Methods: 180 samples from four ecoregions (Chaco Húmedo, Chaco Seco, Espinal, and Pampa) were analyzed. 1H NMR fingerprints (700 MHz) provided 30 identified metabolites via MCR-ALS, while pollen profiles were determined through melissopalynology. Data were explored using PCA and OPLS-DA, and integrated via multi-block fusion. To evaluate the independent predictive capacity of metabolic profiles, machine learning (ML) algorithms —including Random Forest (RF) and SVM— were applied specifically to the NMR datasets.

Results: Data fusion significantly enhanced characterization compared to individual platforms. OPLS-DA models successfully discriminated the four ecoregions and even identified sub-regional clusters, such as Entre Ríos province, within the Espinal. Key regional biomarkers were identified: Chaco Húmedo was associated with higher Valine and Acicarpha tribuloides pollen, while Espinal showed elevated Raffinose, Isomaltose, and Nigerose. Regarding predictive power, ML models applied exclusively to NMR data demonstrated high efficiency. Random Forest achieved the best performance with an overall accuracy of 85% and ROC-AUC of 0.891, successfully classifying samples without requiring individual palynological analysis for prediction.

Conclusions: While the fusion of orthogonal datasets provides a superior baseline for regional characterization, this research demonstrates that ML models can maintain high classification accuracy using only 1H NMR profiles. This offers a scalable, high-throughput framework for honey authentication, resolving complex traceability issues while significantly reducing analysis time and labor costs compared to traditional melissopalynology.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1233 B Modulation of host tryptophan metabolism by a Mediterranean vs. a low-fat diet is associated with cognitive function in older adults with mild cognitive impairment

PRESENTING AUTHOR: ***Tomas Meroño, University of Barcelona-CIBERFES, Spain***

CO-AUTHORS: *Lorena Iglesias Vejar, Miriam Martínez-Huélamo, Elena M. Yubero-Serrano, Marta Cubedo-Cullere, Meryl Cruz, Marina Mora-Ortiz, Anna Guadall, Alex Sanchez-Pla, Pablo Pérez-Martínez, Cristina Andrés Lacueva, Tomás Meroño*

SUB-THEME: Foodomics and food security

INTRODUCTION AND OBJECTIVE: Precise modulation of tryptophan metabolism through diet may promote brain resilience. This study aimed to evaluate the effect of a Mediterranean diet (MD), with or without probiotic supplementation, compared with a low-fat diet, on host- and microbiome-derived tryptophan metabolites and their association with cognitive function in older adults with mild cognitive impairment (MCI).

MATERIAL AND METHODS: Randomized, crossover clinical trial including 47 older adults with MCI, who completed three different dietary interventions during 24-weeks period: MD with probiotics, MD with placebo, and a low-fat diet. Dietary intake was assessed using a validated-food frequency questionnaire and a 3-day 24h dietary records. Cognitive function was assessed using a neuropsychological battery test. Dietary tryptophan intake was quantified from the dietary records. Tryptophan metabolites were measured using a targeted metabolomics exposome-based UPLC-MS/MS method. Linear mixed-effects models adjusted for age, sex, and intervention sequence were applied, with false discovery rate (FDR) correction using an exploratory threshold. Multivariate tests were used for association analyses.

RESULTS: kynurenine, 5-hydroxytryptophan, 3-hydroxykynurenine were increased in the MD interventions (FDRadjusted p-value<0.05). In addition, both MD interventions reduced N-acetyl-N-formyl-5-methoxykynurenamine (AFMK) levels, while MD+probiotics showed an increase in phenyllactic acid (FDRadjusted p-value<0.25). A direct association was observed between 5-hydroxytryptophan and visuospatial domains, while there was an inverse association between AFMK levels and executive functions. Dietary tryptophan from nuts was positively associated with 5-hydroxytryptophan and 3-hydroxykynurenine levels, as well as tryptophan from vegetables with kynurenine levels. Energy from SFA was negatively associated with kynurenine levels.

CONCLUSIONS: Balancing fats and including tryptophan from key food groups in older adults induced changes in tryptophan metabolites. Precise dietary modulation of tryptophan metabolism may constitute a nutritional target within the gut–brain axis for improving cognitive function.

1234 A Unveiling the Chemical Diversity of Southeast Asian Herbal Teas: Comparative Profiling of Whole-Plant Metabolites and Traditional Infusions

PRESENTING AUTHOR: ***Hiroki Naito, The University of Osaka, Japan***

CO-AUTHORS: *Hiroki Naito, Lulu Eki Daysita, Sivamoke Dissook, Walter Alvarez Laviña, Eiichiro Fukusaki, Sastia Prama Putri*

SUB-THEME: Foodomics and food security

Herbal teas derived from a diverse array of plants have long been consumed in Southeast Asia. However, the compositional data of local resources are fragmented, and the diversity of candidates makes it difficult to determine which species to prioritize. This necessitates a systematic approach to selecting underexplored species and evaluating their chemical profiles, comparing their inherent potential with their delivery in actual consumption forms. This study aims to uncover the chemical potential of these herbal teas and evaluate their functional delivery through traditional brewing.

To achieve this, we employed a literature-driven screening to select 20 herbal teas (10 flowers and 10 leaves). The selected samples were analyzed using gas chromatography-mass spectrometry (GC/MS)-based wide targeted primary metabolite profiling, and liquid chromatography-mass spectrometry (LC/MS)-based targeted analysis to specifically profile functional compounds. Furthermore, to assess actual intake efficiency, we compared the relative abundances in hot water extracts with those in comprehensive solvent (using the Bligh-Dyer method) extracts of pulverized samples.

GC/MS analysis identified 135 compounds. Multivariate analyses, including PCA and OPLS-DA, revealed that flower teas are characterized by specific accumulations of sugars and amino acids compared to leaves, despite strong species-dependent variations. Through LC/MS analysis, various functional compounds, including catechins, isoquercetin, and γ-aminobutyric acid , were detected, revealing species-specific distributions. Additionally, *Peperomia pellucida* (pepper elder) exhibited approximately nine times higher campesterol (a phytosterol known for its cholesterol-lowering effects) levels than other tested herbs. Results revealed highly variable extractability among compounds, with traditional brewing yielded less than 10% of specific functional compounds compared to solvent extraction in certain plants. These findings provide a chemical basis for

understanding the bioactive potential of Southeast Asian herbal teas and offer crucial insights for developing novel consumption strategies to maximize them.

1235 B High-throughput metabolite profiling of yeast culture supernatant using PESI-MS

PRESENTING AUTHOR: ***Takeo Taniguchi, The University of Osaka, Japan***

CO-AUTHORS: *Nobuyuki Okahashi, Prihardi Kahar, Takanari Hattori, Chiaki Ogino, Hidenori Takahashi, Fumio Matsuda*

SUB-THEME: Metabolic engineering

[Introduction] High-throughput profiling of metabolites in crude biological samples such as culture media remains a challenging problem in metabolomics studies. Probe electrospray ionization-mass spectrometry (PESI-MS) enables rapid direct analysis of matrix-rich samples without time-consuming sample preparation. In this study, using yeast culture supernatants as a model, we investigated the feasibility of rapid metabolite profiling using PESI-MS.

[Materials and Methods] Saccharomyces cerevisiae and 13 non-conventional yeasts were cultured for 24 h in SD medium containing 2, 5, or 10% glucose. Equal volumes of ethanol were mixed with spent medium supernatant and served for PESI-qTOF/MS. 28 metabolites (amino acids, organic acids and sugar alcohols) were measured per sample in 15 seconds.

[Results] To establish a high-throughput PESI-MS method, the measurement stability, acquisition time, and the dynamic range were evaluated. Measurement stability was assessed using the same probe by 10 repeated 30-s analysis of SD medium supplemented with 28 metabolites at 100 μM, where 22 metabolites showed relative standard deviations below 10%. 23 metabolites achieved a relative standard error below 5% in a 6-s acquisition, enabling the further reduction of the measurement time. The dynamic range was investigated by analyzing SD medium with 1 μM to 100 mM standards. The linear range was confirmed from 100 μM to 10 mM for 25 metabolites. Using the developed method, 126 yeast culture supernatants from 14 strains under 3 culture conditions in triplicate were analyzed in 15 seconds (6 seconds for both negative and positive modes, with 2.4 seconds for polarity switching). Principal component analysis revealed clustering of 13 strains according to glucose concentration of the medium, whereas Komagataella phaffii formed a distinct cluster associated with elevated secretion of α-ketoglutarate and its derived amino acids. These results highlight the utility of PESI-MS for rapid metabolite profiling of crude microbial culture supernatants.

1236 A omics-guided engineering of a hyperpigmented Haloferax volcanii strain producing bacterioruberin with anticancer properties

PRESENTING AUTHOR: ***Ansgar Poetsch, Nanchang University, China***

CO-AUTHORS: *Sofia Frecha, Mercedes Elizalde, Roberto Paggi, Maria Ines Gimenez, Guadalupe Romero Turati, Micaela Cerletti*

SUB-THEME: Metabolic engineering

Introduction & Aim: Bacterioruberin is a C50 carotenoid from haloarchaea with potent antioxidant properties and emerging anticancer potential. Proteomics of a conditional LonB mutant in *Haloferax volcanii* identified phytoene synthase (PSY) as a substrate of this membrane protease. PSY increased 50–70 fold, correlating with bacterioruberin-enriched pigment accumulation. However, the LonB mutant grows poorly, limiting its biotechnological application. Previous evidence suggested that PSY's C-terminus (Ct) is responsible for its stability. The aim of this work is to characterize a PSYCt mutant for growth, bacterioruberin yield, and biological activity in tumoral cells.

Methods: *H. volcanii* H26 (WT) and strain PSYCt, devoid of the C-terminal degron, were grown in CAB medium at 42 °C. Growth was monitored by OD600 and carotenoids were extracted from stationary-phase cells and quantified at 474 nm. Dried extracts were resuspended in DMSO. Antiproliferative activity (0–1.5 μM) was evaluated by MTT assay in Vero cells, used as non-tumoral control cells, and Huh-7 and HepG2 cells (liver tumor) at 24, 48, and 72 h. Apoptosis was analyzed by acridine orange/ethidium bromide staining (AO/EtBr) and confocal microscopy.

Results: PSYCt showed stable bacterioruberin production. Total carotenoid yield was 4-fold higher in PSYCt compared to WT, with no detectable growth differences. At 0.25 μM and 48 hs, the extract had no effect on Vero cells but reduced Huh-7 and HepG2 viability by 50%. By AO/EtBr staining, at 0.5 μM apoptotic Huh-7 cells doubled relative to control and at 1.5 μM, nearly 40% were apoptotic.

Conclusions: Proteomics uncovered that not increasing PSY synthesis but preventing its degradation is a viable strategy to achieve higher PSY concentration and consequently enhanced bacterioruberin production in *H. volcanii*. Pigment extracts

selectively reduced tumor cell viability and induced apoptosis, highlighting PSYCt as a promising platform for the sustainable production of bacterioruberin as a natural pigment with potential anticancer applications.

1237 B Metabolomic Profiling of Pseudomonas putida Grown on Ethylene Glycol as the Sole Carbon Source

PRESENTING AUTHOR: ***Igor Sepulveda Rodrigues, USP-RP, Brazil***

CO-AUTHORS: *Ricardo Roberto da Silva, Maria Eugenia Guazzaroni*

SUB-THEME: Microbial metabolomics

The exponential rise of global PET production that exceeds 80 million tons annually overwhelms waste management systems, leading to massive accumulation in oceans, landfills, and soil, resulting in severe impacts on ecosystems. Current waste management technology is not able to solve this rising environmental problem. Ethylene glycol (EG) is a byproduct of PET degradation that limits microbial growth and constrains scalable bioremediation and upcycling. It has been shown previously that Pseudomonas putida tolerates and can use EG as a carbon source. Our group has been characterizing a new P. putida strain (BJA5) that shows rare growth capability and comparatively higher tolerance to ethylene glycol compared to previously described strains. The main hypothesis is that successful growth depends on efficient conversion of EG into glyoxylate and on intracellular glyoxylate accumulation, triggering transcription of the gcl operon, enabling carbon assimilation for biomass formation. This project aims to expand biological solutions for plastic waste by elucidating how P. putida tolerates and grows on ethylene glycol (EG) as the sole carbon source. We performed growth curve experiments across EG concentration ranges and mixed carbon gradients, showing prolonged lag phases and patterns consistent with delayed pathway inductions. Metabolite profiling using Liquid (L) and Gas (G) Chromatography (C) coupled to mass spectrometry (MS) has shown that the latter allowed the detection of downstream intermediates of EG metabolism, especially glyoxylate. The next steps of that study include monitoring glyoxylate accumulation through the growth curve using GC-MS, the creation of knckout of the gene gclR, known to repress the expression of gcl and the overexpression of gene glcDEF know to encode an enzyme that catalyses the oxidation of glicolate in to glioxilate. Overall, our preliminary metabolomics validation and genomic investigation afforded a extrategy that may support the observation of an extended lag phase, and provide strain-specific metabolic signatures.

1238 A Factors Shaping the Captured Actinomycete Metabolome: Taxonomy, Culture Media, and Extraction

PRESENTING AUTHOR: ***Michaela Plechatá, Institute Of Microbiology CAS Prague, Czechia***

CO-AUTHORS: *Aninda Mazumdar, Tommaso Stefani, Zdeněk Kameník*

SUB-THEME: Microbial metabolomics

Comprehensive characterization of actinomycete metabolomes is challenged by the strong dependence of detectable chemical diversity on cultivation conditions, downstream sample processing, and chemical analysis. Here, we present a systematic high-throughput approach to assess how biological and analytical variables shape the observable metabolome across taxonomically diverse actinomycetes.

Ten strains representing distinct genera (Streptomyces, Nocardia, Nonomuraea, Mycobacterium, Amycolatopsis, Couchioplanes, Saccharomonospora, Kribbella, Actinoplanes, and Micromonospora) were cultivated in 10 chemically diverse media and subjected to four solid-phase extraction strategies (acid/base pretreatment combined with reversed-phase or mixed-mode ion-exchange sorbents), generating over 400 extracts. Samples were analyzed by LC–MS/MS (timsTOF). Data processing in MZmine 4, including blank subtraction, yielded 6,964 high-quality microorganism-associated features, of which approximately 15% were annotated using GNPS2, in-house spectral libraries, MetaboScape, and SIRIUS.

To enhance metabolite annotation, metabolomic data were integrated with genome mining results from antiSMASH 8.0. For example, a Couchioplanes strain harbored a biosynthetic gene cluster with high similarity to the sibiromycin pathway, and the corresponding metabolite signal was detected under specific cultivation conditions. Comparative analysis of the full dataset - including media-, taxonomic-, and extraction-strategy-resolved feature distributions visualized using UpSet plots - revealed that culture medium exerted the strongest influence on detectable chemical diversity (~3,300 unique features), followed by taxonomic variation (~3,100) and extraction strategy (~2,600). Detailed metabolite distributions across the different extraction approaches will be presented.

All annotated data will be deposited in CMMC-KB to facilitate community access and enable biological context for untargeted metabolomics datasets, allowing users to link detected compounds in any sample to actinomycete producing strains.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1239 B Phenotype-Guided Multiomics Reveals Differences in Furaquinocin Biosynthesis in Streptomyces cinnamonensis

PRESENTING AUTHOR: ***Sofia Camila Bravo, Ruhr University Bochum & Max Planck Institute, Germany***

CO-AUTHORS: *Frank Peeters, Christian Rückert-Reed, Daniel Petras, Frank Schulz*

SUB-THEME: Microbial metabolomics

Streptomyces are well known for their ability to produce a wide range of bioactive secondary metabolites. Differences in culture coloration between wild-type and industrial monensin-producing Streptomyces cinnamonensis strains motivated an untargeted multi-omics approach combining whole-genome sequencing and metabolomics.

Comparative genomic analysis using Nanopore whole-genome sequencing revealed multiple mutations and polymorphisms between the strains. Untargeted metabolomics using reverse-phase and HILIC chromatography coupled to Orbitrap and QTOF HRMS, respectively, allowed the detection of several metabolites produced by both strains.

One of the main differences identified was found in hybrid biosynthetic gene clusters related to furaquinocin and endophenazyne biosynthesis, including variations in copy number and mutations. Furaquinocins are meroterpenoid compounds containing a quinone core linked to an isoprenoid moiety, and some congeners have shown bioactivity, including antitumoral activity against HeLa cells. Comparative metabolomics enabled the simultaneous detection of most known furaquinocin types and revealed significant differences in their distribution and oxidation state between strains.

Interestingly, a mutation in an accessory gene within this pathway could explain the differences observed in the metabolic profile and reveal its previously unknown role in this biosynthetic gene cluster. Overall, the combination of metabolomic and genomic analyses improved the understanding of this biosynthetic pathway, demonstrating the value of complementary omics approaches.

1240 A Nano-flow SQUAD LC-HRAM enables ultrasensitive, multi-matrix bile acid quantitation and discovery

PRESENTING AUTHOR: ***Julian Saba, Thermo Fisher Scientific, Canada***

CO-AUTHORS: *Rob Harlan, Bashar Amer, Cynthia Grim, Rahul R. Deshpande, Nichole Reisdorph, Katrina Doenges, Michael Armstrong, Susan S. Bird*

SUB-THEME: Microbial metabolomics

Bile acids (BAs) are central regulators of metabolic signaling and host-microbiome interactions, yet their comprehensive analysis remains challenging due to structural similarity and broad concentration ranges. Although Simultaneous Quantitation and Discovery (SQUAD) workflows enable integrated targeted and untargeted metabolomics, sensitivity limitations at conventional flow rates restrict detection of low-abundance BAs. Here, we introduce a nano-flow SQUAD LC-HRAM workflow that overcomes these constraints, enabling ultrasensitive BA quantitation alongside expanded metabolite discovery. The method integrates a Thermo Scientific Vanquish Neo nano-flow LC system with a Thermo Scientific Orbitrap Excedion Pro mass spectrometer to enhance sensitivity and metabolome coverage across diverse biological matrices.

Method development employed unlabeled and stable isotope–labeled bile acid standards to support absolute quantitation. Chromatographic separation was performed at 1 µL/min using an easySpray PepMap column (150 mm × 75 µm, 2 µm particles). The nano-flow SQUAD strategy combined high-resolution full-scan MS^1 quantitation with concurrent data-dependent MS^2 acquisition using AcquireX to improve fragmentation coverage and confidence in unknown annotation.

Nano-flow chromatography delivered an order-of-magnitude increase in bile acid signal intensity compared to conventional analytical-flow LC, enabling sub-femtomole detection sensitivity. This enhanced performance supported confident measurement of low-abundance BAs across matched fecal, cecum, brain, plasma, and urine samples, including C57BL/6J mice subjected to route-dependent cannabis administration. Concurrent untargeted analysis expanded bile acid–related feature detection, particularly low-abundance conjugated and microbiome-associated species, with the largest gains observed in fecal and cecal matrices.

Across all sample types, the workflow maintained quantitative robustness while enabling simultaneous discovery from a single injection. Nano-flow SQUAD LC-HRAM thus provides a scalable and ultrasensitive platform for comprehensive, multi-matrix bile acid profiling in complex biological studies.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1241 B Derivatization-Driven Functional Group Molecular Networking Expands Detection and Annotation of Polar Microbiota Metabolites

PRESENTING AUTHOR: ***Zdenek Kamenik, Institute of Microbiology Czech Acad Sci VAT CZ61388971, Czechia***

CO-AUTHORS: *Tommaso Stefani, Anna Michl, Martin Schwarzer*

SUB-THEME: Microbial metabolomics

Polar metabolites produced or modified by the gut microbiota play essential roles in host physiology, yet their comprehensive analysis remains challenging due to poor retention in conventional reverse-phase LC–MS workflows. Chemical derivatization using 3-nitrophenylhydrazine (3-NPH) facilitates detection of carbonyl-, carboxyl-, and phosphate-containing metabolites but has primarily been applied in targeted analyses.

Here we present a strategy combining 3-NPH derivatization with functional group–driven molecular networking to enable large-scale untargeted analysis of microbiota-associated metabolites. Because derivatization introduces a shared structural moiety into metabolites containing targeted functional groups, their MS/MS spectra acquire common fragmentation features. When analyzed using molecular networking in GNPS, this results in clusters driven by the presence of the derivatized functional group rather than the structural similarity of the original metabolites, enabling systematic exploration of metabolite families.

