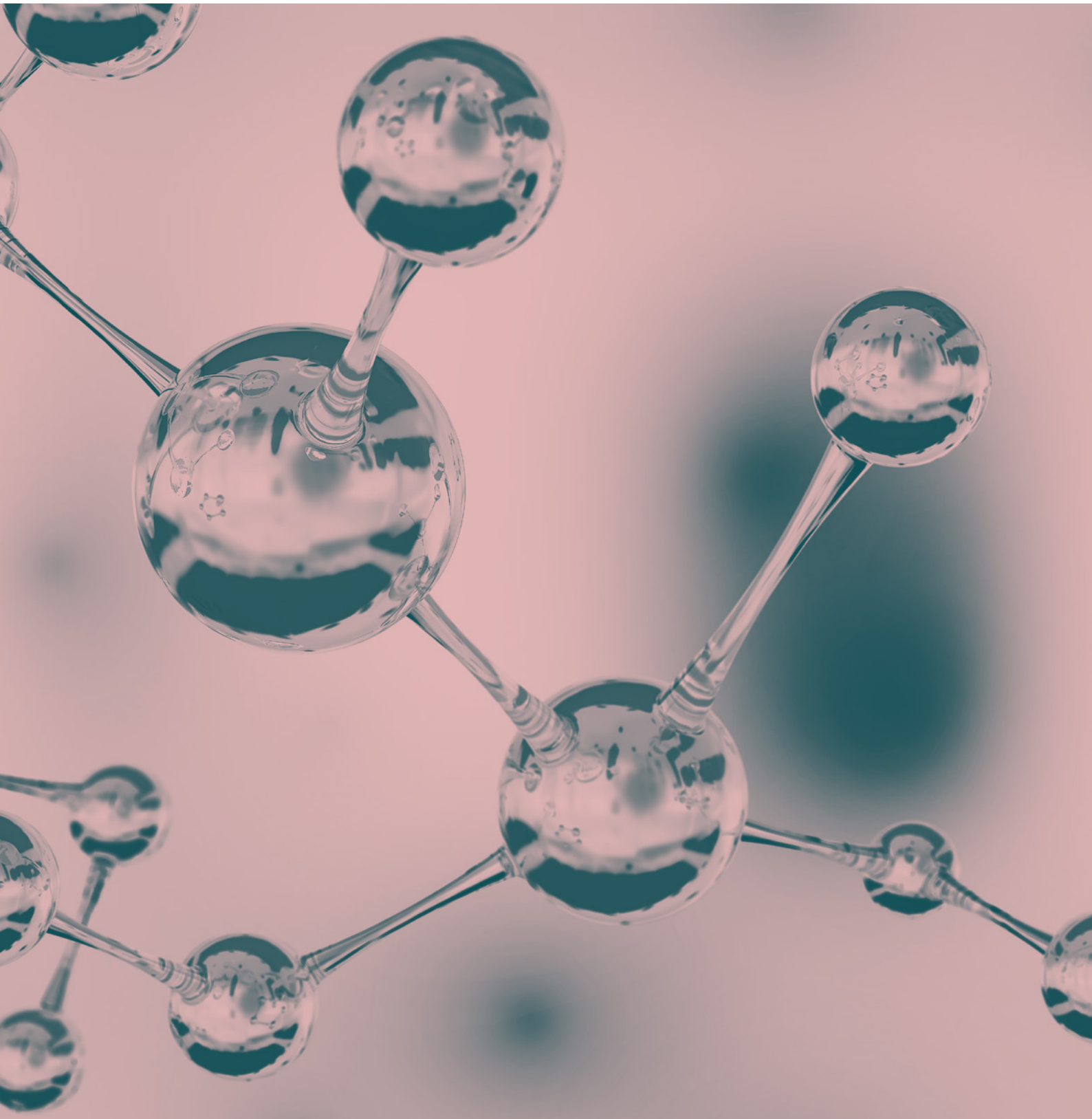
IECM 2025 Conference

The 4th International Electronic Conference on Metabolomics

13–15 October 2025 | Online



Program and Abstract Book

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The 4th International Electronic Conference on Metabolomics

13–15 October 2025 | Online

MDPI

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Prof. Dr. Chi Chen

Session Chairs

Dr. Ala F. Nassar
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Table of Contents

Welcome from the Chairs

Dear Colleagues,

We are delighted to welcome you to the **4th International Electronic Conference on Metabolomics (IECM2025)**, an event set to bring together leading researchers, innovators, and practitioners in the rapidly evolving field of metabolomics. This returning conference builds on the resounding success of our first 3 conferences in 2016, 2017, and 2018 and promises to provide an exceptional platform for intellectual exchange, collaboration, and exploration of cutting-edge advancements.

Metabolomics offers unparalleled insight into metabolic homeostasis and dysfunction within biological systems since metabolites (the metabolome) often more directly reflects physiological status than genes (the genome) or proteins (the proteome). As an indispensable tool in systems biology, metabolomics enables both comprehensive profiling of the metabolome and precise characterisation of functional metabolites, driving innovation across diverse domains in science and medicine.

Organized and proudly sponsored by *Metabolites* (ISSN 2218-1989), a peer-reviewed journal dedicated to advancing knowledge in this field, this conference will feature **six dynamic sessions** exploring diverse aspects of metabolomics:

- **Clinical Metabolomics: Unlocking Insights for Endocrinological Disorders;**
- **Pioneering Technological Frontiers in Metabolomics;**
- **Breaking Barriers with Advanced Data Analysis in Metabolomics;**
- **Precision Nutrition Meets Metabolomics: Transforming Human and Animal Health;**
- **Metabolomics in Drug Discovery: Shaping the Future of Pharmacology;**
- **Microbial Metabolites: Novel Pathways to Health and Therapeutics.**

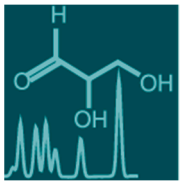
Accepted abstracts will be published in the *Journal Proceedings*, and participants presenting unpublished findings will have the opportunity to submit manuscripts for inclusion in a **Special Issue of Journal *Metabolites***, with a **significant discount on APC charges** after peer review.

On behalf of the dynamic *Metabolites* editorial board and our dedicated editorial team, we warmly invite you to join us for this exciting event. Whether through presenting your latest research or engaging with the diverse community of metabolomics professionals, your participation will enrich the collective discourse and help shape the future of this vibrant field.

We look forward to welcoming you to this virtual conference and celebrating the incredible advancements in metabolomics together.