To evaluate this approach, we analyzed a library of 650 polar and gut microbiota-associated metabolites. Using 3-NPH derivatization, 57% of the library was detected, corresponding to 67% of compounds containing derivatizable functional groups, including short-chain fatty acids, organic acids, amino acids, and phosphate-containing metabolites. To address the lack of spectral libraries for derivatized metabolites, we generated a combinatorial SMILES library of derivatized structures from ~10,000 microbial metabolites curated from MiMeDB using a Python-based workflow and performed in silico annotation using SIRIUS.

Application of this workflow to fecal samples from mice with distinct microbiota revealed clear separation of groups in PCA using only derivatized features, highlighting the biological relevance of the approach. The resulting molecular network contained 4006 nodes organized into 294 clusters, of which 8% of nodes and 21% of clusters were annotated using spectral libraries, with additional annotations obtained through in-silico analysis.

Together, these results demonstrate that combining functional group derivatization with molecular networking provides a scalable strategy for expanding the detectable chemical space of polar metabolites in untargeted metabolomics.

1242 A Time-restricted feeding modulates anxiety in a sex-specific manner in mice with neuropathic pain

PRESENTING AUTHOR: ***Vinko Palada, University of Helsinki, Finland***

CO-AUTHORS: *Manqing Wen, Wenjing Dai, Eija Kalso, Vinko Palada*

SUB-THEME: Model organisms

Introduction: Evidence suggests that neuropathic pain (NP) and comorbid mood disorders are associated with circadian abnormalities. This suggests a role for chronotherapies, such as time-restricted feeding (TRF), to alleviate pain and comorbid anxiety. We investigated the effects of TRF on pain and anxiety-related behaviours in spared nerve injury (SNI) mice with NP and diurnal changes in the hypothalamus transcriptome, a key hub for modulating anxiety, and for controlling feeding and metabolism. Methods and results: SNI male and female C57BL/6JRj mice received TRF during dark active phase or ad libitum feeding (ALF) for three weeks post-surgery. Behavioural tests, von Frey, hot plate, light-dark box and open field, were performed at baseline and post-surgery; hypothalamus tissues were dissected in morning (zeitgeber time (ZT) 2-6) and afternoon (ZT 8-12). Male, but not female, SNI mice under TRF showed significantly reduced anxiety-like behaviours (LDT: $p = 0.0259$, OFT: $p = 0.0054$). Differential expression gene (DEG) analysis identified 33 DEGs in the hypothalamus in male SNI mice between TRF and ALF in the afternoon, with enriched anxiety-related, mitochondrial and circadian genes. Conclusion: These findings support TRF as a potential therapeutic approach to alleviate comorbid anxiety in NP.

PLANTS, FOOD, ENVIRONMENT AND MICROBES

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1243 B — Integrating Indigenous Knowledge with Emerging Technologies: AI-driven metabolomics of selected South African medicinal plants

PRESENTING AUTHOR: ***Kgalaletso Othibeng, University Of Johannesburg, South Africa***

CO-AUTHORS: *Fidele Tugizimana*

Zamani Cele

Justin van der Hooft

SUB-THEME: Natural products

Medicinal plants lie at the intersection of biodiversity, Indigenous Knowledge Systems (IKS), and modern science, offering rich sources of natural products with significant therapeutic potential. In South Africa, Melianthus comosus and Hypoxis hemerocallidea are widely used in traditional medicine for anti-venom, wound-healing, and anti-inflammatory purposes, yet their phytochemical maps are poorly known, thereby limiting the applications of the plants and discovery of novel natural products. There is also limited understanding of the underlying biochemistry of these plants with regards to the specialized metabolites they possess: their biosynthetic routes, regulations, biological roles and functions in the plant. Thus, this study applied computational metabolomics to profile the chemical diversity of the leaf and root extracts from both plants and correlate metabolite patterns with their reported medicinal uses. Methanolic and aqueous plant extracts were analysed on advanced liquid chromatography-mass spectrometry (LC-MS) platforms and computational workflows such as feature-based molecular networking, MS2Query, Sirius and DreaMS were used to annotate and evaluate the acquired data. The results revealed rich and diverse metabolite landscapes dominated by flavonoids, hydroxycinnamic acids, tannins, sugars, and lipid-like compounds. Clear species- and tissue-specific differences were observed: M. comosus showed higher flavonoid abundance, supporting its traditional use in anti-inflammatory and anti-venom remedies, while H. hemerocallidea was enriched in lipid-like molecules, consistent with wound-healing and protective properties. Biological assays further showed that the extracts differed in potency and inhibitory performance. Overall, all extracts showed markedly stronger inhibition of 5-lipooxygenase (5-LOX) compared with (COX-2), suggesting a preferential effect on the lipoxygenase pathway. This pattern is consistent with the broader importance of 5-LOX and COX-2 as complementary inflammatory targets in medicinal plant research. This work provides the first integrated computational metabolomics and bioactivity-based comparison of M. comosus and H. hemerocallidea, linking phytochemical diversity with traditional use and identifying candidate chemical classes for future isolation and pharmacological validation.

1244 A — NMR metabolomic analysis of Moringa oleifera (Lam.) tissues grown from two in vitro methods

PRESENTING AUTHOR: ***Gerhard Prinsloo, University Of South Africa, South Africa***

CO-AUTHORS: *Elmien Coetser, Elsa S. du Toit*

SUB-THEME: Natural products

Moringa oleifera Lam. is a valuable food source but also used treat a wide range of medicinal ailments, and therefore important for its potential in drug discovery. This plant produces a plethora of biologically active molecules, and this study provides insight into the production of compounds and how they are affected by production in different cell culture methods. The metabolic response of plants can be used in optimising plant growth systems by aiming at the production of compounds of interest. Two in vitro methods were compared in this study; a conventional solid media method and a temporary immersion system (TIS). The callus and shoot tissues of these cultures were subjected to 1H NMR-based metabolomic analysis to determine the metabolite differences between material produced by thw two tissue culture methods. The growing environment influenced the production of gamma-aminobutyric acid (GABA), chlorogenic acid and glutamine. This is an indication that in vitro methods and different plant growth regulator treatments can be used to manipulate the production of metabolites of plant tissues. The manipulation of the metabolome allows researchers and producers to optimise culture systems for the production of specific compounds.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1245 B Metabolomics and multicriteria optimization for the selection of a Psilocybe strain with high biotechnological potential

PRESENTING AUTHOR: ***Juan Pablo Yasmo Perez, National University of Colombia, Colombia***

CO-AUTHORS: *Carolina Chegwin Angarita, Carolina Vega Oliveros, Daniel Andres Pardo Rodriguez, Leonardo Castellanos Hernandez, Monica Patricia Cala Molina, Monica Constanza Avila Murillo*

SUB-THEME: Natural products

Background: Fungi of the genus Psilocybe produce psychoactive tryptamines such as psilocybin and psilocin, which have attracted attention due to their therapeutic potential. However, their metabolomic diversity remains insufficiently characterized and may contribute to strain-dependent biological activity and biotechnological applications.

Methods: Eleven Psilocybe spp strains were evaluated using an integrated approach combining biomass production in liquid culture, acetylcholinesterase (AChE) inhibition assays, and LC–MS/MS-based untargeted metabolomics. Strain selection was performed through multicriteria decision analysis using the Analytic Hierarchy Process (AHP). Strain F-56 was selected for further evaluation using factorial design experiments to assess the effects of salt concentration, pH, nitrogen source, and inoculum size. In addition, kinetic studies were performed at 5, 7, and 11 days using two nitrogen sources yeast extract and malt extract which had been previously associated with high and low acetylcholinesterase (AChE) inhibition and psilocybin production, respectively.

Results: Biomass production ranged from 32 to 50 mg with high reproducibility (RSD

Conclusions: The integration of metabolomics and multicriteria analysis enabled the selection of a Psilocybe strain with high biotechnological potential. The observed decoupling between growth and secondary metabolism highlights the importance of culture conditions. Ongoing kinetic metabolomic analyses will further elucidate metabolic dynamics and support optimization strategies.

1246 A Potential DNA gyrase inhibitors identified in an endophytic fungus extract of Alternaria alternata P02PL2

PRESENTING AUTHOR: ***Sizwe Ndlovu, North-West University, South Africa***

CO-AUTHORS: *Aviwe N. Matandela, Sphamandla E. Mtambo, and Wonder P. Nxumalo*

SUB-THEME: Natural products

The escalating threat of multidrug-resistant (MDR) bacterial pathogens underscores the urgent need for novel antimicrobial agents. Here, we combined a one-strain many compounds (OSMAC) and untargeted metabolomics approaches to investigate the bioactivities and the chemical space of the endophytic fungus Alternaria alternata P02PL2, isolated from a medicinal plant, Sclerocarya birrea. The antibacterial activities were assessed against contemporary clinical isolates, MDR Pseudomonas aeruginosa 5625574, methicillin-resistant Staphylococcus aureus (MRSA) 5627679, and a collection culture of S. aureus ATCC 25923 for reference. We first tested for bioactivities across eight growth media to optimise the production of bioactive secondary metabolites. The bioactive fungal extract fractions exhibited minimum inhibitory concentrations (MICs) ranging from 0.25–0.5 mg/mL. This was followed by an untargeted metabolomics analysis, which identified 41 secondary metabolites. Virtual screening, molecular docking, and 200-ns molecular dynamics simulations targeting S. aureus and P. aeruginosa DNA gyrase revealed lead compounds with superior binding affinities (e.g., Fluorescein: -9.2 kcal/mol for P. aeruginosa; Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro: -8.8 kcal/mol for S. aureus) compared to reference ligands. Binding free energy calculations highlighted strong affinities, with 5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-bis(3,4,5-trihydroxyoxan-2-yl)chromen-4-one (-42.16 kcal/mol) and Fluorescein (-41.42 kcal/mol) outperforming the P. aeruginosa reference ligand evo (-19.80 kcal/mol), and 80s (-48.74 kcal/mol) leading for S. aureus. Per-residue energy decomposition and quantum chemical analyses further elucidated key interactions and reactivity profiles. These results position A. alternata P02PL2 as a promising source of DNA gyrase inhibitors to combat antimicrobial resistance.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1247 B Metabolite Studies in Microbiome Manufacturing: Investigating the metabolism of Bacteroides, Clostridium, and Prevotella spp. co-cultures to improve probiotic production

PRESENTING AUTHOR: ***Jessica O'Loughlin, The University of Edinburgh, United Kingdom***

CO-AUTHORS: *Robin Sison, Kristine Parson, Catherine McNay, Sarah Windsor, Louise Horsfall, Karl Burgess*

SUB-THEME: Natural products

Many industrial bacterial processes are carried out in monoculture conditions, but the constraints of these environments can limit their efficiency and product biomolecule complexity. One solution to this is to use a co-culture approach, where additional microbial species can have complementary and synergistic interactions to make bioreactor conditions more reliable and robust. However, understanding the interactions between different species in these conditions is required to maintain stable microbial communities and the lack of this is currently a major limiting factor to the widespread adoption of this method. Therefore, the aim of my research is to elucidate metabolite interactions between three gastrointestinal bacterial species - Bacteroides cellulosilyticus, Clostridium beijerinckii, and Prevotella herbatica - to advance our knowledge about how co-culture methods can used in biological manufacturing, in this case, focusing on probiotic production. Monoculture analyses of these species reveals distinct and potentially beneficial metabolic interactions between them in co-culture conditions. Where B. cellulosilyticus' polysaccharide-degrading quantities may allow the release of additional sugars into the media that P. herbatica and C. beijerinckii can consume, promoting their respective lipid turnover and fermentative metabolic characteristics. Different co-culture methods are used to provide complementary insights into how these bacteria interact. Here, direct co-culturing of the bacteria in the same environment allows changes to the extracellular metabolome to be mapped alongside fluctuations in bacterial populations over time, providing an overall picture of the dynamic interactions that may occur in industrially-relevant conditions. However, in-direct, two-way co-culture combinations allow secreted interactions to occur and species-specific intracellular insights to be obtained, letting us see at a phenotypic level how and why certain species may dominate or decline different environments.

1248 A Profiling of Oud (Agarwood) by AEC-MS and TD-GCxGC-MS: Linking Resin Composition to Smoke Emissions

PRESENTING AUTHOR: ★ ***Luciana da Costa Carvalho, University of Oxford, United Kingdom***

CO-AUTHORS: *Laura McGregor, James S. O. McCullagh, and Abdossalam M. Madkhali*

SUB-THEME: Natural products

Agarwood (oud) is a resin-impregnated wood produced naturally by Aquilaria trees in response to injury. Oud chips are burned as incense and its extracts used as perfume across the Arabian Peninsula, Asia, and parts of Africa. In the wild, oud formation can take 25+ years, making it rare and valuable. To meet increasing demand, commercial production of oud relies on artificial stimulation (mechanical, biological or chemical) to induce resin formation in cultivated trees, or artificial means. These approaches contribute to perceived variations in the quality and composition of oud products, but their impact on resin chemistry, and subsequent smoke composition, remains poorly understood.

In order to investigate these variations, we applied untargeted metabolomics using anion-exchange chromatography-mass spectrometry (AEC-MS) and thermal desorption two-dimensional gas chromatography–mass spectrometry (TD-GCxGC-MS) to compare six commercial oud from two suppliers (A and B). AEC-MS analysis of methanolic extracts revealed differences between suppliers and across grades within each supplier. These were driven by differences in abundance for 88 discriminatory compound-features. Nine compound-features were identified via comparison with authentic standards, including myoinositol and pyroglutamic acid (enriched in B3), trehalose-6-phosphate (enriched in A2), hydrocinnamic acid (enriched in A3 and B3), hydroxyacetone (enriched in A3 and B1) and lactose (enriched in A1, A2 and B1). TD-GCxGC-MS analysis of smoke emissions from oud chips revealed distinct volatile profiles, despite their similar aroma to the human nose. Supplier B samples exhibited greater chemical diversity driven by enrichment in methoxy-phenol derivatives, alkylated biphenyls and polycyclic aromatic hydrocarbons associated with lignin combustion and of potential health concern.

Taken together our findings suggest that differences in oud production influence both extract chemistry and combustion emissions. By linking resin composition to smoke by-products, this study provides a framework for investigating oud authenticity, comparative product chemistry, and exposure-relevant compounds generated during its traditional use as incense.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1249 B Examination of dietary strategies for enhanced nitrogen efficiency in dairy cows on rumen metabolism, milk composition and functionality

PRESENTING AUTHOR: ***Nisha Suthar, University College Cork, Ireland***

CO-AUTHORS: *Eoin Wims, Michael Dineen, Raghunath Pariyani, Denis Lynch, Lorraine Bateman, Anita Maguire, James O'Mahony, Tom O'Callaghan*

SUB-THEME: Other

The agri-food sector faces a major global challenge in reducing greenhouse gas emissions, particularly methane and nitrogen-related emissions, in dairy production systems while maintaining productivity and product quality. Government policies aimed at reducing nitrogen losses and protecting water quality are placing pressure on pasture-based dairy systems to reduce nitrogen inputs and improve nitrogen use efficiency (NUE). Typically, cows demonstrate poor NUE. Three potential avenues for improving NUE in dairy systems include reducing external inputs such as nitrogen fertilizer, pasture sward and species strategies, and optimising dietary protein levels. Excessive dietary protein increases nitrogen excretion as urea, contributing to environmental concerns such as nitrate leaching and ammonia volatilization. This study investigates strategies to improve NUE in dairy systems and their effects on milk and rumen metabolism using 1H-NMR and gas chromatography, as well as their impact on milk composition and processibility. To date, we have demonstrated that reducing nitrogen fertilizer application rates did not significantly affect the rumen metabolome. Lowering dietary nitrogen through different crude protein concentrate supplements significantly altered only three of 44 identified metabolites (acetone, propionate and hippurate). Similarly, inclusion of multispecies sward systems significantly influenced three of the 42 identified metabolites (acetone, dimethyl sulfone and hippurate) and seven of the 22 fatty acids analysed. Importantly, these dietary treatments did not negatively affect milk functionality and processability indicators. These results provide initial due diligence on potential consequences of the adoption of these on-farm practices, providing industry stakeholders valuable insights. The "PASTURE-NUE" project now aims to map nitrogen metabolism in dairy cows fed contrasting pasture-based diets differing in white clover and plantain. By analysing biofluids, including rumen, omasum, blood, milk, urine and faeces to gain system-level understanding of nitrogen digestion, absorption, utilization and excretion, helping identify biological mechanisms underlying NUE and to support more sustainable dairy production systems.

1251 B Metabolic responses of beech leaves to abiotic and biotic stress: towards the early detection of tree health decline.

PRESENTING AUTHOR: ***Diana Vinchira-Villarraga, University of Birmingham, United Kingdom***

CO-AUTHORS: *Costanza Cagnina, Gerrard English, Jacqueline Rosette, Lisa Ward, Juan Suarez-Minguez, Robert W. Jackson*

SUB-THEME: Plant defense, pathogens and interactions

Plant resilience to biotic and abiotic stress often involves metabolic reprogramming, yet early detection of these responses in forest ecosystems remains challenging. To investigate stress-induced metabolic changes, beech trees were exposed to drought, pathogen infection, or wounding for 12 weeks. Leaf metabolic profiles were acquired using untargeted HPLC–MS/MS. The study aimed to characterise stress-specific or shared metabolic responses and evaluate their potential to support early, non-destructive hyperspectral monitoring of tree canopies. The metabolic profiles of beech leaves subjected to drought or pathogen (Phytophthora pseudosyringae) stress revealed that trees undergo differential metabolic reprogramming to adapt to both conditions. This phenomenon occurs early in response to pathogen infection, two weeks post-infection, and persists for up to 12 weeks. In comparison, abiotic stress is not detectable in leaves until 8 weeks, suggesting a later response than that observed with pathogen infection. Notably, drought stress response appears to be stronger than that induced by pathogen infection, as a larger number of metabolites varied in drought-stressed trees compared with control trees (Control vs Drought >1400; Control vs Pathogen >200). In the dataset, 569 features were identified as associated with discrimination between control and stressed conditions. Most of these features were exclusive to drought stress (55%) or pathogen infection (28%), while only a minority were shared between both conditions. Changes in prenol lipids and flavonoids (including biflavonoids) characterised the response to P. pseudosyringae, whereas the accumulation of amino acids such as phenylalanine, tyrosine, and tryptophan represented key markers of drought stress. Future correlation analyses will link metabolomics data with hyperspectral measurements to determine whether specific changes in chemical classes are associated with spectral signals. This approach could support the early, non-destructive detection and monitoring of tree health in forest environments.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1252 A Untargeted metabolomics of tomato exposed to shiso VOCs uncovers perillaldehyde-derived metabolites and the recipient leaf response

PRESENTING AUTHOR: ***Yoshihide Matsuo, Suntory Global Innovation Center Inc, Japan***

SUB-THEME: Plant defense, pathogens and interactions

Perilla (Perilla frutescens var. crispa, “Shiso” in Japanese) is a herb cultivated in East Asia for centuries and is known empirically for strong weed-suppressing and antimicrobial activity. Perillaldehyde, the principal monoterpene of shiso essential oil, is generally regarded as the main active component. Yet what perillaldehyde becomes inside a neighbouring plant that takes it up from the air remains poorly described at the metabolome level.

We exposed Solanum lycopersicum cv. Micro-Tom to shiso VOCs in a chamber and profiled leaf metabolites by CE-MS and LC-MS, combining targeted quantification with untargeted feature analysis. The clearest signal was a marked accumulation of perillic acid — the carboxylic acid corresponding to perillaldehyde — indicating that tomato leaves enzymatically oxidise the inhaled monoterpene rather than only absorbing it. The untargeted layer additionally surfaced a series of shiso-specific features whose accurate masses and retention behaviour are consistent with further perillaldehyde-derived conjugates and oxidation products; structural assignment is in progress.

Beyond the perillaldehyde pathway, the recipient leaf metabolome shifted more broadly. Coordinated changes appeared in antioxidant metabolites, amino acid pools and several central primary metabolites, suggesting that shiso VOCs perturb redox balance and core carbon use alongside the monoterpene biotransformation. Tomato therefore both oxidises the inhaled monoterpene and remodels its own metabolism in response.

The work reframes shiso's well-known activity as more than an external deterrent — its VOCs leave a chemical record inside neighbouring plants. Identifying the new perillaldehyde-derived metabolites should clarify whether tomato treats shiso volatiles as a substrate for detoxification, a signal that reorganises metabolism, or both.

1253 B Herbivore-Derived Signals Trigger Genotype-Dependent Defense Metabolic Reprogramming in Eucalyptus: Evidence for HAMP-Mediated Immunity.

PRESENTING AUTHOR: ***Javiera Nahuelcura Valenzuela, Universidad de Concepción, Chile***

CO-AUTHORS: *Javiera Nahuelcura, Areli Jara, Nicolás Fernández, Catalina Bravo, Ignacio Salinas, Andy J. Pérez*

SUB-THEME: Plant defense, pathogens and interactions

The eucalyptus weevil, Gonipterus platensis, is a major threat to Eucalyptus plantations, with marked differences in susceptibility across species and hybrids. While Eucalyptus nitens exhibit relatively high resistance, Eucalyptus globulus is more susceptible, and interspecific hybrids display intermediate phenotypes. However, the molecular basis underlying these differential defense responses remains poorly understood, particularly in the context of herbivore-specific signaling.

Here, we investigated whether elicitation with a foregut extract from G. platensis, a source of herbivore-associated molecular patterns (HAMPs), can activate downstream defense metabolic responses in Eucalyptus species. Plants of E. globulus, E. nitens, and their hybrid were subjected to elicitation and compared with mechanical damage controls (DAMP) in a time-course experiment comprising seven sampling points. Untargeted LC–MS metabolomics (UHPLC–ESI–QTOF) was employed using complementary HILIC and C18 separations to maximize metabolome coverage. Multivariate analyses (PCA and OPLS-DA) revealed clear genotype-dependent metabolic reprogramming following elicitation.

E. nitens displayed a pronounced and sustained response, characterized by the downregulation of roseoside and the significant upregulation of hydrolyzable tannins, including glucogallin and higher galloylglucose derivatives (trigalloyl-, tetragalloyl-, and pentagalloylglucose). These changes are consistent with the activation of defense pathways associated with herbivore deterrence and oxidative stress mitigation. The hybrid genotype exhibited a similar but attenuated response, whereas E. globulus showed no significant metabolic shifts, suggesting a limited capacity to mount HAMP-triggered defense responses in this specific interaction. Together, these results provide evidence that G. platensis-derived elicitors act as HAMPs capable of inducing genotype-dependent defense metabolic reprogramming in Eucalyptus. This work highlights the relevance of herbivore-specific molecular cues in shaping plant immunity and demonstrates the power of metabolomics to resolve downstream chemical defense responses in forest species.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1254 A Metabolic characterization of the response to infections caused by Geotrichum citri-aurantii in the peel of susceptible and resistant mandarin varieties.

PRESENTING AUTHOR: ***Hugo do Carmo, CENUR LN, Universidad de la Republica, Uruguay***

CO-AUTHORS: *Inés Moltini, Elena Pérez, Joanna Lado, Andrés López-Radcenco, Guillermo Moyna*

SUB-THEME: Plant defense, pathogens and interactions

In citriculture, fruits are susceptible to various pathogens during postharvest stages, leading to significant economic losses. Among the main diseases, sour rot caused by Geotrichum citri-aurantii stands out. The breeding program of the Instituto Nacional de Investigación Agropecuaria (INIA) identified different degrees of resistance to this pathogen in mandarin varieties. NMR-based metabolomics provides an opportunity to identify metabolites and mechanisms involved in the resistance response. In this study, two mandarin varieties, Nova (resistant) and Clementina (susceptible), were analyzed in response to infection by Geotrichum citri-aurantii. For each variety, 20 biological replicates were obtained, and flavedo samples were collected from uninoculated control fruits, as well as from inoculated fruits at both the inoculation site and the diametrically opposed side. 1H NMR spectra of the aqueous fraction of peel extracts were acquired at 500 MHz. After preprocessing, the spectral data matrix was analyzed by principal component analysis (PCA), orthogonal projections to latent structures discriminant analysis (OPLS-DA), and univariate analyses of metabolites whose signals allowed integration. These analyses identified a higher abundance of citrate and malate in response to infection in both varieties. These metabolites are associated with alterations in sugar and carbohydrate metabolism and in pathways related to organic acids. When comparing infected varieties, naringin levels were increased in Nova, highlighting the role of this flavonoid as an important defense metabolite. In Clementina, levels of osmoprotectant alkylamines such as dimethylglycine and choline were increased. Overall, pathogen exposure mainly affected primary metabolism, limiting nutrient and carbon source availability while generating a chemical environment that restricts pathogen growth. These adaptations reflect metabolic reprogramming oriented towards defense and homeostasis maintenance, phenomena that could be exploited in the development of new mandarin cultivars with improved resistance against phytopathogenic microbes.

1255 B Variation in plant chemical diversity is transmitted to a higher trophic level

PRESENTING AUTHOR: ***Thomas Dussarrat, Bielefeld University, Germany***

CO-AUTHORS: *Thomas Dussarrat, Elizabeth Aiken, Jacqueline C. Bede, Caroline Müller*

SUB-THEME: Plant defense, pathogens and interactions

Plants and insects have co-evolved over millions of years to develop complex chemical interactions that shape ecosystem dynamics. Extensive efforts have enabled the identification of a wide array of metabolites involved in plant defence. In contrast, less is known about how far plant chemodiversity is reflected in the herbivore's chemical composition. In this study, we tested whether phloem-sucking herbivores, i.e. aphids, can capture plant intraspecific chemodiversity (i.e. the chemical richness, diversity and disparity of a plant) and assessed the extent to which aphid chemistry is linked to the diet.

Aphids (Macrosiphoniella tanacetaria) were placed in four feeding conditions, each consisting of four plants: (i) Tanacetum vulgare plants all belonging to the same chemotype (i.e. same leaf terpenoid profiles), (ii) plants of two distinct chemotypes, (iii) plants of four distinct chemotypes and (iv) three T. vulgare plants of three distinct chemotypes plus one plant of Chrysanthemum indicum. After eight days, aphids and their respective diet (leaves of all four plants) were subjected to untargeted metabolomics.

Dietary variation induced a clear chemical distinction between aphids, illustrating their ability to capture intra- and interspecific plant chemodiversity. For example, the terpenoid diversity of the diet was mirrored in the aphids. Overall, nearly 300 aphid chemical features significantly tracked dietary variation. These features included phenolics and amino acids, which can be taken up with the plant phloem, and fatty acids and terpenoids, which may be captured from the leaf surface. Besides, diets comprising multiple chemotypes or species tended to exhibit high chemical redundancy, thus resembling the chemodiversity of diets with low chemotype numbers. Overall, these findings show that variation in the chemodiversity of plants can be transmitted even to phloem-feeding herbivores and encourage a shift in our perspective from biodiversity alone to the preservation of ecosystem chemodiversity.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1256 A Metabolomics at the core of Mandarin (Citrus reticulata) breeding and production

PRESENTING AUTHOR: ★ ***Ignacio Migues, Facultad de Química, Universidad de la República (UdelaR), Uruguay***

CO-AUTHORS: *Ignacio Migues, Fernando Rivas, Guillermo Moyna, Horacio Heinzen*

SUB-THEME: Plant metabolomics

Mandarin production (Citrus reticulata) faces several challenges, including the time and resources required for sensory evaluation of new hybrids, the threat of phytopathogens such as citrus canker (Xanthomonas citri), and unstable yields due to alternate bearing. Traditional breeding and management often depend on late-stage observations. The metabolomic approach could provide objective tools to predict outcomes earlier and offer a comprehensive view of mandarin tree productivity. The present work outlines some applications of metabolomics in fruit acceptability, fruit setting, and fruit development, in relation to tree physiology, the management practices applied, and susceptibility/resistance to phytopathogens.

After 1H NMR metabolic profiling of extracts from pulp, peel, leaves, and ovaries, targeted analysis focused on specific chemical families (GC-MS for epicuticular waxes; LC-MS/MS for leaf polyphenols and gibberellins in the ovaries) helped describe markers of fruit characteristics and tree physiology.

In the case of early variety selection, high-field 1H NMR untargeted analysis of fruit pulp enabled the construction of a chemometric model using the sugar-to-acid ratio that predicts consumers' fruit acceptability. The method was transferred to a 60 MHz benchtop NMR for easy application in cultivar selection and field quality control. Cultivars whose fruit waxes contained certain alkanes, isoalkanes, and aldehydes in peel waxes are markers for canker resistance of the fruit, while higher alcohol levels suggest susceptibility. The study of the leaf flavonoids allowed the identification of narirutin as a marker for the non-productive "off" phase in alternate bearing, aiming to boost productivity. For better fruit setting and productive outcome, the evolution of the gibberellins' cascade was studied through LC-MS/MS in 'Afourer' ovaries, showing that the GA1 pathway rules fruit set under different pollination conditions.

Combining untargeted discovery with targeted validation enlightens the mandarin tree and fruit system metabolome. Turning chemical differences into practical metrics helps improve variety selection and farm management.

1257 B Phytochemicals as authenticity markers: Semi-targeted workflow integrating molecular networking and MRM for differentiating organic and conventional Brassicaceae seeds

PRESENTING AUTHOR: ***Daniela Andrea Ramirez, IBAM CONICET - FCA UNCuyo, Argentina***

CO-AUTHORS: *Roberto Felipe Bafumo, Florencia Pía Alloggia, Alejandra Beatriz Camargo*

SUB-THEME: Plant metabolomics

Introduction: Research-driven methodologies help ensure organic traceability and certification for seeds, the primary input of any food production process. Advanced analytical tools, by characterizing intrinsic composition and identifying key chemical markers, serve to distinguish production systems and support quality standards, particularly omics-based methods. Phytochemicals, as plant secondary metabolites, are strong candidates for tracking the origin of food products, including their organic or agroecological origin.

Aim: To discriminate between Brassica seeds of different origins —organic/agroecological vs. conventional (chemically treated)—through phytochemical profiling and semi-targeted LC-MS/MS metabolomics workflow integrated with multivariate analysis and molecular networking.

Methods: Glucosinolates (GLS) in cauliflower and radish were the chosen phytochemicals. Agroecological and conventional seeds (N=3/group) were pretreated and analyzed using reversed-phase LC-QqQ(LIT)-MS/MS. The pseudo-targeted protocol utilized product ion scans (m/z 259, 97) in ESI- mode for chemical family screening. Profiling employed Multiple Reaction Monitoring (MRM) with 22 GSL transitions. Precursor Ion-IDA-EPI data enabled molecular networking by grouping shared MS/MS fragments, providing class-level context for discriminants. MRM areas were processed for uni- and multivariate analysis.

Results: Fragment-based filtering and spectral alignment reduced raw PrecIon-IDA-EPI signals by 75% across both species, enriching glucosinolate-like features for downstream analysis. Molecular networking organized these features into GLS-related molecular families, revealing structural patterns associated with agricultural origin. Most discriminant nodes were associated with indolic clusters, whereas targeted MRM confirmed specific metabolites within a set of compounds. In cauliflower, aliphatic GLS such as glucoiberverin and glucoraphanin were discriminant markers. Whereas indolic GLS,

including 4-methoxy- and 4-hydroxyglucobrassicin, dominated in radish. Higher indolic glucosinolates in organic samples align with their stress-responsive role.

Conclusion: We developed a workflow integrating family-level and targeted phytochemical profiling to compare intrinsic differences among agricultural inputs. Detected glucosinolates acted as indicators of production origin and stress-related metabolic responses, supporting horticultural seed authenticity assessment.

1258 A LC-HRMS-based metabolomic profiling of bioactive compounds in strawberries under controlled atmosphere storage

PRESENTING AUTHOR: ***Magdalena Irazoqui, Udelar, Uruguay***

CO-AUTHORS: *Sofía Barrios, Patricia Lema*

SUB-THEME: Plant metabolomics

Strawberries are a rich source of bioactive compounds, including phenolic acids, flavonoids, and other metabolites associated with antioxidant activity. Understanding how postharvest conditions affect their metabolic profile is relevant for assessing fruit quality and nutritional value. This study aimed to investigate the metabolomic profile of minimally processed strawberries (cv. 'INIA Ágata') stored under different controlled atmosphere conditions and temperatures.

Samples obtained from factorial storage experiments with varying O_2, CO_2, temperature, and storage time were frozen, freeze-dried, and extracted with methanol prior to analysis. Metabolomic profiling was performed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Data acquisition was carried out using a dependant data acquisition (IDA) strategy, combining TOF-MS survey scans with MS/MS fragmentation of selected precursor ions in both positive and negative electrospray ionization modes.

Untargeted metabolomic screening enabled the detection of several hundred metabolic features, with approximately 100 compounds detected in negative mode and more than 200 in positive mode above the defined intensity threshold. Data processing included peak detection, retention time alignment, blank correction, and quality filtering based on quality control samples and internal standard normalization.

This workflow provides a robust platform for exploring metabolomic changes in strawberries and supports further studies aimed at linking metabolic profiles with storage conditions and physiological responses during postharvest storage.

1259 B Metabolomic Fingerprinting of Peruvian Cocoa: Tracing Chemical Markers Linked to the Flavor Profile in fine flavor cocoa.

PRESENTING AUTHOR: ★ ***Vanessa Jessenia Mayorga Martino, Pontificia Universidad Católica del Perú, Peru***

CO-AUTHORS: *Vanessa Mayorga, Deybis Paucar, Nubia Martínez, Víctor Sal y Rosas, Alfredo Ibáñez, Madina Mansurova*

SUB-THEME: Plant metabolomics

Peru is recognized for its high genetic diversity of Theobroma cacao and the production of fine-flavor cocoa with complex aromatic profiles. However, the chemical basis underlying regional variability and post-harvest processing effects in Peruvian cocoa has been only partially explored. In particular, the identification of metabolite markers associated with geographic origin and post-harvest processing conditions is still limited.

In this study, an untargeted metabolomics approach was applied to characterize the chemical diversity of fine-flavor cocoa from three regions of Peru: Cusco, San Martín, and Amazonas. Fresh beans (at pod opening) and dried beans obtained after fermentation and drying were collected from two independent producers per region. Metabolite profiling was performed using GC-MS and LC-MS, and the resulting datasets were explored through multivariate statistical analysis to investigate metabolic variability associated with geographic origin and post-harvest processing.

Distinct metabolomic fingerprints showed strong discriminatory power and may serve as chemical markers for regional differentiation. In addition, the comparison between fresh and dried cocoa beans revealed substantial metabolic shifts associated with fermentation and drying processes; including compounds potentially involved in the formation of aroma and flavor precursor metabolites.

The results of our study may lead to the broad applicability of the proposed untargeted metabolomics workflow for cocoa characterization and differentiation, supporting future strategies in traceability, quality assessment, and the valorization of regional varieties.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1260 A 1H-NMR metabolomic profile based on the nontargeted metabolites in the leaves of selected Mormodica species

PRESENTING AUTHOR: ***Lufuno Ethel Nemadodzi, University Of South Africa, South Africa***

CO-AUTHORS: *Gudani Millicent Managa*

SUB-THEME: Plant metabolomics

Background: Momordica balsamina L. (Cucurbitaceae), commonly known as balsam apple, or African pumpkin, is a vegetable with high nutritional value, which is mostly used as food in sub-Saharan Africa. It has also been largely used in traditional medicine to treat several diseases, such as malaria, fevers, and diabetes. In South Africa, it is organically grown, and the leaves are consumed by the VhaVenda tribe in the Vhembe district, Limpopo Province. It forms part of the list of top 8 edible African leafy vegetables, such as "pumpkin leaves, mallow jute, Amaranthus, nightshade, spider plant, Chinese cabbage, blackjack". Traditionally, it is used to cure hypertension and maintain blood pressure, specifically among the elderly. However, there is little scientific information available on the use of advanced scientific tools to comprehend the biochemical structure of M. balsamina. Methodology: A 1H-nuclear magnetic resonance tool was used to identify the untargeted metabolites released by M. balsamina, and spectra were phase-corrected and binned with MestReNova and statistically analyzed with SIMCA 18.0.2. Results: The findings revealed the presence of metabolites such as 2-deoxyadenosine, which is known to inhibit glucose-stimulated insulin release. In addition, 2-deoxyuridine with antiviral properties, 2-Deoxyinosine with antitumoral activity, amino acids (2-Hydroxy-3-methylvalerate, 2-Furoylglycine), and 3-Chlorotyrosine associated with oxidative stress in chronic renal failure. Imidazole, which possesses anti-cancer activity, anti-microbial properties, and has been widely used to treat a wide range of illnesses, was also detected. Furthermore, choline (flavor-agent metabolite), tiglylglycine (body mass-reducer metabolite), cis-Aconitate, citraconate, citrate, and citrulline were identified. Conclusion: It is concluded that the inclusion of consumption of M. balsamina in the diet and its consumption will help improve human health and aid a range of ailments.

1261 B Age-Related Metabolic Reprogramming in Eucalyptus grandis × Eucalyptus camaldulensis Bark Revealed by GC-MS-Based Metabolomics.

PRESENTING AUTHOR: ***Thais Cataldi, ESALQ-USP, Brazil***

CO-AUTHORS: *Thais Regiani Cataldi, Felipe Alexsander Rodrigues da Silva, Andressa Fernanda Ducatti Gois, Ilara Gabriela Frasson Budzinski, Mônica T. Veneziano Labate, Ciro Abbud Righi, Welington Luiz de Araujo, Carlos Alberto Labate*

SUB-THEME: Plant metabolomics

Understanding metabolic shifts associated with tree age is essential for improving forest management and biomass production in Eucalyptus species. This study investigated age-related changes in primary metabolism in the bark of a hybrid clone of Eucalyptus grandis × Eucalyptus camaldulensis (COP-1277) cultivated in an agroforestry system during the summer season. An untargeted metabolomics approach based on gas chromatography–mass spectrometry (GC-MS) was employed to characterize primary metabolites and compare 3-year-old and 10-year-old trees.

Primary metabolites were extracted, derivatized, and analyzed by GC-MS, followed by identification using retention time and spectral matching. A total of 45 metabolites were identified in bark tissue. Among them, 16 metabolites were exclusive to 3-year-old trees, 9 were exclusive to 10-year-old trees, and 20 were common to both age groups. Metabolites were classified into sugars (25%), sugar alcohols (10%), carboxylic acids (30%), amino acids (5%), and other compounds (30%), including fatty acids, phenols, flavonoids, and lactones.

Multivariate analysis using partial least squares discriminant analysis (PLS-DA) demonstrated clear metabolic separation between age groups (R^2 = 0.81899; Q^2 = 0.46728). Based on VIP scores (>1), nine metabolites were identified as significantly contributing to age discrimination. Younger trees showed higher abundance of metabolites associated with active primary metabolism and energy production, including carboxylic acids and intermediates linked to glycolysis and the tricarboxylic acid cycle. In contrast, older trees exhibited increased levels of metabolites related to carbohydrate accumulation and structural reinforcement.

These results reveal a clear age-dependent metabolic reprogramming in bark tissue, reflecting a shift from growth-oriented metabolism in younger trees to structural maintenance and carbon storage in older trees. GC-MS-based metabolomics proved to be a robust tool to characterize physiological transitions associated with secondary growth in Eucalyptus. This study was supported by FAPESP Thematic Project (2021/01012-9).