Dr. Reza Salek
Event Chair
School of Clinical Medicine,
University of Cambridge, UK

Prof. Dr. Chi Chen
Event Chair
Department of Food Science and
Nutrition, University of Minnesota, USA



metabolites
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Metabolites (ISSN 2218-1989) is an international, peer-reviewed open access journal of metabolism and metabolomics. Metabolites publishes original research articles and review articles in aspects of metabolites and metabolism relevant to the fields of metabolomics, metabolic biochemistry, computational and systems biology, biotechnology and medicine. We strongly suggest that all submissions have a substantial focus on the measurement, analysis or biological roles of metabolites, metabolic biomarkers or metabolic pathways.

Metabolites encourages scientists to publish their experimental and theoretical results in as much detail as possible. Therefore, there is no restriction on article length. Sufficient experimental details must be provided to enable the results to be accurately reproduced. Electronic material representing additional figures, materials and methods explanation, or supporting results and evidence can be submitted with the main manuscript as supplementary material.

Impact Factor: 3.7 (2024); 5-Year Impact Factor: 4.1 (2024)

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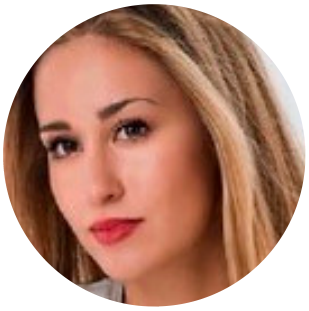

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Prof. Dr. Cecilia Calado
Chemical Engineering Department, ISEL- Instituto Superior de Engenharia de Lisboa, Polytechnic University of Lisbon,
Lisbon, Portugal

Invited Speakers

Dr. Lin Huang

Department of Clinical Laboratory Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China

Dr. Vasu Gautam

Norton Healthcare, Norton Neuroscience Institute, Louisville, USA

Dr. Simone Rochfort

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CAS Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

Program at a Glance

	Day 1	Day 2	Day 3
Morning	**Session 2.** Technological Advances in Metabolomics		**Session 6.** Microbial Metabolites with Novel Functions in Diseases and Health **Session 1.** Clinical Application of Metabolomics with Special Reference to the Endocrinological Arena
Afternoon	**Session 3.** Advanced Metabolomics and Data Analysis Approaches	**Session 5.** Applications of Metabolomics in Pharmacology and New Drug Development	**Session 4.** Metabolomics for Precision Nutrition in Humans and Animals

IECM 2025 Program

13th October 2025 (Monday)

Session 2. Technological Advances in Metabolomics

Time: 9:30 (CEST, Basel) | 3:30 (EDT, New York) | 15:30 (CST Asia, Beijing)

CEST Time	Speaker	Title
9:30–9:35	Welcome from the Event Chair Dr. Reza Salek	
9:35–9:40	Welcome from the Session Chair Dr. Madhu Basetti	
9:40–10:00	**Prof. Dr. Xinyu Liu** Invited Speaker	Comprehensive analysis of gut microbiota related metabolites and their relationship with health
10:00–10:20	**Dr. Lin Huang** Invited Speaker	Metabolomics based on laser desorption/ionization mass spectrometry
10:20–10:40	**Dr. Yuping Cai** Invited Speaker	Discovery of unknown metabolites and metabolic reactions by mass spectrometry-resolved stable-isotope tracing metabolomics
10:40–10:55	**Diogo Serrano** Selected Speaker	Comparative Evaluation of MIR and NIR Spectroscopy for Monitoring Kidney Disease Biomarkers
10:55–11:10	**Aviral Singh** Selected Speaker	Tackling noise and redundancy in LC-MS metabolomics data using an AI-powered analysis workflow
11:10–11:25	**Simone Serrao** Selected Speaker	Integrated Metabolomics and Lipidomics from Dried Blood Spots: Extraction Optimization and Short-Term Evaluation

Session 3. Advanced Metabolomics and Data Analysis Approaches

Time: 14:00 (CEST, Basel) | 8:00 (EDT, New York) | 20:00 (CST Asia, Beijing)

CEST Time	Speaker	Title
14:00–14:05	Welcome from the Session Chair Prof. Hunter N.B. Moseley	
14:05–14:25	**Dr. Xiaoyang Su** Invited Speaker	Assessment of the uncertainty in metabolic flux analysis
14:25–14:45	**Prof. Kim Seongho** Invited Speaker	Advancing Similarity Measures for Library-Based Compound Identification in Metabolomics
14:45–15:00	**Hao Xu** Selected Speaker	LipidIN 2.0: an end-to-end intelligent analysis platform for 4D lipidomics and glycolipidomics
15:00–15:15	**Leonardo Tenori** Selected Speaker	Deriving three one-dimensional NMR spectra from a single spectrum
15:15–15:30	**Inês Correia** Selected Speaker	Kinetics of the serum metabolic fingerprint to predict mortality of critically ill patients
15:30–15:45	**Qingqing Mao** Selected Speaker	ClearAIF: An R-Based Computational Pipeline for DIA Metabolomic Data Processing and Reporting
15:45–16:00	**Erik Huckvale** Selected Speaker	Machine Learning Predicted Pathway Annotations Greatly Improves Pathway Enrichment Analysis of Metabolomics Datasets
16:00–16:10	Flash Poster Presentation (5 mins per person)	**Grace Sun** A New Enzymatic Distance Concept Enables Machine Learning Regression of Metabolite Chemical Representation Features to Distance in Metabolism **Hunter Dlugas** Mass Spectral Matching for Compound Identification in Metabolomics: An Open-Source Python/Shiny Package

14th October 2025 (Tuesday)

Session 5. Applications of Metabolomics in Pharmacology and New Drug Development

Time: 14:00 (CEST, Basel) | 8:00 (EDT, New York) | 20:00 (CST Asia, Beijing)

CEST Time	Speaker	Title
14:00–14:05	Welcome from the Session Chair Dr. Ala F. Nassar	
14:05–14:35	**Prof. Dr. Cecília R.C. Calado** Keynote Speaker	Comparison of omics platforms for biomarkers discovery to predict mortality at an Intensive Care Unit
14:35–14:50	**Alessia Vignoli** Selected Speaker	NMR-based Metabolomic Investigation Of The Effects Of Alzheimer's Molecular Stressors On Neuroblastoma Cells
14:50–15:05	**Emma Rowan** Selected Speaker	Water fleas as "canaries in the coal mine" to monitor pharmaceutical pollution using metabolic perturbations as indices
15:05–15:20	**Rebeca André** Selected Speaker	Omics-Based Insights into the Hypocholesterolemic Effects of Fucus vesiculosus Extract on Intestinal Cells
15:20–15:35	**Charlotte Lemoine** Selected Speaker	HepG2 cells may not be reliable allies for the study of hepatic gluconeogenesis
15:35–15:55	Flash Poster Presentation (5 mins per person)	**Afaf Fizazi** May succination be involved in cardiotoxicity? **Vanessa dos Santos Silva** In Silico Metabolomic Analysis of Saxitoxin and Its Analogs **Muhammad Waqas** Metabolomics-Guided Profiling of Azadirachta indica Reveals Novel Bioactive Signatures for Anticancer Drug Development **Raúl González-Domínguez** Characterization of the bioactive profile of beach-cast seaweeds using a quantitative and large-scale metabolomics approach