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1262 A Exploring the metabolic diversity of Uruguayan sweet potato varieties through targeted and untargeted approaches

PRESENTING AUTHOR: ***Mikaela Rajchman, Facultad de Medicina, Universidad de la República, Uruguay, Uruguay***

CO-AUTHORS: *Rafael Velazco, Sofía Comas, Aníbal M. Reyes, Mauricio Mastrogiovanni, Joanna Lado, Esteban Vicente, Rafael Radi, Adrián Aicardo*

SUB-THEME: Plant metabolomics

Sweet potato (Ipomoea batatas) is one of the top five vegetables in Uruguay in terms of both production area and commercial volume. Recent research and plant breeding efforts have led to the development of new varieties that exhibit enhanced agronomic properties and quality attributes linked to consumer preferences, such as flavor, texture, appearance, and, more recently, health-related attributes. This has increased interest in cultivars with unique characteristics, particularly orange-fleshed sweet potatoes known for their β-carotene content and purple-fleshed varieties rich in anthocyanins, which have potential applications in various industries and are linked to health-promoting effects. Understanding these varieties is crucial for recognizing their unique properties and improving their market value. This study aimed to characterize and compare the metabolic profiles of different sweet potato genotypes using targeted and untargeted metabolomics approaches. Five distinct genotypes were chosen: F23A2.2 (purple-fleshed), C2003.6 (orange-fleshed), INIA Rubí 63 (white-fleshed), and two purple-fleshed clones with marbled flesh patterns (A1807.19 and C1831.6). Samples underwent freeze-drying, grinding, and homogenizing before extraction procedures, which were adapted according to the metabolites of interest. Analyses included spectrophotometric assessments for total phenolic content, anthocyanins, and carotenoids. Under optimized conditions, F23A2.2 sweet potato had the highest content of phenolic compounds and anthocyanins, followed by C1831.6 (purple- and orange- fleshed) and thirdly A1807.19 (purple- and white- fleshed). Additional LC-UV/Vis analyses were performed, monitoring various wavelengths for specific compounds (phenolics, flavonols, carotenoids, and anthocyanins). Metabolite identification included low and high resolution LC-MS/MS analyses. Annotation strategies, such as MZMine and MS-DIAL workflows, along with feature-based molecular networking in GNPS, and MS-DIAL analyses using different spectral libraries, were employed for comprehensive metabolite characterization. Integrating spectrophotometric, chromatographic, and mass spectrometry methods provides a thorough overview of the metabolite profiles of these sweet potato genotypes, enhancing the understanding of their nutritional and functional potential.

1263 B Beyond Pest Control: GC–MS Metabolomics Reveals Insecticide-Induced Metabolic Reprogramming in Coffee Seedlings

PRESENTING AUTHOR: ***Frederico Garcia Pinto, Federal University of Viçosa, Brazil***

CO-AUTHORS: *Carlos G. da Cruz, Brena R. M. Ikehara, Natália R. de Almeida, Erick A. A. Rocha, Wellington L. de Almeida, Flávio L. Fernandes, Frederico G. Pinto*

SUB-THEME: Plant metabolomics

Soil-applied insecticides are widely used in coffee production, yet their impact on plant metabolism remains poorly characterized. Here, we employed a GC–MS-based metabolomics approach to investigate metabolic alterations in coffee seedlings treated with insecticides used against the coffee leaf miner (Leucoptera coffeella).

Seedlings were treated via soil application with thiamethoxam, thiamethoxam + cyproconazole, dinotefuran, dinotefuran + pyriproxyfen, imidacloprid, and flupyradifurone, with untreated plants as controls. After 80 days, leaf samples were collected and subjected to GC–MS analysis followed by statistical evaluation.

Distinct metabolic profiles were observed across treatments. Neonicotinoid treatments were associated with increased levels of free sugars, particularly metabolites linked to the galactose pathway, alongside a consistent reduction in inositol levels, suggesting a shared metabolic cost. In contrast, flupyradifurone induced a distinct metabolic response, characterized by the marked accumulation of the neuroactive alkaloid N-methylphenylethanolamine, tentatively identified by spectral library matching.

These results demonstrate that soil-applied insecticides trigger specific and compound-dependent metabolic reprogramming in coffee seedlings. This study highlights the potential of metabolomics to uncover unintended biochemical effects of agrochemicals, providing new insights into plant–insecticide interactions beyond pest control.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1264 A NMR-Based Metabolomics for the differentiation of Sorghum genotypes and the enhancement of bioethanol production

PRESENTING AUTHOR: ***Lucia Pimenta, Universidade Federal de Minas Gerais, Brazil***

CO-AUTHORS: *Jéssika Thayanne da Silva, Pablo Batista Mendes, Maria Lúcia Ferreira Simeone, Valéria Aparecida Vieira Queiroz, Cícero Beserra de Menezes, Cristiane de Carvalho Guimarães, Alan Rodrigues Teixeira Machado*

SUB-THEME: Plant metabolomics

Sorghum bicolor has been improved by crossing inbred lines, a breeding strategy to enhance starch accessibility and fermentation efficiency for bioethanol production. This study assessed NMR-based metabolomics as a tool to distinguish among four sorghum genotypes (SC084, CMSXS180, BR501, and BRS305), including inbred lines and hybrids obtained from controlled crosses, with different tannin content and origin, and to correlate their metabolic profiles with bioethanol yield. The study also correlated their metabolic profiles with bioethanol yield and evaluated the influence of sorghum lipid content on ethanol production efficiency. Sorghum grains, analyzed both degreased and in natura, underwent NMR analysis, phenolic quantification, and fermentation performance testing. Ethanol yields ranged from 277.21 to 317.94 mg g^{-1}, with SC084 yielding the lowest and BR501 the highest. Notably, defatted, tannin-free grains exhibited greater fermentation efficiency compared to in natura grains; reduced lipid content increased relative starch content, thereby improving ethanol yield. The tannin-free genotypes, BR501 and CMSXS180, achieved the highest ethanol yields, while SC084 consistently produced the lowest yield, correlating with its elevated total phenols (37.26 mg GAE g^{-1}) and condensed tannins (94.89 mg g^{-1}). ^{1}H NMR experiments, combined with multivariate data analysis, showed clear distinctions among the genotypes and identified two principal separation patterns. The first separation, along PC1, corresponded to genotype differentiation, with loadings revealing bins in the δ 6.80–6.96 region, characteristic of aromatic protons from phenolic compounds such as flavan-3-ols, which may negatively influence fermentation efficiency, as indicated by Pearson's correlation analysis. The second separation, along PC2, distinguished the in natura samples from the defatted samples. NMR spectra also enabled the identification of approximately 13 metabolites. In particular, ^{1}H NMR analysis of CMSXS180 revealed aromatic signals compatible with tyrosine derivatives, p-hydroxybenzaldehyde, p-hydroxyphenylacetic acid, and dhurrin. Overall, this approach provides a robust framework for selecting sorghum genotypes with enhanced fermentation performance.

1265 B LC-MS-based untargeted metabolomics reveals genotype-dependent downstream DAMP-related response in aerial tissues of Eucalyptus cuttings during vegetative propagation.

PRESENTING AUTHOR: ***Ignacio Salinas, Universidad De Concepción, Chile***

CO-AUTHORS: *Catalina Bravo, Nicolás Fernández, Javiera Nahuelcura, Andy J. Pérez*

SUB-THEME: Plant metabolomics

Globally, plantations of Eucalyptus species are extensively utilized for pulp production, with Eucalyptus globulus and Eucalyptus nitens being the most widely planted species in Chile. Hybrid breeding programs have generated E. nitens × E. globulus materials with improved frost tolerance and wood quality. However, these genotypes display contrasting physiological responses during large-scale vegetative propagation.

In this study, we employed LC–MS-based untargeted metabolomics to characterize the temporal metabolic dynamics in aerial tissues of small cuttings obtained from 10 genotypes, including parental species and hybrids with contrasting historical performance during clonal propagation. Cuttings were sampled at days 7, 10, 13, 16, and 20 after planting. Multivariate analyses (PCA and OPLS-DA) were applied to explore metabolic variation and identify discriminant features.

Our results revealed that the constitutive metabolome of contrasting genotypes is significantly differentiated by hydrolyzable tannins, including trigalloyl-glucose and tetragalloyl-glucose. Over time, genotypes with lower propagation performance exhibited a marked accumulation of these compounds, whereas in higher-performing genotypes their levels remained stable or decreased.

Rather than indicating a direct involvement in root formation, these patterns are consistent with a genotype-dependent activation of defense-related metabolic pathways in aerial tissues during the early stages of vegetative propagation. Hydrolyzable tannins are well-known components of plant defense, contributing to oxidative stress responses and protection against biotic challenges. Their differential accumulation may therefore reflect distinct strategies of resource allocation between defense and developmental processes under propagation-associated stress conditions.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

These findings highlight the importance of considering systemic metabolic responses during clonal propagation and provide candidate biomarkers associated with stress and defense phenotypes. Ongoing analyses of basal tissues will be essential to establish potential links between aerial metabolic reprogramming and root initiation processes.

1266 A LC-HRMS/MS Chemical Profiling of Anthocyanins in Uruguayan Purple Sweet Potato Genotypes

PRESENTING AUTHOR: ***Anibal M. Reyes, Facultad de Medicina, UDELAR, Uruguay***

CO-AUTHORS: *Mikaela Rajchman, Rafael Velazco, Adrián Aicardo, Cecilia Chavarría, Joanna Lado, Esteban Vicente, Rafael Radi, Mauricio Mastrogiovanni*

SUB-THEME: Plant metabolomics

Purple-fleshed sweet potatoes cultivars of Ipomoea batatas (L.) Lam. (Convolvulaceae) are distinguished by their deep violet flesh and rich nutritional profile. Beyond standard dietary fiber, minerals, and vitamins, they are a significant source of anthocyanins. These polyphenolic pigments function as bioactive compounds with documented health benefits, positioning these cultivars as promising functional foods. This study utilized Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS/MS) to analyze the anthocyanin content in hydroalcoholic extracts of purple sweet potato genotypes developed by the National Institute of Agricultural Research (INIA, Uruguay). To optimize metabolite detection and identification, untargeted metabolomics experiments were conducted using a High Resolution QTOF system via Data-Dependent Acquisition (DDA). Various instrument parameters—including Spray Voltage, Maximum Candidate Ions, Collision Energy, and the Zeno Trap—were evaluated to refine the analytical output. The data was processed using a Feature-Based Molecular Networking (FBMN) workflow through MZmine3 and GNPS2. The results indicated that while the total number of features remained consistent across acquisition methods, the ratio of features to library hits varied, with the optimized method yielding a superior 1:5 identification rate. A total of 24 anthocyanin compounds were identified with Confidence Levels 2 (probable structure, Schymanski scale) and 3 (tentative candidates, Schymanski scale) in two specific genotypes: A1807.19 and F23A2.2. Analysis revealed distinct chemical profiles: A1807.19 was primarily composed of cyanidin glycosylated derivatives, whereas F23A2.2 showed a predominance of peonidin glycosylated derivatives. Both genotypes exhibited minor detection of pelargonidin derivatives. The identified anthocyanins existed in non-acylated, monoacylated, and diacylated forms, primarily conjugated with caffeic, ferulic, p-hydroxybenzoic, and vanillic acids. These findings align with established profiles of Asian cultivars, confirming the high phytochemical potential of Uruguayan genotypes.

1268 A A multi year metabolomics study reveals the influence of genotype, season and storage on fruit metabolome.

PRESENTING AUTHOR: ***Heike Schwendel, New Zealand Institute for Bioeconomy Science Limited, New Zealand***

CO-AUTHORS: *B.H. Schwendel, L. Turnbull, H. Lloyd, O. Angelin-Bonnet, D. Hunter, R. Diack, S. Mair, N. Gapper, J. Johnston, L. Favre*

SUB-THEME: Plant metabolomics

The commercial success of an apple cultivar is determined by many factors, including genetics, growing environment, harvest time, storage treatment and post-storage conditions. While individual factors such as soil mineral content, days after flowering, and crop load have been investigated in detail, their effects on the final consumer product are often variable and not reliably reproducible. This study presents a multiyear trial usin metabolomics approach to investigate the composition of three apple cultivars ('Gala', 'Braeburn' and a 'Gala'-'Braeburn' cross), grown within the same orchard in New Zealand, with apples sampled repeatedly from the same trees. Semi-polar and non-polar compounds in apple flesh have been analysed throughout the growing, harvest and storage periods, representing 10 timepoints and four storage treatments (n=362). Crop load was also implemented as an influencing factor to determine how orchard management practices affect fruit quality post storage.

Initial analysis of the semi-polar metabolite data revealed a strong seasonal effect, followed by a clear distinction among the three genotypes, with the 'Gala'-'Braeburn' cross having an intermediate metabolic profile between its parents. The non-polar metabolite data showed similar seasonal effects, but no clear distinction between the three cultivars, with 'Braeburn' and 'Gala'-'Braeburn' cross having similar metabolic profiles and only 'Gala' distinguishing out. Storage time over four months appeared to affect non-polar compounds more than semi-polar compounds, with cultivars fully separated in semi-polar data, while non-polar data only separated 'Gala'. Individual storage treatments appeared to have no distinct effect on semi-polar and non-polar compounds with cultivars distinctively different from the others. The effect of crop load was especially observed for semi-polar compounds of the 'Gala'-'Braeburn' cross.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

The different responses of each cultivar to growth period and storage, as well as the interactions between influencing factors (e.g. cultivar and crop load), highlights the need for an integrated approach to optimise fruit.

1269 B A Chemometric and AI-based Approach for Characterizing Taste in Sweet Basil (Ocimum basilicum)

PRESENTING AUTHOR: ***Shashikant Kotwal, Rutgers University-New Brunswick, United States***

CO-AUTHORS: *Francisca Giaverini, Utsav Kumar, John McLaughlin, Rong Di, Qing-li Wu, James E. Simon*

SUB-THEME: Plant metabolomics

Sweet basil (Ocimum basilicum) is a widely consumed culinary and medicinal herb rich in diverse specialized metabolites that contribute to its flavor, aroma, and bioactivity. Basil aroma is linked to volatile terpenes, but the molecular basis of sweet and bitter taste remains poorly understood. Here, we present a multi-platform workflow integrating untargeted metabolomics, molecular networking, artificial intelligence-based taste prediction, and molecular docking to investigate taste-associated compounds found in sweet basil. Untargeted metabolomics using UHPLC-UV/DAD-QTOF/MS enabled mzmine-DreaMS molecular network visualization for structurally related metabolites including flavonoids, phenolic acids, lignans, and terpenoids. Chemical diversity and relative metabolite abundance across fractions were observed through differences in molecular families within the network. Compounds were identified by MS2 using mzmine, MetaboKit, MSDIAL, and SIRIUS, then screened via Flavor Analysis and Recognition Transformer (FART) to predict bitter, sweet, umami, and tasteless metabolites. Further, molecular docking of ligand interaction with human bitter taste receptor, TAS2R14, shows intracellular binding pocket favorable to specialized metabolites identified in this study. This integrated workflow accelerates our understanding of taste and may be incorporated in plant breeding programs or the flavor industry.

1270 A Advancing genus-level resolution in the Piptocarphinae subtribe through chemical data

PRESENTING AUTHOR: ***Marilia Gallon, University of São Paulo, Brazil***

CO-AUTHORS: *Marcelo Monge, Norberto Peporine Lopes*

SUB-THEME: Plant metabolomics

Indistinctive morphological differences, frequent trait convergences, and inconsistencies between morphological characters and molecular evidence often pose challenges to plant systematics and taxonomic classifications. Within the family Asteraceae, the delimitation of the tribe Vernonieae has remained relatively stable since its original description, while its subtribal circumscription has been far more complex and repeatedly revised. As a continually evolving taxonomic group, the subtribe Piptocarphinae currently includes nine genera and approximately 150 species distributed mainly across tropical regions of South America. The subtribe includes genera such as Piptocarpha, Critoniopsis, Joseathus, Pollalesta and Piptocoma, with the genus Piptocarpha accounting for more than one-third of the total species diversity. Herein, we integrate chemical data into plant classification to better resolve relationships among genera and improve taxonomic classification. Using a liquid chromatography-mass spectrometry (LC-MS)- based metabolomic approach, we investigated the chemical profiles of 58 Piptocarphinae species, including individuals of the genera Piptocarpha, Critoniopsis and Pollalesta. Venn diagrams and multivariate analyses were used to establish the main differences among genera. The Venn diagram showed a greater abundance of exclusive features in the genus Piptocarpha (ca. 58%), followed by Critoniopsis (ca. 14% of exclusive features) and Pollalesta (ca. 8% of exclusive features), with approximately 6% of the features shared among all genera. Principal Component Analysis (PCA) revealed no clear clustering patterns among the different genera, indicating a high degree of chemical overlap, which is expected given the close phylogenetic and evolutionary relationships within the subtribe. However, Partial Least Squares Discriminant Analysis (PLS-DA) confirmed a segregation of the species according to their respective genera, suggesting that despite overall similarity, genus-level chemical signatures can still be distinguished when group identity is considered. Moving forward, a more detailed characterization of the metabolites that are differentially distributed across genera may provide valuable chemical markers to support and refine plant taxonomic classifications.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1271 B Large-Scale Metabolomics of Understudied Plant Lineages for Natural Product Discovery

PRESENTING AUTHOR: ***Kateřina Kučerová, IOCB Prague, Czech Republic***

CO-AUTHORS: *Tito Damiani, Iuliia Nikolskaia, Pierre-Marie Allard, Emmanuel Defossez, Vlastík Rybka, Melanie-Jayne Howes, Tomáš Pluskal*

SUB-THEME: Plant metabolomics

The Plant Kingdom represents a vast source of chemical diversity, yet phytochemical research has historically focused on a limited number of model species, leaving most plants chemically unexplored. This project aims to discover novel plant natural products and molecular scaffolds from understudied plant lineages by combining large-scale LC–MS-metabolomics screening and a range of recently-developed computational metabolomics tools for the subsequent data analysis.

To prioritize chemically unexplored taxa, we combined automated mining of scientific literature and natural product databases and selected plant genera with fewer than 15 publications and fewer than 15 reported natural products. Using these criteria, we identified 6450+ understudied plant genera for further investigation. Plant material was collected using standardized sampling protocols from the Digital Botanical Garden Initiative, which systematically record sample collection metadata (e.g., taxon name, geographical location, and collection conditions). Using this workflow, we have already collected 5800+ samples from over 3330 distinct plant species, covering 1247 genera, 218 families and 64 orders.

So far, 1876 plant extracts from nearly 1000 species were analyzed using LC-MS/MS. Raw data were preprocessed using MZmine, resulting in a dataset of 379,639 spectral signals. The data were further processed by DreaMS, a machine learning model that learns relationships between molecular structures from MS/MS fragmentation patterns by embedding spectra into a chemical representation space (Bushuiev R. et al., Nature Biotechnology, 2025). These embeddings were used to calculate novelty scores based on the distance of each spectrum in the learned chemical representation space, enabling prioritization of spectral features that are chemically distinct and rarely observed across the dataset. By overlaying additional prioritization criteria, such as precursor m/z above 300 and DreaMS-derived novelty scores, we identified candidate metabolites with potentially unexplored chemical scaffolds.

Currently, we are working on the isolation and structure elucidation of selected candidate metabolites.

1272 A Elevated CO_2 Under Climate Change Affects the Metabolite Profiles of Quercus robur Leaves

PRESENTING AUTHOR: ***Sophie Powell, University of Birmingham, United Kingdom***

CO-AUTHORS: *Diana Vinchira-Villarraga, James E. McDonald, Robert W. Jackson, Mojgan Rabiey*

SUB-THEME: Plant metabolomics

Climate change, driven by elevated carbon dioxide concentration (e[CO2]), is predicted to significantly impact the health of trees. Important tree species, such as Quercus robur (English oak), are threatened by emerging pathogens which could be exacerbated by climate stress. Trees utilise metabolites to enhance tolerance to stressors, including pathogens, by boosting defence responses and increasing resilience. However, it's hypothesised that climate change, specifically e[CO2], will cause a variation in the tree metabolic profiles which could lead to increasing disease susceptibility. At the Birmingham Institute for Forest Research – Free Air Carbon dioxide Enrichment facility (BIFoR-FACE) we are investigating the effects of e[CO2] on oak leaf metabolite profiles and assessing interactions between key metabolites and oak microbial communities. At BIFoR-FACE, open arrays of oak-dominant forest are exposed to elevated CO_2 (+20% e[CO_2], ~520 ppm), simulating atmospheric concentrations projected for 2050. Oak leaves from 12 trees were collected at 3 timepoints across the growing seasons of 2016, 2019, and 2024 from e[CO2] and ambient arrays. Untargeted Liquid Chromatography – Mass Spectrometry (LC-MS) was used to determine whether e[CO2] drives changes in the leaf metabolome composition. Variations in metabolite profile were beginning to emerge between the two CO2 concentrations (PERMANOVA: $p = 0.0715$), with most shifts occurring between timepoints (year $p=0.0001$, month $p=0.0113$), a trend mimicked in ITS and 16S rRNA gene microbiome analysis. Assessing variably important features highlighted an increased abundance of flavonoids in e[CO2] conditions. Further analysis identified a cluster of procyanidins enriched under e[CO2]; including procyanidin B1 and A1 (T-Test: $p = 0.022$). These compounds are associated with plant defence responses and stress. Current experiments are exploring how increased procyanidin concentration impacts the oak leaf microbiome. These investigations are crucial for predicting future tree health and developing innovative climate-change based strategies to safeguard oak trees from climate change.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1274 A — HPLC–STOCSY Reveals Two New Lanaroft-Type Biflavonoids from Selaginella plana and S. padangensis

PRESENTING AUTHOR: ***Wilailak Kaewsri, Chulabhorn Graduate Institute, Thailand***

CO-AUTHORS: *Wilailak Kaewsri, Ugyen Lhamo, Yudis Ananda Putra, Wanlaya Thamnarak, Napat Sitthimonchai, Somsak Ruchirawat, Nopporn Thasana*

SUB-THEME: Plant metabolomics

Biflavonoids (BFVs) possess diverse biological activities, e.g., anticancer, anti-diabetes, and anti-inflammatory activities. However, dereplicating BFVs remains challenging with standalone LC-MS because their structural isomers have different biflavonoid linkages and share highly similar fragmentation patterns, thereby increasing the risk of misannotation. In our study, the integrative high-performance liquid chromatography (HPLC)-statistical total correlation spectroscopy (STOCSY) was introduced to enable cross-identification of BFVs across incompletely separated fractions in two Selaginella species, namely Selaginella plana and S. padangensis. The workflow began with the initial screening of two Selaginella crudes by matching HPLC retention time against an in-house BFVs. Subsequently, STOCSY analysis was performed within S. plana to validate the HPLC results. The resulting unmatched correlated signals guided the selection of the target fraction for extensive isolation and structural identification, leading to the discovery of planaflavone A from S. plana. A comparable HPLC RT observed in S. padangensis further guided the target isolation of another undescribed BFV, namely padangeflavone A. The integrative strategy could accelerate compound discovery by enabling discrimination of BFV isomerisms that are indistinguishable by LC-MS.

1275 B — Baseline metabolome profiling of five understudied Capsicum cultivars, using GC-TOF-MS, UPLC-QTOF-MS, and 1H-NMR

PRESENTING AUTHOR: ***Jethro Broughton, University of South Africa, South Africa***

CO-AUTHORS: *Jethro Broughton, Cassandra Upton, Gerhard Prinsloo*

SUB-THEME: Plant metabolomics

The Capsicum genus has five domesticated species, with over a thousand phenotypically distinct cultivars that have been bred by both commercial and enthusiast farmers. Chillies, the fruit of Capsicum plants, have been used throughout history as traditional medicine to treat various gastral and adrenal diseases, provide pain relief, and even reduce depression. Despite their diversity, only a few commercially grown cultivars have had their metabolome profiled. This study aims to profile the metabolome of five understudied cultivars, using GC-TOF-MS, UPLC-QTOF-MS, and 1H-NMR, to determine their bioactive potential and metabolite variation. The assessed cultivars are Capsicum annuum var. Glabriusculum (Dunal) Heiser and Pickersgill cv. CAP524, Capsicum baccatum L. cv. Sugar Rush Peach, Capsicum chinense Jacq. cv. 7-Pot Godstopper, Chocolate 7-Pot Douglah, and Yellow Habanero. Currently, GC-TOF-MS samples have been processed and profiled, revealing a total of 1294 metabolites across all cultivars using chloroform as a solvent, and 464 metabolites using methanol as a solvent. The 1H-NMR samples have been processed and profiled, with CAP524 currently being the only annotated cultivar. The 1H-NMR profiles were manually annotated using Chenomx and automatically annotated using Colmar's Query1D, cross-checking the profiles with the BMRB, HMDB, and MMCD databases. The three 1H-NMR CAP524 samples flagged a total of 774 metabolites across the three databases, 97 of which were shared across all three samples and databases. A total of 28 of these metabolites were also flagged in the GC-TOF-MS profiles. Notable metabolites included: L-Glutamine, Betaine, Spermidine, L-Isoleucine, and L-Cysteine. The UPLC-QTOF-MS samples have been collected and are currently being processed and profiled. Currently, CAP524 has been shown to have beneficial bioactive metabolites, making it a suitable candidate for further studies. The other cultivars are currently being profiled to identify their potential value as candidates for future studies.

1276 A — Computational metabolomics identifies flavonoids as discriminants between Plectranthus hadiensis and Plectranthus ambiguus leaf tissues.

PRESENTING AUTHOR: ***Akhona Myoli, University Of Johannesburg, South Africa***

CO-AUTHORS: *Edwin N. Madala, Justin J.J. van der Hooft, Fidele Tugizimana*

SUB-THEME: Plant metabolomics

Plectranthus ambiguus (known as iBoza elincane in isiZulu) and Plectranthus hadiensis (known as iLozane in isiXhosa) have garnered interest in their pharmacological potential and use as traditional medicine in South Africa. However, prior phytochemical studies have largely focused on the classical screening methods targeting abietane diterpenoids (terpenoids), a chemical class that is well-known for characterising these species. Consequently, this has created knowledge gaps on the metabolomic landscape of the two plants, restricting our understanding of their phytochemical complexity and other

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

important bioactive compounds. Thus, reported herein is a comprehensive metabolomics-based approach to investigate and compare the broader metabolite profiles of P. hadiensis and P. ambiguus, beyond diterpenoids. This untargeted, computational metabolomics work integrated machine learning models with feature-based molecular networking and MS2query in the analysis of both species' leaf extracts. As expected, prenol lipids (i.e terpenoids) were detected and identified as one of the major chemical classes that differentiate the two plants. However, this trend was extended to the flavonoid chemical class. Across both leaf tissues of P. ambiguus and P. hadiensis, flavonoids were detected at varying distributions, where glycosylated flavones were more abundant in P. hadiensis while glycosylated flavanols and methylated flavones were found in higher levels in P.ambiguus. To the best of our knowledge, this is the first study to apply such an integrated computational metabolomics framework in the chemical profiling of P. ambiguus and P. hadiensis, offering novel insights into their phytochemical relationships and species-specific chemical signatures. This comparative analysis contributes to bridging indigenous knowledge with modern analytical science, expanding the current understanding of Plectranthus phytochemistry beyond classical diterpenoid profiling.

1277 B 1H-NMR and LC-MS-based metabolomics of Amaranthus caudatus and A. hypochondriacus species

PRESENTING AUTHOR: ★ ***Niel Snyman, Unisa, South Africa***

CO-AUTHORS: *Gerhard Prinsloo, Nolitha Nkobole*

SUB-THEME: Plant metabolomics

Background/Aim: Amaranth is a leafy vegetable that is mainly used as a food source and regarded as a nutraceutical crop, as it is a rich source of minerals and plant metabolites associated with many health benefits. Several studies that have been conducted so far on Amaranthus mainly focused on the physiological parameters, morphological properties, and nutritional composition, leaving a huge gap in the plant's metabolomics profile of its leaves. Understanding the chemical profile of Amaranthus leaves depends on growing circumstance and environmental factors, as these compounds have been connected to the plants' health-promoting properties. Therefore, the main aim of this chapter is to use the metabolomics approach of ^{1}H-NMR and LC-MS based spectroscopy to compare the metabolite differences in A. caudatus and A. hypochondriacus leaves.

Results: With NMR analysis, 31 compounds were reported in this study, and these compounds were mostly similar in both Amaranthus species. Common metabolites that were reported with using NMR analysis in this study were amino acids (leucine, valine, threonine, GABA, alanine, tryptophan, etc.); sugars (glucose, sucrose, maltose, mannitol, and trehalose); organic acids (malic acid, mannonic acid and citric acid) and phenolic acids (gallic acid, vanillic acid, and ferulic acid). LC-MS further detected chlorogenic acid, citric acid, feruloyltyramine, phenylalanine, L-tryptophan, rutin, quercetin, kaempferol 3-O-rutinoside, and 2,3,23-Trihydroxy-30-nor-12,20(29)-oleanadien-28-oic acid in both A. hypochondriacus and A. caudatus species.

Conclusion: This study revealed that not only are amaranth leaves rich in minerals and proteins but that the plants contain important phytochemicals that have medicinal properties. Future studies should include growing these two species under greenhouse conditions to determine what the difference in compounds will be between open-field cultivation and greenhouse conditions. Also, by including biostimulants in future studies to determine the effect it will have on metabolomic profiles and on the medicinal properties in the same plant species.

1278 A Untargeted Metabolomics Identifies Climate-Driven Markers of Grape Development in Tropical 'Syrah' under Double Pruning

PRESENTING AUTHOR: ***Fernando da Costa, University of São Paulo, Brazil***

CO-AUTHORS: *Alvaro L. L. Cassago, Mateus M. Artêncio, Guilherme J. Zocolo, Luísa Tannure*

SUB-THEME: Viticulture & wine growing

Viticulture has traditionally flourished in temperate zones, but Brazil's vast territory enables fine wine production in diverse climates. Ribeirão Preto, in São Paulo state, has a tropical climate with hot, rainy summers and dry winters. A local winery cultivates Vitis vinifera L. cv. 'Syrah' using the double pruning technique, shifting grape production to the dry winter season. This mitigates summer rain impacts, improves grape development, and enhances wine quality. This study aimed to characterize the metabolic dynamics of both leaves and grapes of 'Syrah' under double pruning in a tropical environment and to identify potential biomarkers associated with grape quality and optimal harvest timing. Untargeted LC-MS metabolomics was applied to 370 leaf and 187 grape extract samples collected in 2022-2023, and multivariate statistical analyses were used to correlate metabolic profiles with climatic variables. OPLS-DA models revealed a clear clustering of samples according to collection month, with July consistently showing the most chemically distinct profile, while March–April and May–June formed

separate groups. Key metabolites contributing to this discrimination included flavonols such as quercetin, kaempferol, and isorhamnetin in leaves, and anthocyanins such as cyanidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside in grapes, reflecting different stages of fruit development. Several unknown features also showed high VIP scores, indicating their potential as relevant metabolic markers. Canonical correspondence analysis demonstrated strong associations between climatic variables and metabolic profiles, highlighting solar radiation as a major driver of grape composition, particularly during ripening, while leaf metabolism showed stronger environmental coupling earlier in the season. Notably, this is the first report of rotundone in 'Syrah' grapes under double pruning in tropical conditions. These findings demonstrate the value of integrating leaf and grape metabolomics to better understand vine physiology, support improved harvest decision-making, and reinforce the potential of tropical viticulture for producing high-quality wines.

1279 B Metabolomic and Sensory Characterization of Texas Cabernet Sauvignon and Tannat Wines Using Untargeted LC–MS/MS Profiling

PRESENTING AUTHOR: ***Diana Zamora-Olivares, University of Texas at Austin, United States***

CO-AUTHORS: *New insights into Texas wine chemistry! Our metabolomics-driven study shows clear chemical and sensory differences between Texas Cabernet Sauvignon/Tannat and international benchmarks. Supporting a data-driven understanding of Texas terroir.*

SUB-THEME: Viticulture & wine growing

The U.S. is the fourth-largest wine-producing country, and Texas has emerged as a rapidly growing contributor, now ranking fifth nationally. With a total economic impact of $13.1B, the Texas wine and grape industry represent a vital agricultural sector whose expansion is driven by improved wine quality, loyalty to Texas-grown products, and a strong culture of wine tourism. Despite this growth, Texas wines such as Cabernet Sauvignon and Tannat remain underrepresented in the scientific literature, and no prior studies have integrated chemical and sensory analyses for these important varieties. Understanding the metabolomic signatures of these wines is increasingly relevant as the state faces significant environmental challenges that shape grape and wine composition.

In this study, we characterized the physicochemical, sensory, and metabolomic profiles of Texas Cabernet Sauvignon and Tannat wines and compared them with wines from internationally recognized benchmark regions. Twenty-six Texas wines (13 Cabernet Sauvignon, 13 Tannat) from the Texas High Plains and Texas Hill Country AVAs were analyzed alongside Cabernet Sauvignon from California and France, and Tannat from Uruguay. Chemical analyses included pH, titratable acidity, alcohol, color, antioxidant capacity, phenolic composition, and untargeted metabolomics using high-resolution LC–MS/MS. Multivariate statistical analyses revealed significant regional differences in acids, phenolics, and aroma-related metabolites, which aligned with distinct sensory characteristics. Both varieties showed clear chemical and sensory differentiation between Texas and benchmark regions. Significant subregional variation was also observed within Texas; High Plains wines were generally associated with more favorable aromatic and sensory attributes compared to Hill Country wines.

This work provides the first integrated chemical–sensory dataset for Texas Cabernet Sauvignon and Tannat. By combining metabolomics with chemometrics, it establishes foundational reference points to support winemakers, guide future regional research in Enology and Viticulture.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1310 A Methods for Daily, Dried Urine Spot Metabolomics at Scale Using 35-day kits, Wearables Integration, and Personalized Surveys

PRESENTING AUTHOR: ***Chad Pozarycki, Deleon, United States***

CO-AUTHORS: *Chad Pozarycki, Jose Luis Rodriguez Andrade, Nellie Kolon, Antonio Varagnolo, Grant Reidy, Brody Dowling, Victor Henriksson, Amanda Stockton, Facundo Fernandez*

SUB-THEME: At-home sample collection

Longitudinal metabolomics studies are typically challenging due to inconvenience of sampling methods and sample preservation requirements, thus increasing sampling cost for the study and necessitating lengthy participant onboarding and frequent researcher-participant interaction. In this work, we demonstrate and discuss aspects of daily dried urine spot analyses on a cohort of 16 users over at least 22 days per user (average 55.5 days/user). In addition, we incorporate daily survey data and wearable device data with our workflow. Participants collect samples at home with a cotton swab passed through the urine stream and dried under ambient conditions. The protocol takes seconds, requires no equipment beyond the swab, and removes cold-chain logistics from return shipping. Swabs ship by standard mail in batched intervals to a central laboratory for analysis. Capillary electrophoresis with laser-induced fluorescence (CE-LIF) separates and detects 10 amino acids relevant to recovery and metabolic state. An automated pipeline handles peak identification, alignment, and relative quantification across analytical batches. Daily surveys capture sleep, perceived exertion, soreness, mood, and nutrition adherence. We integrate wearable data from consumer platforms covering heart rate variability, sleep staging, training load, and resting metrics. Users do not collect all the same survey questions or use the same wearables. The full workflow aligns three data streams sampled at different cadences while maintaining data integrity across the cohort. Practical considerations include participant onboarding, compliance over multi-week windows, missing data handling, sample stability in transit, and batch effects across analytical runs. This kind of workflow enables longitudinal metabolomics data collected at scale, with cost and convenience characteristics suited to daily time resolution studies that can last years and to populations not amenable to clinical sampling. Daily collection compliance averaged 85.6% over the study window, and sample stability across return-shipping intervals showed very few issues.

1311 B A dilute-and-shoot method for high-throughput glucose quantitation in fermentation media using an acoustic injection system

PRESENTING AUTHOR: ***Katherine Steward, SCIEX, United States***

CO-AUTHORS: *Jonas Rombout, Lieselotte Vermeersch, Samuel Hanon, Paul RS Baker, Lloyd Cool, Kevin J Verstrepen*

SUB-THEME: Bioprocessing

Accurate and frequent monitoring of glucose concentrations is essential for optimizing microbial fermentation and maximizing biosynthetic yield. Conventional glucose assays are typically enzymatic or rely on derivatization and chromatographic separation, which limit analytical throughput. Acoustic ejection mass spectrometry coupled with an open-port interface (OPI) offers a chromatography-free alternative capable of rapid, high-throughput analysis.

The objective of this study was to evaluate the performance of an acoustic injection system for quantitative glucose analysis in fermentation media and to benchmark its accuracy and reproducibility against a validated HPLC-IR method.

A dilute-and-shoot workflow was developed using ^{13}C-glucose as an internal standard. Eight calibration standards spanning 0–0.4% (w/v) glucose were prepared with a constant ^{13}C-glucose concentration of 0.1% (w/v). Quantitation was based on the ratio of glucose to ^{13}C-glucose signal intensities. The method was applied to spent synthetic complete (SC) fermentation media from 16 genetically modified *Saccharomyces cerevisiae* strains. For each strain, three biological replicates were analyzed in triplicate following transfer to an MS-qualified 384-well plate. Sample throughput was approximately one analysis every 2.5 seconds.

The calibration curve showed excellent linearity over the tested concentration range (r^2 = 0.9995), with six replicate injections per standard. The ^{13}C-glucose internal standard showed stable signal intensity across all samples. Measured glucose concentrations in unfermented media (0.054% w/v) were consistent with the nominal glucose concentration added prior to fermentation (0.05% w/v). Comparison with validated HPLC-IR analysis demonstrated strong agreement, with a linear regression explaining 99% of the variance (R^2 = 0.9947).

This study demonstrates that the acoustic injection system enables rapid, robust, and quantitative glucose analysis in fermentation media using a simple dilute-and-shoot workflow. The method provides orders-of-magnitude higher throughput

than traditional chromatographic approaches while maintaining strong quantitative accuracy, supporting its utility for real-time or high-throughput fermentation monitoring in metabolomics-enabled bioprocessing.

1312 A Multi-omics analysis of Chinese hamster ovary cells reveals ferroptosis and organic acids as limiting factors in productivity

PRESENTING AUTHOR: ***Karl Burgess, University of Edinburgh, United Kingdom***

CO-AUTHORS: *Luke Johnston, Darina Stoyanova, Bart Pander, Jeff Keen, Mark Rendall, Leon Pybus, Karl Burgess*

SUB-THEME: Bioprocessing

Biopharmaceuticals are an invaluable class of medicines used to treat refractory diseases, including cancer, genetic diseases and autoimmune conditions. The biopharmaceutical market constitutes one of the fastest growing and most valuable sectors of the global healthcare market. Around 50% of all biopharmaceuticals are synthesised via Chinese hamster ovary (CHO) cell bioprocesses. The majority of biopharmaceuticals produced in CHO cells are monoclonal antibodies (mAbs), an increasingly popular biopharmceutical modality.

We applied a combination of liquid chromatography-ion mobility spectrometry mass spectrometry, 1H nuclear magnetic resonance spectroscopy and rapid LC-MS proteomics to the analysis of a commercial CHO cell line during mAb production. Metabolomics revealed four endogenously-produced organic acids with significant inhibitory effects on CHO cell growth dynamics: acetate, butyrate, formate and malate. Of the identified panel of inhibitors, formate displayed the greatest reduction in overall cell growth and was subsequently chosen for a follow-up analysis to investigate the effects on mAb productivity and metabolic changes induced in the presence of excess formate via 1H NMR metabolomics. Formate was found to have a cell-specific productivity enhancing effect in this screening study, and altered important metabolic pathways. This highlighting the complex effects potential inhibitors may exhibit in CHO cell bioprocessing. Proteomic analysis revealed pathways associated with induction of ferroptosis and shed light into the mechanisms that are activated when ROS scavenging chemicals are utilised. Enzymatic inhibition ameliorated ferroptosis, indicating that targeting redox metabolism and preventing its dysregulation provides better outcomes than attempting to reduce downstream effects. We also report the generation of a ferroptosis-resistant CHO cell line through directed evolution.

1313 B Following the Atoms: High-Resolution Dual-Element Stable Isotope Analysis for Advanced Metabolic Flux Studies

PRESENTING AUTHOR: ***Rahul Ravi Deshpande, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Ayush Midha; Bashar Amer; Isha Jain; Susan Bird*

SUB-THEME: Flux analysis

Introduction: Flux analysis quantifies metabolic pathway activity by tracking the flow of carbon or nitrogen atoms through cellular networks. Stable isotope tracers, particularly ^{13}C, have significantly advanced understanding of metabolic regulation. However, most studies rely on targeted analyses and single-element tracers. Untargeted workflows and dual-element labeling experiments (e.g., ^{13}C and ^{15}N) pose analytical challenges due to overlapping isotopic patterns that require ultra-high-resolution and high mass accuracy. Here, we evaluate high-resolution Orbitrap Hybrid mass spectrometer for untargeted analysis of metabolites labeled simultaneously with multiple stable isotopes.