15th October 2025 (Wednesday)

Session 6. Microbial Metabolites with Novel Functions in Diseases and Health

Session 1. Clinical Application of Metabolomics with Special Reference to the Endocrinological Arena

Time: 9:30 (CEST, Basel) | 3:30 (EDT, New York) | 15:30 (CST Asia, Beijing)

CEST Time	Speaker	Title
9:30–9:35	Welcome from the Session Chair Dr. Giovanni Ribaudo	
9:35–9:55	**Prof. Gregorio Peron** Invited Speaker	The "TrichEco" project: developing 2.0 biostimulants trough the solid-state fermentation of agrifood byproducts
9:55–10:10	**Alisa Pautova** Selected Speaker	Aromatic Microbial Metabolites and Biomarkers in Cerebrospinal Fluid for the Diagnosis of Secondary Bacterial Meningitis
10:10–10:25	**Ambrin Farizah Babu** Selected Speaker	Fermented and Unfermented Whole-Grain Rye Modulate and Methylated Quaternary Ammonium Compounds Systemically in High-Fat Diet-Fed Mice
10:25–10:40	**Rui Su** Selected Speaker	Profiling developmental dynamics of aromatic amino acid fermentation in infants
10:40–10:50	**Ms. Xue Liang** Metabolites Publisher	Introduction about Metabolites
10:40–10:50	Exploring *Metabolites:* Journal Overview Ms. Xue Liang, Publisher of *Metabolites*	
10:50–10:55	Welcome from the Session Chair Prof. Dr. Giuseppe Paglia	
10:55–11:15	**Dr. Yan Ni** Invited Speaker	Targeted bile acid identification and biomarker discovery in obesity-associated metabolic diseases
11:15–11:30	**Eleonora Bossi** Selected Speaker	Exercise-induced metabolic changes of cardiac rehabilitation after acute myocardial infarction: Insights from dried blood spot profiling
11:30–12:00	**Dr. Yunping Qiu** Keynote Speaker	Longitudinal metabolomics and lipidomic uninfected and HIV treated human plasma signatures over ten years reveal stability of metabolome differences

Session 4. Metabolomics for Precision Nutrition in Humans and Animals

Time: 14:00 (CEST, Basel) | 8:00 (EDT, New York) | 20:00 (CST Asia, Beijing)

CEST Time	Speaker	Title
14:00–14:05	Welcome from the Session Chair Prof. Dr. Chi Chen	
14:05–14:25	**Prof. Rachel Kopec** Invited Speaker	Navigating Nutritional Complexity with Metabolomics Insights
14:25–14:45	**Prof. Tiffany Weir** Invited Speaker	Exploring the Role of the Gut Microbiome in the Bioavailability of Blueberry Polyphenols
14:45–15:05	**Dr. Simone Rochfort** Invited Speaker	Transition cow health, lipidomic and metabolomic insights
15:05–15:25	**Dr. Vasu Gautam** Invited Speaker	Gut Microbial Signatures associated with Triglyceride abnormalities in people with HIV: implications for cardio metabolic Disease
15:25–15:40	**Alba Simon Melchor** Selected Speaker	Impact of Nutrition on Blood Metabolic Biomarkers in Elderly Adults with Type 2 Diabetes: A Metabolomics Approach
15:40–15:55	**Richa Singh** Selected Speaker	Breed-Dependent Metabolic Signatures in Bovine Milk Under Thermal Stress: Insights from Sahiwal, Tharparkar, and Karan Fries Cattle

15:55–16:10	**Monica Trif** Selected Speaker	Metabolomics-Driven Ingredient Selection and Formulation for Targeted Health Outcomes
16:10–16:20	Flash Poster Presentation (5 mins per person)	**Giuseppe Tancredi Patane** Anthocyanin-Enriched Fraction from Callistemon citrinus Modulates Leydig Cell Function: Insights into Cytotoxicity and Metabolic Impact **Dr. Ardila Melendez** Feeding Patterns with Circadian Misalignment and Their Relationship with Lipid and Glycemic Metabolic Responses in Healthy Adults
16:20–16:25	Closing remarks from the Event Chair Prof. Dr. Chi Chen	

Session 1. Clinical Application of Metabolomics with Special Reference to the Endocrinological Arena

sciforum-142102: Longitudinal metabolomics and lipidomic uninfected and HIV treated human plasma signatures over ten years reveal stability of metabolome differences

Yunping Qiu [1,*], Min Cai [1], Jennifer Jao [2], Qibin Qi [3] and Irwin J. Kurland [1]

[1] Department of Medicine, Albert Einstein College of Medicine, New York City, New York, 10461, USA
[2] Division of Infectious Diseases, Department of Pediatrics & Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
[3] Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 10461, Bronx, NY, USA

Metabolomics has been applied for biomarker discovery and disease mechanism understanding. For clinical studies, especially for large cohort collection, it will take a long time. Therefore, the samples will be stored for different time durations. In this study, we investigated 10-HIV infected and 10 age and gender matching non-infected individuals. We applied widely targeted metabolomics screening (WTSMS) and widely targeted lipidomics screening (WTLS) assays comprising a “Chem2000” (~600 potential small molecules and ~1400 lipid species) to analyze yearly collected samples. In this way, we could investigate the potential degradation along 10-year storage, and the stability of metabolome variations in the healthy controls and HIV infected patients.

Plasma samples were extracted with methanol (metabolites) or ethanol (lipids), each spiked with internal standards. Metabolites were separated on an Ace C18-PFP column, while lipids were analyzed on a CSH-C18-PFP column using a Sciex 6500+ Q TRAP. Data was processed with Multiquanta and analyzed in SIMCA-p, and Genedata Expressionist.

There are 240 small metabolites, and 878 lipids were detected with CVs less than 30% in the QC samples from the WTSMS and WTLS assays. Distinct separations were detected constantly between HIV positive and HIV-negative patients at samples collected in different years. Among all these differentially expressed metabolites or lipids, 164 shared compounds were constantly detected, 34 of them were lower, and 130 were higher in HIV patients compared with non-HIV controls. The analyte changes showing stability over the years include down-regulated lysophospholipids, succinate, tryptophan, and up-regulated phospholipids, carnitines, and pantothenic acid.