Methods: HEK cells were cultured under varying oxygen tensions (50%, 21%, and 1.5%) and incubated with $^{13}C_6$-glucose, $^{15}N_2$-glutamine, or both tracers. Metabolites were separated using a mixed-mode HILIC-AEX column on a UHPLC system. A nano-flow ion pairing chromatography method was also evaluated to enhance sensitivity for polar metabolites. Data were acquired on an Orbitrap Hybrid MS featuring extended dynamic range, enhanced sensitivity, and reduced in-source fragmentation.

Preliminary Data: Initial validation using unlabeled and U-^{13}C E. coli extracts demonstrated accurate isotopologue discrimination at ≥120K resolution, enabling separation of ^{13}C and ^{15}N contributions. Nano-flow ion pairing chromatography increased sensitivity for polar metabolites by up to three orders of magnitude while reducing solvent consumption.

Both targeted and untargeted stable isotope workflows in enabled quantification of carbon and nitrogen labeling patterns across oxygen conditions and correlation to metabolic pathway alterations. These results demonstrate a robust platform for high-resolution, dual-tracer fluxomics using Orbitrap Hybrid MS.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1314 A High-Speed Polarity Switching for Comprehensive Untargeted Metabolomics in Short Analytical Runs

PRESENTING AUTHOR: ***Sunandini Yedla, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Brandon Bills, Rahul R. Deshpande, Bashar Amer, Susan S. Bird, Rafael Melani*

SUB-THEME: High-throughput metabolomics

High-resolution mass spectrometry is widely used for untargeted metabolomics and lipidomics but often requires separate positive and negative ionization runs, increasing analysis time. Conventional polarity switching, due to electronic settling delays, decrease scan density impacting quantitative robustness. The modified Orbitrap Tribrid mass spectrometer with improved ion synchronization and ion transfer delivers 40% higher duty cycle, enabling rapid polarity switching short runs without compromising scan density.

A Lipton black tea bag steeped 1min in water, centrifuged, and filtered was analyzed using polarity switching with 15- and 5-minutes gradients on C18, 150 mm and 50 mm columns connected to modified and existing Orbitrap Tribrid mass spectrometers coupled to HPLC system with water/methanol + 0.1% formic acid as solvents. Data were evaluated in FreeStyle 1.8 and Compound Discoverer 3.5.

Raw data was analyzed to evaluate instrument scan rate performance focusing on whether the short gradient provided sufficient data points for confident metabolite identification under polarity-switching. Chromatographic peaks with at least 5 data points and with consistent analyte presence across all the points were considered valid. The modified Orbitrap Tribrid mass spectrometer demonstrated a substantially higher scan rate than the existing Orbitrap mass spectrometer. At 60K resolution, the modified instrument acquired 2,274 and 932 MS1 scans during long and short gradients, respectively, compared with 1,488 and 608 scans on the existing system. Epicatechin ($C_{15}H_{14}O_6$ $[M+H]^+$ 291.0863) illustrates this improvement, with 11 and 6 data points across the chromatographic peak for long and short runs respectively, on the modified system versus 7 and only 3 data points on the existing system, Similar trends were observed across multiple compounds. Despite a 66% shorter runtime, the modified system retained 73% annotations compared to a 15-minute run on the existing system.

For Research Use/Purpose Only. Not for Diagnostic Procedures.

Fast polarity switching enables high-throughput metabolomics

1315 B Daily urinary amino acid profiles track training load and recovery dynamics in a longitudinal cohort study

PRESENTING AUTHOR: ***José Andrade, Deleon, United States***

CO-AUTHORS: *Jose Luis Rodriguez Andrade, Chad Pozarycki, Nellie Kolon, Antonio Varagnolo, Grant Reidy, Brody Dowling, Victor Henriksson, Amanda Stockton, Facundo Fernandez*

SUB-THEME: High-throughput metabolomics

Longitudinal metabolomics studies are challenged by sampling cost, participant burden, and sample preservation requirements. Here we report findings from a cohort of 16 active adults who collectively completed 671 daily dried urine spot collections paired with daily wellness surveys and wearable device data, with observation windows of 22–150 days per participant. Urine samples were analyzed by capillary electrophoresis with laser-induced fluorescence (CE-LIF) targeting 10 amino acids relevant to nitrogen balance, recovery, and neurochemical state.

Primary regression analyses were run with user fixed effects, examining whether CLR-transformed amino acid levels on day "t" predicted self-reported fatigue, soreness, and sleep quality at lags of t+1 to t+3. Glutamine and proline emerged as robust predictors, replicated across different statistical tests. Elevated urinary glutamine predicted greater self-reported physical and mental fatigue in t+1 and t+2 across all users reporting them ($n = 5$, $\beta = +0.54$, $q < 0.001$), consistent with glutamine excretion marking peak catabolic state following exercise. Proline predicted a decrease in self-reported physical tiredness, muscle soreness, and mental fatigue from t+1 to t+3, across all users reporting them ($n = 7$, β range -0.51 to -1.05, all $q < 0.001$). This is consistent with proline's role in the connective tissue repair phase of recovery.

Event study analysis around high-load training days confirmed these temporal signatures. For the 6 users with Garmin or Whoop wearables, on average, glutamine surged at t+1 to t+2 post-workout while proline peaked at t+3 to t+4. Reverse-direction analyses showed that recovery metrics such as Garmin's body battery predicted alanine levels the next morning ($\beta = -0.006$, $q < 0.001$).

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

These findings support daily urinary metabolomics as a practical complement to wearable monitoring for tracking training-recovery dynamics at timescales inaccessible through weekly or monthly sampling.

1316 A — High-Speed, High-Resolution DESI Imaging of murine brain and porcine liver tissues on a Benchtop Folded-Flight-Path Mass Spectrometer for the detection of metabolites and lipids

PRESENTING AUTHOR: ***Hernando Olivos, Waters Corporation, United States***

CO-AUTHORS: *Mark Towers, Helen Yates, Philip Young, Lisa Towers, Joanne Ballantyne*

SUB-THEME: Imaging MS

DESI Mass Spectrometry Imaging (MSI) can easily enable mapping of metabolites and drugs in biological tissues. As interest in single-cell level imaging grows, there is an increasing demand for imaging metabolites (and lipids) critical to cellular functions. However, reducing the pixel size decreases the amount of analyte, making it more challenging to detect many metabolites. This reduction of pixel size also reflects on the significant increase of number of pixels and acquisition time. The workflow described here enables much higher throughput, achieving acquisition speeds of up to 100 spectra (or pixels) per second.

Murine brain and porcine liver sections (18 µm) were analyzed at 5–100 Hz using a benchtop folded-flight-path mass spectrometer (XEVO™ MRT MS) paired with a DESI XS source. Across this range, the instrument maintained mass resolution up to 100,000 FWHM and stable mass accuracy in both untargeted and targeted modes. Low-flow DESI operation (

Also, due to the ionization nature of DESI, the analysis of small molecules metabolites such as the common metabolites of TCA cycle, aminoacids, and such can be detected along with lipids and higher molecular weight species, for example. The workflow described here enables much higher throughput of these metabolites and lipids that could have implications on larger cohorts of samples to achieve statistical significance.

Novel aspect: Demonstration of DESI imaging at up to 100 Hz with high mass and spatial resolution on a benchtop folded-flight-path mass spectrometer.

1317 B — Spatiotemporal Mapping of Defense-Related Metabolites in Eucalyptus nitens Leaves Following Elicitation with Gonipterus platensis Oral Secretions by MALDI-MSI

PRESENTING AUTHOR: ***Areli Jara, Universidad de Concepción, Chile***

CO-AUTHORS: *Javiera Nahuelcura, Nicolás Fernández, Catalina Bravo, Ignacio Salinas, Andy J. Pérez*

SUB-THEME: Imaging MS

Infestation by the defoliating insect Gonipterus platensis represents a major threat to Eucalyptus plantations in Chile, particularly affecting Eucalyptus globulus, a highly susceptible species. In contrast, Eucalyptus nitens exhibits a higher degree of resistance, making it a relevant model to investigate spatially resolved chemical defense mechanisms. Understanding the in-situ distribution of specialized metabolites in this species is essential to elucidate how defense responses are organized at the tissue level.

In this study, we employed matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to characterize the spatiotemporal metabolic response of E. nitens leaves following elicitation with oral secretions from G. platensis. A time-course experiment was conducted using intact mother plants, with leaf samples collected at multiple time points after elicitation. Transverse cryosections were prepared and coated with 2,5-dihydroxybenzoic acid (DHB) by vacuum sublimation prior to MSI analysis.

Preliminary results revealed a spatially resolved and time-dependent redistribution of defense-related metabolites in leaf tissues. In particular, signals putatively assigned to glycosylated phenolics and roseoside, were predominantly localized in secretory cavities and adjacent mesophyll regions in non-elicited tissues, whereas a relative decrease in signal intensity was observed at early time points following elicitation. Conversely, ions consistent with hydrolyzable tannins exhibited increased intensity in specific tissue domains, suggesting compartmentalized metabolic reprogramming. These changes were most evident within the first hours post-elicitation and tended to stabilize at later time points.

Overall, these findings provide initial evidence that elicitation with G. platensis oral secretions induces a spatially structured metabolic response in E. nitens leaves, involving the redistribution of specialized metabolites associated with plant defense. This work highlights the potential of MALDI-MSI to resolve the tissue-specific deployment of chemical defenses and supports its application for studying plant–herbivore interactions at the molecular level.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1318 A Deep Structural Profiling of Bufonid Secretions: Overcoming Isomeric Co-elution through Ion Mobility

PRESENTING AUTHOR: ***Fabiola Almeida Garcia, University of Sao Paulo, Brazil***

CO-AUTHORS: *Isel Pascual Alonso, Roberto Alonso Bosch, Rodrigo Moreira da Silva, Jacqueline Nakau Mendonça, Norberto Peporine Lopes*

SUB-THEME: Ion mobility spectrometry

Bufadienolides are complex polyhydroxysteroids (C24) characterized by a steroidal core with an unsaturated six-membered α-pyrone ring at the C17 position. While these compounds exhibit significant pharmacological potential, their characterization via conventional LC-MS/MS is frequently compromised by co-elution complexes, where constitutional isomers exhibit identical retention times (tR) and m/z values [1–3]. In this study, we employed Trapped Ion Mobility Spectrometry (TIMS) coupled with UPLC-Q-TOF mass spectrometry to resolve the co-elution bottleneck in the secretions of Peltophryne fustiger, Peltophryne florentinoi, Peltophryne peltocephala, and Rhinella ornata. Analysis of pooled quality control (QC) samples revealed a critical acquisition constraint: high-abundance steroids dominated the DDA-PASEF duty cycle, leading to the stochastic undersampling of minor components. This phenomenon results in a precursor masking effect where the limited dynamic range of the instrument fails to trigger MS/MS events for low-abundance isomers [4]. To address these constraints, we utilized the differential isomeric distribution across taxa. In specific species, the relative intensity of certain isomers is sufficiently elevated to facilitate the triggering of fragmentation precursors that remain latent in the QC pool, enabling their direct MS/MS characterization or unambiguous detection as discrete mobility features. While ion mobility applications in complex natural mixtures remain limited [4], this study represents the first use of TIMS-MS/MS to resolve isobaric isomeric bufadienolides co-eluting within a single chromatographic peak directly from crude venom secretions. This strategy allowed for the discrimination of 5 distinct sets of isobaric isomers. These components were baseline-resolved (Rs≥1.5) based on their unique collision cross-section (CCS) values (N2 drift gas, 1/K0 0.45–1.45 Vs/cm2) [5]. Furthermore, the mobility dimension facilitated the discrimination between constitutional isomers and in-source fragments (ISF), as ISF components co-migrated with parent ions in the gas phase. This CCS-indexed library represents the first structural mapping of endemic Cuban Peltophryne secretions, demonstrating that species-targeted TIMS-MS/MS is a prerequisite for accurate chemotaxonomic profiling.

1-Sun, X. et al. 2020. 2- Hu, Y. et al. 2011. 3- Zhang, Y. et al. 2016. 4-Carnevale, F. et al. 2022. 5-Gabelica, et al 2019.

Acknowledgments: FAPESP grant 2023/00837-0, 22/11454-1, FCFRP, CAPES and CNPq.

1319 B Ion mobility separation of isomers for microbial metabolomics

PRESENTING AUTHOR: ***Michal Adámek, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic***

CO-AUTHORS: *Tommaso Stefani, Aninda Mazumdar, Ondřej Hřebíček, Zdeněk Kameník*

SUB-THEME: Ion mobility spectrometry

Ion mobility has emerged as a powerful complement to mass spectrometry, offering an additional dimension of separation that may distinguish isomers and isobars. However, its performance in metabolomics has been demonstrated primarily through individual case studies. We aim to address this gap through a systematic evaluation of trapped ion mobility spectrometry (TIMS) in metabolomics with a particular focus on microbially related metabolites. To establish a representative library of isomers, we developed custom Python scripts to identify commercially available isomers and categorize them into “isogroups”. The selection was further curated by integrating bioactivity annotations from PubChem. The resulting library includes nearly 1,000 bioactive compounds organized into 374 isogroups, each containing up to 10 isomers.

The compounds are being analyzed using LC-TIMS-MS/MS to generate high-resolution mass spectrometry and ion mobility data, including CCS values. To evaluate and maximize the resolving power of TIMS, we are optimizing key acquisition parameters, including ramp-time to balance duty cycle and separation efficiency, the number of PASEF-ramps to maintain high-quality MS/MS spectra without compromising sensitivity and TIMS-stepping to ensure sufficient coverage of ion mobility window and capture the full structural diversity of the library.

The resolving power of TIMS was preliminarily evaluated using a representative subset of 41 compounds, selected to mirror the chemical, isomeric, and mass diversity of the library. By optimizing TIMS parameters, we achieved mobility separation across several classes. Full mobility separation was observed for progestogens (e.g., dydrogesterone and tibolone) and saponins (e.g., saikosaponin B2 and D). Partial separation was also achieved for several triterpenoids (e.g., 11-keto-β-boswellic acid, 18α-glycyrrhetinic acid and gypsogenin) and (E)- and (Z)- aztreonam isomers.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

To facilitate interpretation of ion mobility data, we are developing a custom web application, integrating raw datafiles for chromatogram and mobilogram visualization with MZmine-processed data and statistics. This platform enables rapid comparison of retention time and ion mobility behavior across large metabolomics.

1320 A NMR Plasma Lipoproteins on the Bench

PRESENTING AUTHOR: ***Claire Cannet, Bruker, Germany***

CO-AUTHORS: *Kyle Rodriguez, Nils Pompe, Julien Wist, Oscar Millet, Philip Nitschke*

SUB-THEME: Low-cost and field-deployable metabolomics platforms

Introduction: NMR-based lipoprotein and metabolite profiling is well established for clinical and population studies, but its routine implementation has traditionally relied on high-field NMR systems with significant infrastructure requirements. Extending robust and standardized plasma metabolomics to benchtop NMR platforms could broaden accessibility while maintaining analytical quality.

Aim: The aim of this study was to evaluate the analytical performance, reproducibility, and robustness of standardized plasma lipoprotein and metabolite profiling implemented on a benchtop NMR system using models transferred from a clinically validated high-field platform.

Methods:Lipoprotein and metabolite profiling was performed using the LipoQuant Suite on a Fourier 80 benchtop NMR system. Prediction models were directly transferred from the Bruker Avance IVDr 600 MHz platform. Model performance was evaluated using more than 2,400 plasma and serum samples originating from five independent studies and measured across six instruments. Monte-Carlo cross-validation and independent external validation were applied. Quantification accuracy was assessed using NIST-1950 reference materials. Long-term and inter-instrument reproducibility were evaluated across multiple systems. Automated sample handling, including cooling, preheating, and barcode-based tracking, ensured standardized processing.

Results:The transferred models demonstrated strong analytical performance, with correlation coefficients exceeding $R > 0.9$ for 60 parameters and $R > 0.85$ for 82 parameters. Results were highly consistent across instruments, showing no detectable instrument-specific bias and stable performance over time. Quantitative accuracy was confirmed using NIST-1950 reference samples. Long-term reproducibility across multiple instruments supported suitability for longitudinal and routine applications. The fully automated workflow enabled continuous, unattended operation with a total prep-to-result time of approximately 15 minutes per sample.

Conclusion:Standardized plasma lipoprotein and metabolite profiling can be successfully implemented on a benchtop NMR system while preserving reproducibility, quantitative performance, and inter-instrument comparability previously established on high-field platforms. This approach enables robust, automated plasma metabolomics in routine laboratory environments and supports scalable applications in longitudinal and population-based studies.

1321 B Real-Time Metabolomic Monitoring of cells culture by Online High-Resolution Mass Spectrometry

PRESENTING AUTHOR: ***Floriant Bellvert, MetaboHUB, France***

CO-AUTHORS: *Nina Lager Lachaud, Rochelle Kouakou, Christophe Ellero, Xavier Cameleyre, Maud Heuillet, Lindsay Peyriga, Floriant Bellvert*

SUB-THEME: New developments in instrumentation

Fermentation monitoring is essential for bioprocess development, optimization, and control. While conventional parameters such as temperature, dissolved oxygen, pH, and biomass are routinely monitored, metabolites provide direct insight into cellular regulation and metabolic activity. Because metabolic changes can occur rapidly, conventional metabolomic analyses are often insufficient due to their limited temporal resolution and time-consuming workflows. To address this challenge, we adapted a real-time metabolomic workflow for online monitoring of microbial cultures.

We have developed an analytical platform combining a bioreactor with an automated fluidic interface comprising culture and solvent pumps, 6- and 10-way valves, and an in-line filtration valve connected directly to a high-resolution mass spectrometer. In addition, we have developed two software programmes: A Python-based workflow manager has been developed to automate fluidic control and initiate analytical sequences. A second workflow manager has been implemented for the automatic, real-time processing of mass spectrometry data.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

As a proof of concept, metabolites from an Escherichia coli culture were continuously monitored following a 13C-glucose pulse to assess isotope incorporation dynamics. The E. coli culture was analyzed every minute using flow injection mass spectrometry on an Exploris 240 instrument (Thermo Scientific) operated at a resolving power of 90,000 at m/z 200. This setup enabled high-frequency metabolite monitoring and rapid detection of isotopic labeling dynamics. In parallel, an integrated real-time data-processing solution is under development to enable direct visualization, identification, and quantification of metabolites, with the potential for automated feedback control of the fermentation process.

Future developments will focus on improving metabolome coverage by mass spectrometry and implementing quantitative metabolite analysis using internal standards or benchtop NMR coupled in series. This approach is expected to be transferable to other biological systems, including adherent cell cultures.

1322 A Expanding plasma metabolome coverage with enhanced dynamic range scan mode and iterative MS/MS acquisition

PRESENTING AUTHOR: ***Michal Kaczmarek, Thermo Fisher Scientific, Germany***

CO-AUTHORS: *Amer Bashar; Rahul Deshpande; Susan S. Bird*

SUB-THEME: New developments in instrumentation

NIST SRM 1950 is a widely used pooled plasma reference, yet reported untargeted coverage varies across platforms and laboratories. In addition to extraction protocol, acquisition strategy and background control, untargeted plasma metabolomics is constrained by its broad concentration range. In data-dependent MS/MS, high-abundance ions can dominate precursor selection and shorten ion accumulation times, limiting fragmentation of low-abundance metabolites. Enhanced dynamic range (eDR) scan mode is a novel practical lever to reduce this saturation-driven bias and improve sampling of otherwise under-sampled ions. We evaluated whether eDR MS1 acquisition combined with an iterative deep-scan MS/MS strategy improves low-abundance sampling and MSI level 2 annotations across three reversed-phase chemistries (C18, PFP, F5).

Methanol-precipitated SRM 1950 extracts were analyzed in positive and negative ionization mode on a modified Orbitrap hybrid mass spectrometer (120k MS1, 30k MS2) with eDR enabled/disabled. For each column, an iterative sequence (AcquireX deep scan) comprised triplicate full-scan reference injections, a blank, and five sequential MS/MS identification injections using updated inclusion/exclusion lists. Features were grouped with adduct/isotope/in-source fragment handling and blank filtering, and MS/MS spectra were matched to a reference library (≤5 ppm; score ≥60) in Compound Discoverer 3.5 to assign MSI level 2 identifications.