This study revealed stable metabolites/lipids variations in plasma samples collected from HIV-positive and HIV-negative subjects over 10 year time period, which can be useful in monitoring overall metabolic health.

sciforum-134638: Adverse neonatal outcomes predispose to exacerbated metabolic disturbances in childhood obesity

Lucía Jurado-Sumariva [1,*], Álvaro González-Domínguez [1], Otto Savolainen [2], Rikard Landberg [2] and Raúl González-Domínguez [1]

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[2] Division of Food and Nutrition Science, Chalmers University of Technology, Gothenburg, SE-412 96, Sweden

Introduction: The perinatal period is highly sensitive to obesogenic determinants, being consistently reported that adverse birth outcomes are among the most important early risk factors for childhood obesity. Nevertheless, the molecular mechanisms underpinning this greater predisposition remain to be elucidated.

Methods: To get deeper insights into the influence of neonatal conditions in obesity-related metabolic disturbances occurring later during childhood, we applied high-throughput metabolomics (UHPLC-HRMS) to plasma and erythrocyte samples from a cohort of children diagnosed with obesity, from whom birth metrics (i.e., gestational age, weight, and length at birth) were available from their medical records.

Results: The findings of this study evidenced that adverse neonatal outcomes (i.e., low gestational age, weight, and length at birth) may predispose to an unhealthier metabolic status, as reflected in negative associations with anthropometric parameters (e.g., waist circumference) and biochemical markers of insulin resistance (e.g., HOMA-IR) and inflammation (e.g., CRP, inflammatory indices). This was accompanied by exacerbations in a multitude of central metabolic pathways which play a crucial role in obesity pathophysiology, such as energy metabolism, homeostasis of branched chain and aromatic amino acids, regulation of oxidative stress, and biosynthesis of steroid hormones and bile acids.

Conclusion: Accordingly, we hypothesize that even minor variations in neonatal conditions may provoke deleterious molecular programming mechanisms with strong impact on later metabolic risk.

sciforum-130508: Exercise-induced metabolic changes of cardiac rehabilitation after acute myocardial infarction: Insights from dried blood spot profiling

Eleonora Bossi [1,*], Marta Nobile [1], Lorenzo Ticini [1], Federico Paoletti [1], Simone Serrao [1], Antonio Zaza [2], Lia Crotti [1,3], Gabriella Malfatto [3] and Giuseppe Paglia [1]

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[3] IRCCS, Istituto Auxologico Italiano, Department of Cardiology, Cardiac Rehabilitation Unit, S. Luca Hospital, Piazzale Brescia 20, Milan, Italy

Ischemic heart disease remains the leading cause of cardiovascular-related mortality worldwide. Although the benefits of cardiac rehabilitation (CR) through physical exercise after acute myocardial infarction (AMI) are well known, knowledge of metabolic adaptations remains limited. This study investigates the metabolic response to CR in AMI patients implying untargeted metabolomics and lipidomics analysis on dried blood spots (DBS).

Seventeen male subjects after a first AMI were enrolled in a three-week rehabilitation protocol. DBS samples were collected before and after training in three distinct times– first training, mid-protocol and final training. Untargeted metabolomics and lipidomics analyses were performed using ultra-high performance liquid chromatography coupled with mass spectrometry to assess training-induced changes. Statistical analysis was performed using paired t-test (p0.05), with results visualized in volcano plots.

Progressive training adaptations were observed over time, along with changes in energy metabolism and improved physical performance at the end of the CR protocol. Short-term change in lipid profile was also detected. In particular, phosphatidylserine (PS) levels increased significantly post-first training. PS are known to decrease after myocardial infarction, and previous studies suggest that boosting PS levels (e.g. oral supplementation) could be a potential therapeutic strategy by exerting cardioprotective effects. Our results suggest that training enhances PS biosynthesis through potential activation of phosphatidylserine synthase 1 (PSS1), indicating a potential benefit of CR in cardiac repair. Additionally, CR monitoring showed that blood levels of N-acetyl-L-tyrosine (NAT) increase with rehabilitation time. NAT has been shown to induce mitohormesis, an adaptive mitochondrial stress response, in mice and insects and has potential links to physical training in humans.

Comprehensively, our findings suggest that CR induces changes in both the metabolomic and lipidomic profiles of AMI patients, alongside improvements in physical performance. PS and NAT emerged as promising biomarkers for monitoring CR progress and may contribute to potential benefits on AMI recovery.

Session 2. Technological Advances in Metabolomics

sciforum-145959: Comparative Evaluation of MIR and NIR Spectroscopy for Monitoring Kidney Disease Biomarkers

Diogo Serrano [1,*], Paulo Zoio [1,2], Cecília Calado [2] and Luís Fonseca [1]

[1] iBB – Institute for Bioengineering and Biosciences, i4HB – The Associate Laboratory Institute for Health and Bioeconomy, IST – Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal
[2] ISEL- Instituto Superior de Engenharia de Lisboa, Instituto Politécnico de Lisboa, Lisbon, Portugal

Introduction: Chronic kidney disease (CKD) represents a major global health challenge, often remaining undetected until advanced stages due to limitations of current biomarkers, such as blood creatinine, urea, and albumin. Fourier-transform infrared spectroscopy (FTIR), particularly in the mid-infrared (MIR) and near-infrared (NIR) regions, has emerged as a promising alternative for monitoring biochemical alterations associated with CKD, since it can be performed with a simple drop of blood. This work compares the predictive potential of MIR and NIR spectroscopy to assess serum levels of creatinine, urea, and albumin in simulated CKD conditions. Methods: A total of 54 serum-based solutions were prepared, reflecting different CKD stages (0–5). The MIR and NIR spectra of these solutions were pre-processed with baseline correction, normalization, and Savitzky–Golay filters, followed by development of partial least squares (PLS) regression models to predict the target analyte concentration, and discriminant analysis (DA) models to classify "healthy" versus "diseased" and to differentiate CKD stages. Results: PLS regression models showed strong predictive ability for urea concentration using both MIR (R2 = 0.97, RMSE = 10 mg/dL) and NIR spectra (R2 = 0.91, RMSE = 17 mg/dL). Albumin concentration was more reliably predicted by NIR spectra (R2 = 0.93, RMSE = 0.11 g/dL), while creatinine prediction had the best results obtained using the 3rd Overtone NIR spectral sub-region (R2 = 0.91, RMSE = 0.48 mg/dL). PCA-DA models confirmed the capacity of MIR and NIR to classify samples as "healthy" versus "diseased" with accuracies > 90%, although stage classification remained more challenging. Conclusions: Both MIR and NIR spectroscopy presents a strong potential for an economic, rapid and minimally invasive tools for monitoring kidney function related biomarkers like creatinine, urea and albumin. These findings support the integration of FTIR-based platforms in point-of-care diagnostics for early detection and monitoring of CKD progression.