Enabling eDR tripled detected MS1 features, driven by low-intensity ions. Low-to-medium abundance species showed up to 1035% higher signal-to-noise versus conventional full scan. After molecular-entity grouping, eDR yielded 3× more compound entries than full scan without eDR. Under the best chromatography condition, eDR produced 937 MSI level 2 IDs (26% more than standard full scan). Across five identification injections, the iterative workflow triggered library-matched MS/MS on 57% more unique precursors than conventional DDA approach, with many additional annotations originating from MS1 intensities below 1×10^6 that were otherwise unfragmented.

Overall, eDR plus iterative MS/MS substentially expands NIST SRM 1950 plasma coverage by improving MS/MS access to ion-suppressed, low-abundance metabolites.

1323 B Nano Flow Lipidomics: Sensitivity, Reliability, and Practical Implementation

PRESENTING AUTHOR: ***Ciara Sallowicz, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Ciara J. Sallowicz, Thiago Mattos, Rahul R. Deshpande, Bashar Amer, Susan S. Bird*

SUB-THEME: New developments in instrumentation

Introduction: Untargeted lipidomics is commonly performed using high flow liquid chromatography, which provides robust and precise measurements but requires larger sample amounts, consumes solvent, and limits sensitivity. In contrast, nano flow operates at lower flow rates that can improve ionization efficiency, reduce solvent usage, and enable lower on-column loads. Although nano flow methods are established in proteomics, their adoption in lipidomics has been limited due to concerns around robustness, column availability, and method complexity. As nano flow hardware and column options expand, there is increasing interest in understanding how nano flow can be implemented for routine untargeted lipidomics. This work evaluates performance benefits of nano flow lipidomics acquired on a modified hybrid Orbitrap MS and outlines practical considerations for robust implementation.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Methods: Bovine liver total lipid extract spiked with SPLASH internal standards was analyzed using nano flow and high flow liquid chromatography coupled to a modified hybrid Orbitrap MS. Nano flow separations were performed at 700 nL/min on a 75 µm ID C18 column, while high flow separations used a 2.1 mm ID C30 column at 260 µL/min. Full scan MS^1 polarity switching was used for untargeted profiling, and the AcquireX Deep Scan workflow was applied to increase data-dependent MS/MS coverage. A SPLASH dilution series was analyzed in triplicate to evaluate quantitative performance and limits of quantitation. Data was processed using consistent workflows across flow regimes.

Preliminary Data: Nano flow increased lipid annotation depth at lower on-column loads compared with high flow. After class-specific filtering, nano flow produced more high confidence annotations, supporting improved sensitivity. Nano flow conditions also reduced unintentional MS^1 (in-source) fragmentation and improved quantitative performance across lipid classes. Overall, these results indicate that nano flow can increase sensitivity and reliability for untargeted lipidomics while reducing sample and solvent requirements, supporting robust routine implementation.

1324 A Pushing the Limits of Structural Lipidomics: Enhanced Sensitivity and MS^3-Driven Annotation on a Modified High Resolution Mass Spectrometer

PRESENTING AUTHOR: ***Thiago Mattos, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Rahul R Deshpande, Bashar Amer, Brandon Bills, Sunandini Yedla, Rafael D Melani, Susan S. Bird, Thomas Moehring*

SUB-THEME: New developments in instrumentation

Advances in lipidomic analysis require mass spectrometry platforms that deliver high sensitivity, rapid acquisition, and efficient parallel use of multiple mass analyzers. A modified high resolution mass spectrometer was evaluated to meet these demands through improved sensitivity, faster polarity switching, and enhanced parallelization between the two available mass detectors. Bovine liver lipid extracts were used to evaluate the analytical performance of the modified MS and plant leaf extracts were analyzed with the optimized workflow. Samples were spiked with a deuterium-labeled internal standard mix for improved relative quantitative analyses. The lipids were separated using a C30-based reversed-phase chromatography coupled with polarity switching high-resolution MS^1 precursor analyses and data-dependent fragmentation combining higher-energy collisional dissociation (HCD) and collision induced dissociation (CID) with ultraviolet photodissociation (UVPD) for fragmentation of lipids up to MS^n level. Preliminary data show that the limit of detection (LOD) ranged from low picomoles to high femtomoles for all detected internal standards. For instance, 15:0-18:1(d7) phosphatidylcholine (PC) showed an LOD of 25 femtomole on-column in the positive mode, while 15:0-18:1(d7) phosphatidylinositol (PI) showed an LOD of 4 picomole on-column in negative mode. Enhanced parallel operation of the two detectors, together with rapid polarity switching, improved our duty cycle efficiency and markedly increased data density while maintaining quantitative accuracy for lipid measurements. Our MS method consisted of a 3 second cycle time and used HCD- and CID-based MS^2 fragmentation, with triggered CID-based MS^3 fragmentation to resolve coeluting isomeric triacylglycerols, and neutral loss triggered UVPD-based MS^3 to determine regioisomers of PC species. This approach combined with manual data curation allowed for the determination of the exact fatty acyl composition and sn-positions in selected PC species, increasing the number of unique lipid IDs. Finally, using commercially available software we assessed the lipidome of plant extracts and identified over 2000 unique lipids in the dataset.

1325 B Integrated targeted quantitation and deep untargeted plasma metabolomics

PRESENTING AUTHOR: ***Alexander Harder, Thermo Fisher Scientific, Germany***

CO-AUTHORS: *Bashar Amer, Michal Kaczmarek, Claire Dauly, Rahul Ravi Deshpande, Thomas Moehring, Susan S. Bird*

SUB-THEME: New developments in instrumentation

Reliable metabolomics requires mass spectrometry approaches capable of delivering broad untargeted coverage while maintaining high sensitivity and quantitative robustness for targeted analysis. Conventional strategies often require a trade-off between discovery and precise quantitation, which can limit biological interpretation. Here, we describe a workflow that integrates targeted and untargeted analyses within a single experiment to address this challenge. The approach leverages extended dynamic range acquisition to improve metabolome coverage while preserving quantitative performance.

Method development and benchmarking were performed using NIST SRM 1950 plasma, followed by application to plasma samples from a diet-induced obesity (DIO) mouse model. Plasma samples were spiked with isotope-labeled amino acids, extracted with methanol, and analyzed using reversed-phase chromatography coupled to ultra-high-performance liquid chromatography–mass spectrometry. Data acquisition employed MS^1-based strategies, including targeted SIM (tSIM) and

targeted MS^2 (tMS^2), evaluated with and without extended dynamic range acquisition. Data processing supported both targeted quantitation and untargeted feature detection.

The workflow demonstrated reliable quantitation across six orders of magnitude, with limits of detection as low as 0.05 attomoles and lower limits of quantitation near 1 femtomole for selected analytes. Detection limits improved with optimized acquisition modes, particularly when extended dynamic range acquisition was applied. Reduced in-source fragmentation (e.g., ~14% reduction for phenylalanine) contributed to improved spectral clarity and quantitative accuracy. Extended dynamic range acquisition increased MS^1 feature detection approximately sixfold, enhanced MS^2 acquisition more than fourfold, and doubled the number of confidently annotated metabolites. Application to DIO mouse plasma samples enabled improved metabolome coverage, reproducibility, and clear differentiation between dietary groups.

1326 A Sequential same-slide spatial metabolomics and transcriptomics for multimodal tissue phenotyping

PRESENTING AUTHOR: ***Veronika Saharuka, Heidelberg University, Germany***

CO-AUTHORS: *Bianka Berki, James Cleland, Victor Didier, Hsin-Jui Lu, Jakob Meier-Credo, Laura Schütze, Josephine Bargeritz, Edith Heard, Carsten Hopf, Jan-Philipp Mallm, Duncan Odom, Christiane Opitz, Gernot Poschet, Pei-Chi Wei*

SUB-THEME: New multi-omics workflows

Tissue function emerges from the interplay between gene expression and cellular metabolism, yet these modalities are rarely measured together in their native spatial context. While spatial transcriptomics and MALDI-MS imaging have each advanced rapidly, same-section integration remains hindered by protocol incompatibilities that can redistribute labile metabolites and lipids or degrade RNA.

We present a sequential same-slide workflow integrating MALDI-MS imaging with spatial transcriptomics on fresh-frozen tissue sections. Developed jointly between spatial metabolomics and spatial transcriptomics core facilities, the workflow performs MALDI-MS first to preserve endogenous metabolite and lipid distributions, followed by either sequencing-based transcriptomics or imaging-based transcriptomics. We define compatible procedures for MS-compatible embedding, cryosectioning, matrix application, inter-facility transfer, matrix removal, and downstream transcriptomics processing. Spatial alignment is achieved through unsupervised image co-registration, enabling direct comparison of metabolite and transcript distributions within a single histological coordinate system.

We demonstrate the workflow in two biological contexts: liver zonation and brain development. In liver tissue, same-section MALDI-MS and Visium HD recover shared organisation along the periportal–pericentral axis, enabling comparison of metabolic and transcriptional features within defined anatomical zones. RNA integrity and spatial transcript patterns are preserved after MALDI processing, and sequencing-based spatial transcriptomics maintains sufficient data quality for downstream analysis. In brain tissue, we evaluate compatibility of MALDI-MS with Xenium-based spatial transcriptomics on the same slide. Although MALDI sensitivity is reduced on the non-conductive slides required for Xenium, transcript detection and spatial expression patterns remain robust and comparable after MALDI acquisition.

Together, this work establishes a practical framework for same-section multimodal imaging in core-facility environments. By bridging the gap between metabolic and transcriptional modalities on a single slide, the workflow enables discovery of spatially resolved molecular signatures underlying tissue function and disease.

1327 B Identification of a Unique Dietary Antioxidant in Cells and Mitochondria Combining NMR, MS and Ratio Analysis

PRESENTING AUTHOR: ***G.A. Nagana Gowda, Univesity Of Washington, United States***

CO-AUTHORS: *Vadim Pascua, Wentao Zhu, Daniel Raftery*

SUB-THEME: NMR metabolomics

Ergothioneine (ERG) is a naturally occurring dietary antioxidant that has gained attention for its roles in human health and diseases. Despite its biological importance, ERG is rarely detected by MS and has not previously been identified by NMR-based metabolomics. A strong peak in the spectra from red blood cells (RBCs) that varied substantially across samples was observed using ^{1}H-NMR spectra. Analysis using Ratio Analysis of NMR Spectroscopy (RANSY) identified two additional peak multiplets associated with the unknown metabolite. Partial structural elucidation was further supported by multiple ^{1}H-^{1}H and ^{1}H-^{13}C 2D NMR experiments, however the numerous heteroatoms hindered complete structural assignment. LC-MS analyses were conducted on the RBC samples. To narrow the list of potential hits and to aid in connecting the NMR and MS data, two samples with nearly six-fold difference in metabolite concentrations were selected. LC-MS data were acquired in

positive ionization mode, as NMR data suggested the metabolite carried a positive charge due to a trimethylamine group. A mass-to-charge (m/z) peak exhibiting an approximately six-fold intensity difference between samples was identified. Database searches combined with NMR evidence led to the identification of ERG, which was further validated by spiking experiments using an authentic standard. ERG was found in human plasma, whole blood, and red blood cells (RBC), as well as mouse blood and tissues, and its levels varied substantially across biological samples, with particularly high concentrations in human RBCs. A key feature enabling ERG analysis by simple ^{1}H-NMR is its isolated peak from overlap with other metabolites, allowing reliable quantitation. Despite its ubiquity and abundance, ERG has been largely overlooked in prior NMR- and MS-based studies. Our findings demonstrate that ERG can be routinely measured using both NMR and MS, opening new opportunities to incorporate ERG into metabolomics workflows and to further explore its roles in health and diseases.

1328 A Optimizing Plasma Extraction Protocols and the Effect of BMI on Metabolite Quantification

PRESENTING AUTHOR: ***Manida Suksawat, National Phenome Institute, Khon Kaen University, Thailand***

CO-AUTHORS: *Preeyakan Malikrong, Jutarop Phetcharaburanin, Narumon Mahaamnad, Thanaporn Kulthawatsiri, Wassana Jamnongkan, Marisa Attapong, Premkamol Chaipanitkul, Chotika Chatkasem, Peeraya Suksuratin*

SUB-THEME: NMR metabolomics

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for study physiophatological widely utilized for metabolomics studies, however, sample preparation methods significantly influence accurate spectral alignment and chemical shift referencing. This study aims to compare dual-phase extraction (DPE), protein precipitation (PPT), and solid-phase extraction (SPE) methods using glucose as the internal reference in human plasma samples. While PPT and control samples showed consistent glucose peak alignment, DPE and SPE caused glucose resonance shifts, introducing referencing errors that may affect data interpretation. Principal component analysis revealed distinct metabolic clustering based on extraction method, indicating substantial compositional variation. DPE enhanced lipophilic metabolite signals, while PPT and SPE retained small polar metabolites such as tryptophan, trimethylamine N-oxide, and allantoin. BMI- and gender-related differences were observed, with high-BMI male subjects showing increased lipid $-CH_2-/-CH_3$ signals, reflecting metabolic alterations linked to obesity. Heatmap analysis further supported extraction-dependent metabolite pattern variation, especially among lipid and energy-related compounds. These findings emphasize the importance of selecting appropriate extraction methods to ensure accurate chemical shift referencing and metabolite profiling in NMR-based clinical metabolomics.

1329 B NMR Metabolite Fraction Libraries for Quantitative Metabolomic Profiling

PRESENTING AUTHOR: ***Art Edison, University of Georgia, United States***

CO-AUTHORS: *Art Edison, Chris Esselman, Deanna Lanier, Reid Whitley, JoJo Xiang*

SUB-THEME: NMR metabolomics

We have recently developed computational tools that work together to provide the foundation for improved quantitative NMR metabolomics using the complete set of both known and unknown metabolites in a particular biological system. First, we model 1D NMR data in the time-domain using a method called SAND (Spectral Automated NMR Decomposition).1 SAND automatically generates a set of Lorentzian peaks with accurate chemical shifts, amplitudes, and linewidths that are deconvoluted from the mixture spectrum. Second, we use HPLC and SAND processed 1D NMR to create a fraction library from a pooled reference sample. This fraction library reduces overlap, which is severe in NMR metabolomics. It also enables us to generate the complete set of metabolite basis functions, which are the known and unknown NMR-detectable metabolites in the reference sample.2

This poster will introduce both SAND and metabolite fraction libraries and will also outline a new approach for the alignment of SAND NMR profiling data that takes advantage of the tabular domain to improve NMR alignment. We will demonstrate the utility of this overall approach by showing quantitative profiling of metabolites in privet using BATMAN.3 Privet is an invasive plant in Georgia that has proved useful for teaching systems biology to undergraduate and graduate students at the University of Georgia.

1 Wu, Y. et al. SAND: Automated Time-Domain Modeling of NMR Spectra Applied to Metabolite Quantification. Anal Chem 96, 1843–1851 (2024). https://doi.org/10.1021/acs.analchem.3c03078

2 Esselman, C. et al. Metabolite Fraction Libraries for Quantitative NMR Metabolomics. bioRxiv (2025). https://doi.org/10.64898/2025.12.30.696914

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

3 Hao, J. et al. Bayesian deconvolution and quantification of metabolites in complex 1D NMR spectra using BATMAN. Nat Protoc 9, 1416–1427 (2014). https://doi.org/10.1038/nprot.2014.090

1330 A Robust Absolute Quantification in Targeted 1H NMR Metabolomics Across Multiple Human Biofluids Using ACP 2.0

PRESENTING AUTHOR: ***Kyle Rodriguez, Bruker Biospin, United States***

CO-AUTHORS: *Claire Wegner*

SUB-THEME: NMR metabolomics

Introduction: Quantitative targeted metabolomics by 1H NMR spectroscopy depends on reproducible spectral processing and transparent absolute quantification across diverse biological matrices.

Aim: We evaluated a standardized workflow implemented in Advanced Chemical Profiling 2.0 (ACP 2.0) for automated identification and absolute quantification of metabolites in human plasma, urine, feces, and saliva.

Methods: Samples were prepared and measured using standardized protocols to obtain high-quality spectra representative of clinically and biologically relevant metabolite profiles. ACP 2.0 was used for automated spectral processing, targeted metabolite assignment, and fitting-based absolute quantification. Performance was assessed by (i) metabolite identification, (ii) reproducibility across replicate measurements, and (iii) agreement with certified reference values in NIST SRM 1950 (human plasma) and NIST SRM 3667 (human urine). To test matrix complexity, the workflow was applied to cohorts of fecal and saliva samples exhibiting pronounced overlap and matrix effects.

Results: The automated workflow quantified 50 metabolites in feces and 40 metabolites in saliva.

Conclusions Across all matrices, ACP 2.0 produced consistent quantification and was robust to variations in linewidth, baseline distortions, and spectral overlap. The fitting-based strategy supported metabolite quantification in complex biofluids where peak overlap is common, and results were benchmarked against certified reference materials for plasma and urine. Overall, these findings support the use of a standardized, automated 1H NMR workflow for targeted metabolomics applications spanning clinical and microbiome-relevant sample types.

1331 B Untargeted methods in a mass spectrometry core facility: the positives and challenges

PRESENTING AUTHOR: ***Martina Conti, University of York, United Kingdom***

CO-AUTHORS: *Tony Larson*

SUB-THEME: Non-targeted and semi-targeted methods

Untargeted workflows are being increasingly applied across metabolomics, lipidomics, and small molecule analysis by mass spectrometry (LC-MS, GC-MS and imaging MS). In a multi-user environment, the technical challenges of robust data acquisition (instrument calibration, method development, QC monitoring) are relatively easy to address, compared to the effort needed to understand and tailor experimental design and downstream data processing. In our metabolomics core facility within the Bioscience Technology Facility at the University of York (UK), we routinely perform multiple full-service projects in any given week. We face the challenge of needing to make informed decisions about the best workflows with our users and clients, where the analytical targets and desired outcomes may be unknown. As part of our full-service workflow planning, we need to balance instrument usage with open-access requests and have strategies in place to maximise instrument up-time and equitable usage across constantly changing instrument configurations.

Here we present the challenges we have faced in key case studies in the small molecule field including LC-MS analyses on Orbitrap Exploris 480 and Agilent GC-MSD platforms. We will show how experimental planning is carried out with our clients, how our instruments are managed, and how data processing is tailored and combined across different core staff expertise and mixes of vendor and open access software. We will highlight the advantages and disadvantages of these approaches, and how we perform all this in a cost-recovered facility to ensure we maintain the instrumentation and expertise needed to support multiple users.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1332 A Developing biologically-relevant retention time and MS/MS libraries for use in semi-targeted clinical metabolomics studies.

PRESENTING AUTHOR: ***Catherine Winder, University Of Liverpool, United Kingdom***

CO-AUTHORS: *Wanchang Lin, Hannadi Alamri, Shiva Jalili, Warwick B. Dunn*

SUB-THEME: Non-targeted and semi-targeted methods

Introduction: The identification of metabolites requires the matching of experimental data (MS1, MS/MS fragmentation and chromatographic retention time, RT) to these data in metabolomics databases (constructed from scientific literature) and RT-MS/MS libraries (constructed with authentic chemical standards). MS/MS data are available in mass spectral libraries, are typically acquired at single collision energies and are transferable between laboratories. In contrast, the data in retention time libraries are collected using specific experimental conditions that are not transferable between laboratories. The research presented will outline our strategy to develop large-scale biologically-relevant retention time and MS/MS libraries. This will include a comparison of three reversed phase stationary phases (C18, biphenyl, PFP) and a HILIC-amide stationary phase for the analysis of water-soluble metabolites. We will also outline the quality control procedures applied to ensure high quality data were included in each RT and MS/MS library.

Methods: Ten-component mixtures of metabolite standards (2115 water soluble metabolites) were analysed applying four stationary phases (C18, biphenyl, PFP and HILIC-amide). All mixtures were analysed 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 Tracefinder and mzVault.

Summary: The highest number of metabolites were detected consistently across the reversed phase compared to the HILIC stationary phases. No single stationary phase retained all metabolites and different stationary phases provided small numbers of metabolites uniquely detected by that phase; this was observed most when comparing reversed phase and HILIC assays. Multiple adducts were detected for the metabolites analysed and all adducts should be included in MS/MS libraries.