sciforum-142291: Comparison of omics platforms for biomarkers discovery to predict mortality at an Intensive Care Unit

Tiago A.H. Fonseca [1,2], Cristiana P. Von Rekowski [1,2], Inês Correia [1], David R.C.P. Nunes [1], Mariana Duarte [1], Luís Bento [2,3] and Cecília R.C. Calado[1,4,*]

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Introduction: Serum metabolomics, by representing the pathophysiological state of the organism, have been applicable to biomarker discovery in complex clinical status. Despite the great promises of metabolomics, unfortunately conventional analytical platforms, such as the ones based on ultra-high-performance chromatography associated with high resolution mass spectroscopy (UHPLC-HRMS), present limitations when applied to large scale populations, due to their associated high costs, analytical complexity, laborious and time-consuming nature. The current work compared an UHPLC-HRMS platform with an alternative platform based on FTIR-spectroscopy to acquire the metabolic fingerprint of the system to predict mortality at an intensive care unit (ICU). **Methods:** Predictive models of mortality of 16 patients, from which half died at the ICU, were developed based on serum metabolomics acquired by UHPLC-HRMS and FTIR spectroscopy. Models were developed based on principal component analysis followed by linear discriminant analysis (PCA-LDA) from serum acquired 7 days before the clinical outcome, *i.e.,* deceased or ICU discharge. **Results:** After feature selection, it was possible to develop excellent models based on a set of serum metabolites with 100% accuracy for the validation dataset. It was also possible to develop excellent models, based on the molecular fingerprint obtained by FTIR-spectroscopy, that after optimization of spectral pre-processing and band selection, resulted in a 92% accuracy for the validation dataset. **Conclusions**: The FTIR-spectroscopy platform enabled the development of predictive models as good as the ones developed on data obtained from the UHPLC-HRMS platform, while being applicable in rapid, simple, more economical and high-throughput analysis. All these characteristics are of paramount importance for application at large-scale populations, and consequently on the validity of the predictive models.

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sciforum-145916: FTIR Spectroscopy of Allograft Perfusate Separates DCD and DBD Donors: Early Results

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Background: Rapid, objective phenotyping of donor organs is an unmet need in kidney transplantation, particularly when comparing donation after circulatory death (DCD) with donation after brain death (DBD). We tested whether Fourier-transform infrared (FTIR) spectra of the static cold-storage preservation solution collected from kidney allografts capture discriminative biochemical signatures of donor type.

Methods: Perfusates from Celsior-preserved kidney allografts (n=10; 5 DCD/ 5 DBD) were analyzed in the *Fourier transform infrared (FTIR) spectroscopy* mid-infrared (Amide I and fingerprint regions). After standard quality control and normalization, spectra underwent routine derivative processing. Supervised classifiers were trained and assessed with cross-validation to evaluate discrimination between donor types.

Results: FTIR spectroscopy of perfusate revealed donor-type signatures. Pipelines combining derivatives with non-redundant feature selection consistently improved discrimination. The fingerprint region outperformed Amide I. In cross-validation, models achieved high performance, with *area under the curve* up to 0.84–1.00 and accuracies up to 1.00 within this pilot; the best pipeline reached perfect in-cohort discrimination.

Conclusions: Perfusate FTIR spectroscopy provides a rapid, economic label-free readout that could complement pump parameters and metabolomics during organ perfusion. Given the small, matched pilot and heterogeneous spectral quality, larger, standardized, multi-site studies with rigorous internal validation and assessment of instrument/site transferability are required before clinical deployment.

sciforum-141051: Integrated Metabolomics and Lipidomics from Dried Blood Spots: Extraction Optimization and Short-Term Evaluation

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Dried blood spots (DBS) have recently gained attention as a practical alternative to conventional blood collection, offering advantages such as simplicity, minimal invasiveness, and user-friendliness. Their robustness and ease of handling have expanded their applications in both clinical and research contexts, particularly in mass spectrometry–based metabolomics and lipidomics. In this work, we optimized and evaluated extraction parameters, including solvent composition, extraction workflow, and analyte stability to perform combined metabolomics and lipidomics analyses from a single DBS sample using three commercial microsampling devices: Capitainer, Whatman, and Telimmune. Five extraction solutions, selected among those commonly used for metabolite and lipid extraction, were tested to maximize recovery of both polar metabolites and lipids, and short-term stability of analytes at ambient temperature was assessed for up to five days. Among the solvents investigated, pure methanol emerged as the optimal single-solvent condition, providing the best compromise for co-extraction of both metabolite classes. In addition, a two-step protocol combining methanol followed by aqueous extraction significantly improved polar metabolite recovery and reproducibility, particularly in Capitainer and Whatman devices. Stability studies showed that Capitainer preserved metabolite and lipid integrity for up to six days at room temperature, whereas significant variations appeared after three days in Whatman and Telimmune devices, indicating the necessity of cold-chain storage for these platforms. In conclusion, this study identifies methanol-based extraction as the most effective approach for integrated metabolomics and lipidomics analyses from a single spot, and highlights the importance of considering device stability to ensure reliable and reproducible multi-omics analyses in clinical and translational studies.

sciforum-146558: Saliva and Urine Metabolome in Kidney Transplant Recipients

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The aim of this study was to evaluate the biofluid metabolome of kidney transplant recipients and their possible association with periodontitis. This was a cross-sectional, analytical, and descriptive study involving 61 patients, who visited the Dental School of Paulista University. Participants were arranged in four groups: GT: kidney transplant recipients without periodontal disease (n = 11); GTDP: kidney transplant recipients with periodontal disease (n = 12); CG: normal renal function without periodontal disease (n = 19); and GCDP: normal renal function with periodontal disease (n = 19). Saliva and urine samples were collected for further metabolomic analysis. The gas chromatography/mass spectrometry technique was used to evaluate the metabolites. The data obtained were analyzed using principal component analysis (PCA) to determine the main metabolites in the samples; Permanova, that statistically compared the four groups using all metabolites simultaneously; Jaccard similarity index was used to directly compare the similarity of the groups in terms of the presence of metabolites; and a Venn diagram which all salivary and urine metabolites were plotted on to evaluate the distribution of metabolites between groups. Statistica 12, SPSS 20, Past 3.0, Origin Pro 2019, and MetaboAnalys 5.0 statistical programs were used to obtain the results. The study identified 23 and 30 metabolites common to the GT and GTDP groups when the saliva and urine samples were analyzed, respectively; the benzene class and its derivatives were overexpressed. The most important metabolite for determining the pattern of PCA performed in saliva samples was ornithine and that in urine samples was palmitoleic acid. There was no significant difference between the salivary and urinary metabolites when compared among the four groups; however, it was possible to differentiate the specific metabolites for each group and the relationships between them. Metabolites shared in salivary samples of patients with periodontal disease are related to the fermentation process and bacterial metabolism, suggesting that they are potential indicators for periodontal injury.

sciforum-145961: Tackling noise and redundancy in LC-MS metabolomics data using an AI-powered analysis workflow

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Analysis of large-scale data acquired from untargeted high-resolution LC-MS metabolomics experiments requires time and significant manual effort due to the large data volume, high noise levels, and data redundancy. Most existing tools depend on user-defined signal-to-noise thresholds and often lack intuitive graphical interfaces, making them less accessible to users without programming expertise. To address these challenges, we developed a novel, end-to-end LC-MS data analysis algorithm with an AI-powered preprocessing pipeline and a user-friendly interface. Our approach integrates data quality checks, automated peak detection and alignment, and statistical analysis, while maintaining platform-agnostic compatibility and supporting large datasets.

The algorithm uses a one-dimensional convolutional neural network (CNN) classifier to distinguish true peaks from noise, significantly reducing false positives. A separate encoder-decoder CNN architecture accurately segments peaks and computes the area under the curve. These models were trained on over 10,000 synthetic and 5,000 human-annotated experimental data points, achieving over 97% classification accuracy and a mean IOU of 0.92 for peak boundary segmentation. The method achieves over 80% data reduction, lowering computation costs and manual workload.

When benchmarked against available open source data analysis tools such as MZmine, XCMS, and MS-Dial, our algorithm demonstrated superior quantification accuracy and reproducibility. Although it detects fewer total features, these included a higher number of true features — 929 compared to 857 (MS-Dial) and 866 (XCMS), with lower coefficient of variation across replicates. Overall, the AI-powered algorithm combines deep learning with scalable cloud computing to offer a powerful, accessible solution for robust automated analysis of metabolomics data. Our algorithm can facilitate faster identification of hidden patterns, biomarkers, and pathways, as well as data mining of public domain metabolomics datasets.

The algorithm can be freely accessed at https://msone.claritybiosystems.com and is capable of analyzing DDA, DIA and ion-mobility data

Session 3. Advanced Metabolomics and Data Analysis Approaches

sciforum-145813: *ClearAIF*: An R-Based Computational Pipeline for DIA Metabolomic Data Processing and Reporting

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Background:

In a metabolomics core facility setting, supporting a diverse user base requires efficient and comprehensive MS/MS data acquisition while minimizing repeated sample injections. Data-Independent Acquisition (DIA, or All-ion fragmentation, AIF) provides an effective solution by capturing MS/MS data for all precursor ions in a single run, making it well-suited for untargeted metabolomics. While several AIF processing tools are available, opportunities remain to improve the accuracy of the MS/MS spectral deconvolution, the transparency in pseudospectrum reporting, and better integration with community-driven software ecosystems such as *TidyMass*. To address these needs, we developed a new computational pipeline, *ClearAIF*, to enhance the transparency, interpretability, and usability of DIA/AIF-derived data.

Methods:

We developed *ClearAIF*, an R-based, open-source, and modular computational pipeline to reconstruct high-quality pseudo-MS/MS spectra from DIA/AIF data. A distinctive feature of the workflow is a cascade spectral purity plot that transparently reports fragment assignment, which offers an intuitive visualization of precursor–fragment relationships, enabling users to directly assess fragment reliability. The pipeline is also compatible with the *MetID* package within *TidyMass* for metabolite annotation, allowing efficient integration into existing annotation workflows.

Results and Impact:

To maximize spectral information in DIA/AIF datasets, we optimized data acquisition using entropy-optimized collision energies. Using cancer cell lysates as test cases, this approach delivered broader metabolome coverage and reliable reconstruction of high-quality pseudo-MS/MS spectra. When applied to DDA datasets, *ClearAIF* improved the quality of MS/MS spectra, supporting highly credible metabolite annotation and robust library development. By improving the transparency and interpretability of AIF-derived spectra, *ClearAIF* facilitates marker validation and advances efforts in metabolite discovery, offering practical benefits for core facility workflows while providing a flexible and accessible solution for the broader metabolomics community.

sciforum-138623: A New Enzymatic Distance Concept Enables Machine Learning Regression of Metabolite Chemical Representation Features to Distance in Metabolism

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Understanding and reconstructing metabolic pathways can help identify biomarkers and metabolic changes associated with systemic diseases, such as cancer and cardiovascular diseases. However, in most metabolomics datasets, more than half of the experimentally detected metabolites can be unannotated, making their pathway-specific involvement largely unknown. This study suggests that a distance concept can help place an unannotated metabolite into specific parts of a metabolic network by finding its "distance" in relation to known metabolites. We introduce the concept of "enzymatic distance" to quantify the separation of any two metabolites in metabolism in terms of the number of reaction centers involved and the flow of mass between them. Enzymatic distance was scored by two metrics: counts of shared atom mappings and reaction center atoms averaged over the reaction paths separating two compounds. Univariate and multivariate multi-layered perceptron (MLP) regression models were trained and evaluated over 100 epochs and 30 cross-validation iterations to predict enzymatic distance metrics between target metabolites given only their chemical structural features. While atom mappings were robustly predicted by univariate (R2=0.9608 ± 0.0031(sd)) and multivariate (R2=0.9576 ± 0.0028(sd)) regression, reaction centers were predicted significantly less accurately by univariate (R2=0.8761 ± 0.0065(sd)) and multivariate (R2=0.8593 ± 0.0071(sd)) regression. The univariate regression model predicted both metrics more accurately than the multivariate regression (p-value0.001), as predicting both metrics separately renders higher prediction accuracy. Within metabolite compounds, chemical substructures containing oxygen and nitrogen were found to play a significant role in defining enzymatic distance in metabolism, with more complex features enhancing the accuracy of atom mapping predictions. By quantifying and predicting relationships between known and unknown metabolites, unannotated metabolites in the human body can be better interpreted, aiding the detection and interpretation of metabolic diseases.

sciforum-140338: Information-Content-Informed Kendall-tau Correlation Methodology: Interpreting Missing Values as Useful Information

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Introduction: Almost all correlation measures currently available are unable to directly handle missing values.Either missing values are ignored, or they are imputed and used in the calculation of the correlation coefficient. In either case, the correlation values are impacted based on a perspective that the missing data represents no useful information. Missing values occur in real metabolomics data sets for a variety of reasons, but frequently due to specific analytes falling below the limit-of-detection of the analytical instrumentation (left-censored values). These missing data are not missing at random, but represent potentially useful information by virtue of their "missingness" at one end of the data distribution.