1333 B Simultaneous targeted quantification and untargeted discovery of human serum metabolome

PRESENTING AUTHOR: ***Antonis Myridakis, Imperial College London, United Kingdom***

CO-AUTHORS: *Antonis Myridakis, Alexandra Parada-Torres, Sachin Dubei, Elliot Murphy, Meixuan Li, Romina Pacheco-Tapia, Inés Castro-Dionicio, Francesc Puig-Castellví, Olena Potapenko, Hendrik Bartolomaeus, Johannes Holle, Nicola Wilck, Marc-Emmanuel Dumas*

SUB-THEME: Non-targeted and semi-targeted methods

We report a novel metabolomics workflow, based on a hybrid quadrupole-orbitrap-linear ion trap mass spectrometer, enabling concurrent targeted quantification and untargeted profiling of the human plasma metabolome. The method employs a four-injection high-performance liquid chromatography–mass spectrometry (LC–MS) sequence combining reversed-phase (RP) and hydrophilic interaction (HILIC) separations in positive and negative electrospray ionisation modes.

We quantitatively and sensitively target 150 known metabolites via the linear ion trap with calibration curves while simultaneously acquiring high-resolution full-scan (MS^1) and data-dependent MS/MS spectra for untargeted analysis via the orbitrap mass analyser. Full details about sequence structure and appropriate measures to ensure high quality data for both untargeted and targeted acquisitions are presented. All 150 metabolites exhibit linear responses (typically $R^2 > 0.99$) across wide concentration ranges with low limits of detection, enabled by the incorporation of native standards and selected stable-isotope internal standards. The method is validated for precision, accuracy, and matrix effects following standard guidelines.

We are currently applying this pipeline to a kidney transplant patient (KTX) cohort in the frame of IMMEDIATE EU-funded project. While transplantation transforms survival in end-stage renal disease, early signs of subclinical graft dysfunction are often missed by conventional markers. We aim to provide earlier, systems-level insight by capturing dynamic biochemical changes. By evaluating KTX samples, we aim to obtain absolute concentrations for a curated biomarker panel alongside broad untargeted profile. We are integrating these targeted and untargeted layers with clinical endpoints, including graft function, rejection episodes, and immunosuppressant exposure. Ultimately, this analysis will define robust metabolic signatures for early graft injury and outcome prediction, helping to discover and validate markers associated with rejection risk and patient response to immunosuppression.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1334 A Real-Time similarity searching of the mzCloud library for guided unknown characterization of roasted tea metabolites and lipids

PRESENTING AUTHOR: ***Brandon Bills, Thermo Fisher Scientific, United States***

CO-AUTHORS: *Sunandini Yedla, Rahul Deshpande, Bashar Amer, Rafael Melani, Susan Bird*

SUB-THEME: Non-targeted and semi-targeted methods

Metabolomics relies on spectral library matching to convert mass spectrometry signals into chemical identities. However, biological samples contain far more unknown features than compounds with direct library matches. Characterization of unknowns often depends on static acquisition parameters selected prior to analysis, which may limit structural annotation. Real-Time Library Search (RTLS) enables dynamic ddMSn by adjusting parameters using real-time library comparisons. Similarity experiments identify the closest matching compound and directs ddMS3 scans toward matching fragments (to confirm known substructures) and non-matching fragments (for unknown structural elements), improving structural characterization. Historically similarity experiments needed small libraries to prevent lengthy processing times. In this work a large library (offline mzCloud) is utilized to perform similarity searching on tea.

Tea samples were prepared and analyzed using a UHPLC system with a Hypersil C18 column. Samples were analyzed on an Orbitrap IQ-X Tribrid mass spectrometer and a modified Orbitrap Tribrid system. RTLS with fragment ion indexing enabled similarity searching against the offline mzCloud library (>21,000 compounds). MS2 spectra were compared in real time using reverse cosine scoring, triggering ddMS3 on matched and unmatched fragments. Data were processed in Freestyle and Compound Discoverer™.

RTLS similarity searching using offline mzCloud was completed without exceeding allocated search time or slowing acquisition by using fragment ion indexing (scoring only candidates with 4+ matched fragments). When matches were found, MSn data confirmed substructures and supported scoring. For similarity-only matches, MSn data assessed substructure likelihood, and candidates from ChemSpider and MassList were ranked using FISh scoring based on MS2 and MS3 fragment explanation. The modified Orbitrap Tribrid system demonstrated faster and more sensitive ddMS3 acquisition than Orbitrap IQ-X leading to more scans and higher quality spectra. Guiding ddMS3 based on closest matches enabled more relevant data collection supporting substructure confirmation.

1336 A Enhanced metabolite identification and quantitation using continuously scanning Q1 data-independent acquisition

PRESENTING AUTHOR: ***Rebekah Sayers, Sciex UK, United Kingdom***

CO-AUTHORS: *Paul RS Baker, Robert Proos, Cagakan Ozbalci, Max Hecht, Antonella Chiapparino, Thusi Rupasinga, Rebekah Sayers, Jason Causon*

SUB-THEME: Non-targeted and semi-targeted methods

Data-independent acquisition (DIA) workflows are increasingly applied in small-molecule metabolomics because they enable simultaneous qualitative and quantitative measurements. However, conventional fixed-window DIA suffers from limited precursor specificity due to co-isolation of multiple ions, leading to complex fragment spectra and reduced confidence in metabolite annotation. Here, we evaluate a Zenotrap-enabled scanning DIA (ZT Scan DIA) workflow that introduces a continuously scanning Q1 isolation window, providing an additional precursor-specific dimension to DIA data. We further assess a narrow-window implementation designed to enhance specificity for isomeric and closely related metabolites.

NIST SRM 1950 human plasma was protein-precipitated, and the supernatants were analyzed by high-resolution mass spectrometry coupled to an Exion HPLC system. Chromatographic separation was performed using a Waters ACQUITY Premier CSH Phenyl-Hexyl column (2.1 × 100 mm, 1.7 µm) with a 19-minute gradient of 0.1% formic acid in water and methanol. Data were acquired using data-dependent acquisition (DDA), standard ZT Scan DIA. The continuously scanning Q1 isolation was combined with Zenotrap pulsing to maximize MS/MS duty cycle and fragmentation sensitivity. Performance was evaluated based on precursor specificity, spectral complexity, annotation confidence, and metabolome coverage.

ZT Scan DIA improved precursor-to-fragment ion assignment compared with DDA and conventional fixed-window DIA by leveraging the continuously scanned Q1 dimension to facilitate data deconvolution. This resulted in reduced spectral complexity and increased confidence in metabolite annotations. In NIST 1950 plasma, ZT Scan DIA enabled improved differentiation of isobaric and isomeric metabolites and minimized precursor co-isolation without compromising MS/MS data quality or metabolome coverage.

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

Continuously scanning Q1 DIA improves specificity and confidence in untargeted metabolomics by enabling more accurate precursor-fragment associations. Narrow-window ZT Scan DIA further enhances discrimination of closely related metabolites while maintaining quantitative performance. These results demonstrate the suitability of scanning DIA workflows for high-confidence, quantitative small-molecule metabolomics applications.

1337 B Application of Zeno SWATH DIA in multi-tissue metabolomic profiling

PRESENTING AUTHOR: ***Thusitha Rupasinghe, SCIEX, Australia***

CO-AUTHORS: *Qing Zhao, Guo Shou Teo, Chenyuan Huang, Jiong-Wei Wang, Thusitha Rupasinghe, Paul Baker, Markus R. Wenk, Hyungwon Choi*

SUB-THEME: Novel approaches in lipidomics

Untargeted metabolomics has emerged as a method of choice in discovery-driven and hypothesis-generating research. Using a HRMS instrument, untargeted analysis can be performed in either data-dependent acquisition (DDA, as information-dependent acquisition, or IDA) or data-independent acquisition (DIA, or SWATH-MS). Although IDA remains the most widely used approach in the metabolomics field, conventional IDA workflows suffer from inherent limitations due to their stochastic nature in MS/MS spectra acquisition, biased towards abundant precursor ions. In contrast, SWATH-MS acquisition offers a truly unbiased MS/MS spectral collection, enhancing the MS2-level detection of low-abundance metabolites that are otherwise missed in IDA. In this study, we describe a scalable quantitative metabolomics workflow based on sequential application of IDA and SWATH-MS acquisition using the ZenoTOF 7600 system.

Mice were subjected to left anterior descending artery occlusion followed by reperfusion. In total, twelve tissues were collected at four time points post-surgery, including the heart, liver, lung, spleen, kidney, intestine, colon, brain, white adipose tissue (WAT), brown adipose tissue (BAT), skeletal muscle, and plasma. Tissue and plasma samples were extracted using a biphasic methyl tert-butyl ether (MTBE)/methanol (MeOH)/H_2O system to separate organic and aqueous fractions (1).

Metabolic changes in the infarct region of the left ventricle are the primary focus of the myocardial infarction mouse model. For downstream analysis, differentially changed metabolites were identified based on the highest-quality quantifiers. To further validate our biological findings, a list of significantly altered metabolites was examined by mining all high-quality MS1- and MS2-level quantifiers from the Zeno SWATH DIA data and up to three MS2-level quantifiers that passed our filtering pipeline were incorporated into the visualization, alongside their corresponding MS1-level features on the left. A high degree of concordance was observed between MS1- and MS2-level peak features, demonstrating the capability of Zeno SWATH to provide MS2-level quantification with higher confidence.

1339 B MetaboHUB (French national infrastructure in metabolomics and fluxomics for life sciences), towards next generation metabolomics and fluxomics: from population to single cells

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

CO-AUTHORS: *MetaboHUB consortium*

SUB-THEME: Other

Since 2013, French National Infrastructure MetaboHUB (MTH) funded by the French Ministry of Research 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, (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 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.

1340 A Toward Quantitative MSI in Eucalyptus Leaf Tissues: Impact of Matrix Selection on the Ionization of Defense-Related Metabolites

PRESENTING AUTHOR: ***Catalina Bravo Sauterel, Universidad de Concepción, Chile***

CO-AUTHORS: *Javiera Nahuelcura, Ignacio Salinas, Andy J. Pérez*

SUB-THEME: Quantitation in metabolomics

Mass spectrometry imaging (MSI) enables spatially resolved analysis of plant metabolites directly in tissue sections. In Eucalyptus species, extending MSI toward quantitative applications (qMSI) is relevant for elucidating the physiological roles of metabolites involved in abiotic and biotic defense. However, qMSI in plant tissues remains analytically challenging due to matrix effects, chemical heterogeneity, and variability in ionization efficiency among metabolite classes.

Here, we evaluated the impact of matrix selection on ionization performance as a critical step toward qMSI implementation in Eucalyptus leaf tissues. Commonly used MALDI matrices—2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (SA), 1,5-diaminonaphthalene (1,5-DAN), and 3,4-dimethoxycinnamic acid (DMCA), with trifluoroacetic acid (TFA) as an additive—were systematically assessed using MALDI-MS. Two ecologically relevant metabolites were analyzed over a concentration range of 1–100 pmol·μL^{-1}: trans-polydatin, associated with photoprotection and oxidative stress responses, and penta-O-galloyl-β-D-glucose, a hydrolyzable tannin involved in defense against pathogens and herbivorous insects.

For trans-polydatin, CHCA, DHB, and 1,5-DAN produced the highest signal intensities, with DHB providing the most favorable compromise between sensitivity and minimal matrix-derived interference. Its fragment ions, m/z 228 and m/z 229, were detected more frequently than the intact ion, m/z 391. In contrast, the adducts [M+Na]+ and [M+K]+ of penta-O-galloyl-β-D-glucose showed optimal ionization with CHCA, followed by DHB, both with low background interference. These results demonstrate that ionization efficiency strongly depends on matrix chemistry and analyte structure.

Our findings highlight the need for compound-specific optimization for quantitative MSI in Eucalyptus leaf tissues. In this context, tailored or combined matrix approaches may improve sensitivity and reproducibility for metabolites with different physiological functions.

Overall, this study provides a methodological foundation for implementing qMSI in Eucalyptus leaves aimed at resolving the spatial accumulation of metabolites. Future work will extend these evaluations to on-tissue imaging conditions to assess spatial performance and quantitative reliability.

1341 B Comprehensive Quantitative Metabolomics of 2000+ Metabolites in Human Biofluids

PRESENTING AUTHOR: ***David Wishart, Alberta University, Canada***

CO-AUTHORS: *Jiamin Zheng, Lun Zhang, Rupasri Mandal*

SUB-THEME: Quantitation in metabolomics

Metabolomics is reaching an inflection point. Either it can continue relying on traditional, manually intensive non-targeted workflows and remain primarily a niche research discipline, or it can evolve into a truly quantitative, automated, and scalable analytical science capable of driving the next generation of clinical, pharmaceutical, environmental, and forensic applications. While non-targeted metabolomics enables discovery-driven qualitative profiling of up to 1,000 metabolites, targeted metabolomics typically focuses on the precise quantification of smaller panels of 100–200 predefined metabolites for biomarker discovery and clinical diagnostics. Bridging the gap between breadth and quantification remains one of the major challenges in the field. To address this need, we developed a comprehensive LC-MS/MS-based targeted metabolomics platform capable of identifying and quantifying more than 2,000 metabolites, including up to 1,780 individual metabolites and approximately 950 metabolite sums and ratios. The assay combines direct injection mass spectrometry with reverse-phase LC-MS/MS workflows and integrates derivatization, extraction, and selective detection using multiple reaction monitoring (MRM) to maximize analytical sensitivity, reproducibility, and metabolome coverage. The platform requires only 40 μL of sample and is fully adapted to a 96-well plate format, enabling rapid, high-throughput analysis suitable for large-scale studies. The method supports quantitative profiling across 39 chemical classes and has been validated in serum, plasma, urine, and fecal samples. Broad metabolite coverage includes amino acids, lipids, bile acids, organic acids, nucleotides, sugars,

biogenic amines and microbiome-derived metabolites. The assay has already been successfully applied in biomarker discovery and translational research studies, demonstrating its utility for systems-level biochemical profiling and precision health applications. We believe this fast, scalable, and fully automated quantitative metabolomics workflow represents an important step toward making metabolomics a routine and foundational technology for next-generation clinical, pharmaceutical, environmental, and forensic chemistry applications.

1342 A Stable Isotope Labeled Reference Metabolome - Development for Enhanced Single-Cell Metabolomics and Machine Learning-Driven Data Reconstruction

PRESENTING AUTHOR: ***Fiona Tilghman, Medical University Vienna, Austria***

CO-AUTHORS: *Boryana Petrova, Lőrinc Telkes, Arzu Tuğçe Güler, Jeannette Riley Brook, Naama Kanarek*

SUB-THEME: Single-cell metabolomics and lipidomics

Metabolomics captures the downstream effects of gene expression, post-translational regulation and metabolic control, making it a powerful tool to understand developmental and disease states. Extending these insights to single cell (sc) level is essential as cell populations often exhibit substantial metabolomic heterogeneity. This is masked in bulk measurements, where averaging reduces technical noise to some extent but also conceals true cell- to-cell variability. Increasing resolution to sc level also exacerbates the technical challenges inherent to LC-MS-based metabolomics, including instrument noise, matrix effects (ion suppression/ enhancement) and batch effects. Compared to the other omics fields, metabolomics lacks a universal, sequence-like reference system used for unambiguous identification and statistical validation. Key challenges therefore include: (1) assigning detected signals to specific metabolite identities and (2) converting these signals into reliable and comparable quantities across experiments. To address these limitations, we propose the use of a quantified, human reference metabolome as a standardized framework. This reference consists of fully isotopically labeled metabolites that co-elute with endogenous compounds while appearing at distinct masses. As a global internal standard, it enables normalization for not only for technical variability but also in the full matrix, enabling improved metabolite identification, quantification and comparability across conditions.

1344 A High-throughput Single-cell Multi-OMICS Using Standard-flow LC and a Multi-reflecting TOF MS

PRESENTING AUTHOR: ***David Heywood, Waters Corporation, United Kingdom***

CO-AUTHORS: *Qianying Xu, Scarlet Ferrinho, Frederica Orsengio, Robert S Plumb, Lee Gethings, Olivier Cexus, Matthew Spick, Paul Townsend, Melanie Bailey, Clare Mills*

SUB-THEME: Single-cell metabolomics and lipidomics

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

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

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

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

GROUP A: Even Numbers | Sunday and Monday
GROUP B: Odd Numbers | Tuesday and Wednesday

1345 B Isoniazid-based derivatization of carboxylic acids for enhanced LC-MS/MS performance in targeted metabolomics

PRESENTING AUTHOR: ***Guilherme Garcia Rafael, Universidade Estadual de Campinas (Unicamp), Brazil***

CO-AUTHORS: *Carla Beatriz Grespan Bottoli*

SUB-THEME: Targeted methods

Carboxylic acids are key intermediates in central metabolic pathways, including energy production and amino acid metabolism, making them important targets in clinical metabolomics. However, their determination by LC–MS/MS remains challenging due to high polarity, which limits retention under reversed-phase liquid chromatography (RPLC), resulting in early elution and poor resolution. Although hydrophilic interaction liquid chromatography (HILIC) can improve retention, it often presents limited robustness, strong dependence on equilibration conditions, and matrix effects associated with high organic content, compromising reproducibility and quantitative performance. In addition, low ionization efficiency in electrospray ionization (ESI) reduces detection sensitivity. This study describes the systematic optimization of an isoniazid-based derivatization strategy using N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) to enhance the detection of ten carboxylic acids, including malic, lactic, succinic, pyruvic, pyroglutamic, and kynurenic acids, as well as L-arginine, tryptophan, 2,5-dihydroxybenzoic acid, and γ-aminobutyric acid (GABA). Derivatization increases analyte hydrophobicity, improving retention in RPLC, while incorporation of a basic hydrazine moiety enhances ionization efficiency in positive ESI mode. Reaction conditions were optimized using a Design of Experiments approach. A fractional factorial design (2^{6-2}, resolution IV) screened six variables (temperature, time, pH, and concentrations of isoniazid, EDC, and HOBt), followed by a rotational Central Composite Design to define optimal conditions (54 °C, 55 min, 122 mM EDC, 150 mM isoniazid). The optimized protocol significantly improved signal intensity, chromatographic resolution, and signal-to-noise ratio, providing a robust platform for sensitive targeted analysis of carboxylic acids in complex biological matrices.

Acknowledgements: INCT-LK (FAPESP 2025/26623-1; CNPq 408338/2024-5), CNPq 309256/2025-9, CAPES 88887.990353/2024-00.

1346 A Mixed-Mode LC-MS/MS Method for Broad Nucleotide and Nucleoside Profiling

PRESENTING AUTHOR: ***Mohamad Saoud, Heidelberg University, Germany***

CO-AUTHORS: *Fu Xu, Michaela Frye, Stephanie Rössler, Rüdiger Hell, Gernot Poschet*

SUB-THEME: Targeted methods

Nucleotides and nucleosides are essential metabolites involved in energy metabolism, nucleic acid synthesis, genome integrity, and RNA modification pathways. Their comprehensive LC-MS/MS analysis is challenging due to high polarity, structural similarity, and poor retention on conventional reversed-phase columns. Here, we developed a rapid targeted LC-MS/MS method for the detection and quantification of 62 nucleotide- and nucleoside-related metabolites using an Atlantis Premier BEH C18 AX mixed mode column.

Chromatographic separation was achieved within 6 minutes analytical window using ammonium acetate-based aqueous and acetonitrile mobile phases at alkaline pH. The mixed-mode stationary phase combines reversed phase and weak anion exchange interactions, improving retention of phosphorylated metabolites while maintaining compatibility with MS/MS detection. Multiple reaction monitoring was performed on a QTRAP 6500+ mass spectrometer.

The method covers nucleotide triphosphates, diphosphates and monophosphates, deoxynucleotides, canonical and modified nucleosides, oxidized nucleotide species, nucleotide sugars, and DNA damage associated metabolites. Its applicability was evaluated across different biological matrices, including cell pellets, mouse liver and kidney tissue, serum, and urine.

Overall, this method provides a fast, robust, and biologically informative platform for targeted nucleotide metabolomics across diverse sample types, enabling investigation of nucleotide pool composition, purine and pyrimidine metabolism, oxidative nucleotide damage, RNA modification-related metabolites, and alkylation-associated damage markers.