Methods: To use this left-censorship missingness in correlation, we propose the information-content-informed Kendall-tau (ICI-Kt) methodology. We show how left-censored missing values can be included within the definition of the Kendall-tau correlation coefficient, and how that inclusion leads to an interpretation of information being added to the correlation. We also implement calculations for additional measures of theoretical maxima and pairwise completeness that provied additional interprative perspectives to the methodology.

To test and validate the ICI-Kt methodology, we used a variety of simulated datasets with different types and amounts of missingness, as well as real metabolomics experiments from Metabolomics Workbench (MW). These include (but are not limited to) lipidomics of non-small cell lung carcinomia, and small molecule metabolomics of rat stamina (MW ID PR000016); comparing ICI-Kt to Kendall-tau, Spearman and Pearson correlations in each case.

Results: Tests on both simulated and real metabolomics datasets demonstrate that ICI-Kt performs better than Kendall-tau, Spearman, or Pearson correlation methods when left-censored values are present, both in detecting outlier samples and constructing metabolite-metabolite networks.

We provide explicitly parallel implementations in both R and Python packages that enable fast calculations when applied to large datasets.

sciforum-140179: Machine Learning Predicted Pathway Annotations Greatly Improves Pathway Enrichment Analysis of Metabolomics Datasets

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Introduction: In metabolomics data analysis, annotation enrichment analysis (AEA) is a method for detecting over-representation (enrichment) of annotations for metabolite features that have appreciable covariance across an analytical dataset. Enriched annotations, especially enriched pathway annotations, provide biological insight and interpretation of the experimental groups represented in the dataset. AEA applied only to pathway annotations is often called pathway enrichment analysis (PEA). Several knowledgebases have pathway annotations associated with metabolites, including the Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and MetaCyc. However, these three knowledgebases combined have pathway annotations for less than 19,000 metabolites, which is a small fraction of detectable metabolites. For most metabolomics datasets, only 30% to 40% of the detected metabolites have pathway annotations, greatly limiting PEA in detecting enriched pathways. **Methods:** In this work, we have developed an extreme classification model using a dataset constructed from KEGG, Reactome, and MetaCyc combined that can predict metabolic pathways based on metabolite chemical structure. **Results:** Our model scored a Matthews correlation coefficient (MCC) of 0.9036 ± 0.0033. Using predicted pathway annotations, we demonstrate a sizable improvement in PEA results for over 150 experimental datasets downloaded from Metabolomics Workbench. **Conclusions:** Based on our results, we can confirm that significant improvement can be made to PEA for metabolomics experimental datasets using predicted pathways. The improvement to PEA demonstrates the accuracy of the predictions of our machine learning model.

sciforum-141988: Deriving three one-dimensional NMR spectra from a single spectrum

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Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful tool for analyzing complex mixtures due to its ability to manage matrix complexity, provide detailed molecular insights, and preserve sample integrity. Various NMR experiments, such as NOESY, CPMG, diffusion-edited, and J-resolved spectroscopy (JRES), offer complementary insights into biofluids like serum and plasma. For instance, CPMG selectively detects small molecules, diffusion-edited emphasizes signals from macromolecules, and NOESY captures both; while JRES is particularly useful for signal assignment. However, acquiring multiple NMR spectra can be resource-intensive and time-consuming, especially for high-throughput studies. Here we present a simple and efficient strategy to computationally derive CPMG, diffusion-edited, and projected JRES (pJRES) spectra from a single NOESY acquisition using Partial Least Squares (PLS) regression. Serum samples were used as a case study. We used serum NMR data from a total of 1842 individuals enrolled from 18 recruitment centers. The ^{1}H-NMR spectra for all samples were recorded using a Bruker 600 MHz spectrometer operating at 600.13 MHz. The dataset, comprising serum spectra of samples collected in 17 different recruitment centers, was divided into training (80%) and validation (20%) sets. Furthermore, the spectra of 232 samples from one independent recruitment center were used as the independent test set. Predictive models were created applying PLS regression with 1D NOESY spectra used as independent variables for the prediction of CPMG, Diffusion-edited, and pJRES spectra, these latter used as dependent variables. Experimental and predicted spectra were compared, in regions with signal intensities at least three times above the noise level. Evaluation metrics included the median relative error (MRE%), root mean square error (RMSE), coefficient of determination (R^2), and ratio of performance to deviation (RPD). In the independent test set, MRE% values of 6%, 4%, and 13% were achieved for predicted CPMG, diffusion-edited, and pJRES spectra, respectively.

sciforum-145932: Integrating LC-MS and Network-Based Analysis for Untargeted Fecal Metabolomics in Microbiome Research

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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. To investigate the metabolic mechanisms underlying this process, we conducted untargeted LC-MS metabolomics on faecal samples from antibiotic-treated mice.

Faecal metabolite profiling was performed using a Thermo Fisher Scientific UltiMate 3000 HPLC system coupled to a Q Exactive Plus mass spectrometer with electrospray ionization operated in both positive and negative modes. Separation was achieved on a Thermo Hypersil Gold column (1.9 μm, 100 mm × 2.1 mm) with a mobile phase of water (0.1% formic acid) and acetonitrile (0.1% formic acid) at 300 μL/min. The gradient increased from 2% to 100% B over 11 minutes, was held for 4 minutes, and re-equilibrated for 5 minutes.

Raw spectral data were processed through a computational workflow integrating preprocessing, feature extraction, and statistical analysis. Data interpretation employed MZmine, MetaboAnalyst, and network-based tools such as GNPS and Cytoscape for clustering and visualization, with compound annotation guided by spectral library matching and in silico prediction (MS2Query). Preliminary results revealed approximately 1000 metabolite features, with clear separation between antibiotic-treated and untreated groups in PCA analysis. Around 60 discriminant features have been prioritised for subsequent putative annotation.

This study establishes a reproducible workflow for untargeted faecal metabolomics, combining high-resolution LC-MS acquisition with open-source computational platforms. Ongoing work will extend the analysis to longitudinal sampling and integrate parallel microbiome sequencing to assess the durability of FMT effects and provide a systems-level view of host-microbiome interactions.

sciforum-142292: Kinetics of the serum metabolic fingerprint to predict mortality of critically ill patients

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Introduction: Accurate prognosis in intensive care units (ICUs), especially of mortality, remains a persistent challenge, mainly due to the patient's heterogeneity concerning demographic and clinical status, associated with a very dynamic pathophysiological state. The current work explored how the kinetics of the whole serum molecular fingerprint, as acquired by FTIR spectroscopy, can be applied to develop predictive models of mortality in an ICU. **Methods:** The FTIRspectra of the serum of 44 patients, from which 23 deceased, acquired along with the ICU hospitalization, were acquired. Linear discriminant analysis and support vector machine models were developed to predict the patient's mortality, based on serum collected 3 to 7 days before the clinical outcome, i.e., patients' death or patients' discharge. The application of diverse spectra pre-processing methods, feature selection, and kinetics of the spectral features was evaluated. **Results:** It was possible to develop only reasonable predictive models based on whole spectra, with accuracy 75%. These models' prediction was increased by feature selection, that, however, resulted in a maximum accuracy of mortality prediction of 84% and only 3 days before death. To extend the prediction window prior to the patients' death, the kinetics of these spectral features were considered, resulting in an accuracy prediction of 81% when considering the kinetics of the metabolic fingerprint between the 7th and the 5th days before death. **Conclusions**: Based on the kinetics of the serum spectral features, it was possible to develop a good predictive model of mortality 5 days before the patient's outcome. These findings highlight the potential of the applied platform to develop good predictive models, based on rapid, simple, minimally invasive analysis, towards the support of clinical decisions and the management of a relevant clinical infrastructure. **Acknowledgements**: This work was supported by the DSAIPA/DS/0117/2020, PTDC from the Portuguese Foundation for Science and Technology, and by the IPL/IDI&CA2024/R-DICIP_ISEL financed by Instituto Politécnico de Lisboa.

sciforum-145168: LipidIN 2.0: an end-to-end intelligent analysis platform for 4D lipidomics and glycolipidomics

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Accurate, comprehensive, and high-throughput lipid annotation and quantitation remains a long-standing challenge due to the complexity of lipid structures and the limitations of existing analytical pipelines. To address these challenges, we introduce LipidIN 2.0, an end-to-end intelligent analysis platform that integrates annotation, rapid quantitative analysis, and statistical modeling in a platform-independent software solution. LipidIN 2.0 incorporates a **972.1-million dynamic hierarchical lipid fragmentation library**, covering all potential chain compositions, including canonical lipids, glycolipids, and amidated modified lipids. The optimized expeditious querying module delivers unprecedented computational performance, enabling speeds exceeding **one trillion queries per second across large-scale mass spectral databases**. To reduce false annotations, LipidIN 2.0 employs a dedicated module that integrates precise predictions of retention time (Rt) and collision cross-section (CCS), achieving a **median relative error (MedRE) 0.3%**, which results in an estimated 5.7% false discovery rate while enabling the annotation of 8,923 lipids across multiple species. Additionally, the Wide Spectrum Modeling Yield network regenerates lipid fragment fingerprints to enhance both recall and coverage, yielding an estimated 20% improvement in annotation recovery. The platform also features a next-generation quantification module, supporting rapid, accurate, and reproducible lipid quantification. We further demonstrate the broad utility of LipidIN 2.0 across a wide range of lipidomics applications, including large-scale lipid annotation, high-confidence biomarker discovery, and translational studies in clinical cohorts. Together, LipidIN 2.0 represents a significant advance toward reliable, efficient, and comprehensive lipidomics analysis.

sciforum-144792: Mapping Intrinsically Disordered Proteins within Metabolomics Altered Pathways : A Computational Pipeline

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Metabolomics has emerged as a powerful tool for identifying biochemical alterations in disease, revealing key pathways involved in pathophysiology. However, the role of intrinsically disordered proteins (IDPs) in these metabolomics-altered pathways remains largely unexplored, despite their established involvement in signaling, regulation, and drug resistance. This study aims to identify and characterize IDPs present within disease-associated pathways defined by metabolomics datasets, establishing a foundation for future therapeutic targeting. Public metabolomics datasets from the Human Metabolome Database (HMDB) and MetaboLights were screened to identify significantly altered metabolites for selected diseases. Pathway mapping was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) to retrieve associated proteins. These proteins were analyzed for intrinsic disorder using complementary IDP analysis protocols, IUPred3, IDPpred and flDPnn to ensure robustness. **Across the analyzed disease pathways, a total of 186 proteins were retrieved, of which 57 (nearly 31%) exhibited high disorder propensity (more than 30% residues disordered).** Proteins exhibiting disorder were further annotated for biological function and disease relevance using UniProt and STRING. **Among these, 18 IDPs were directly linked to metabolic regulation, 12 to stress response, and 9 to cell signaling.** Several high-disorder proteins were identified in key altered pathways, including those linked to metabolic regulation, stress response, and cell signaling. Notably, many of these proteins lack structural characterization, underscoring their potential as underexplored therapeutic targets. By integrating metabolomics-driven pathway mapping with computational disorder prediction, this approach provides a novel pipeline for prioritizing IDPs in disease biology and lays the groundwork for subsequent virtual screening and drug repurposing efforts.

sciforum-139892: Mass Spectral Matching for Compound Identification in Metabolomics: An Open-Source Python/Shiny Package

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Compound identification is a critical step for mass spectrometry-based metabolomics. Two widely adopted approaches are chemical structure library- and mass spectral library-based compound identifications, both of which rely on calculating similarity scores between a query mass spectrum and reference spectra, either experimentally acquired or generated *in silico*. The use of a highly accurate similarity measure for mass spectral matching is a key component for successful compound identification. To meet this need, we developed a user-friendly Python/Shiny package with a graphical interface for mass spectral matching-based compound identification. The package supports both nominal-resolution mass spectrometry data (e.g., GC-MS) and high-resolution data (e.g., LC-MS/MS). It provides comprehensive preprocessing options, including filtering, weight factor transformation, low-entropy transformation, centroiding, noise removal, and spectral matching, and allows users to customize the order of these steps. The package also incorporates recently proposed entropy-based similarity measures, such as Shannon, Tsallis, and Rényi entropy correlations, alongside traditional cosine and binary similarity measures (1,2). Users can also combine these similarity measures to create mixture similarity measures with custom-defined weights. When using a mass spectral library with known compound names, parameters for specific preprocessing steps can be fine-tuned beyond the default settings. The package supports both untargeted and targeted metabolomics workflows and includes a command-line interface for batch processing. The implementation was validated on two reference libraries, a Webbook-based NIST GC-MS library and a GNPS-derived LC-MS/MS library. On the WebNist GC-MS data, the Cosine Similarity Measure outperformed the three entropy-based similarity measures with respect to accuracy (Cosine: 84.28% [83.75%,84.73%]; Shannon: 80.69% [80.17%,81.25%]; Rényi: 81.09% [80.56%,81.63%]; Tsallis: 81.68% [81.15%,82.19%]), and on GNPS LC-MS/MS data, the Cosine Similarity Measure slightly outperforms the three entropy-based similarity measures with respect to accuracy, although the 95% CIs overlap (Cosine: 69.07% [67.26%,71.01%]; Shannon: 67.92% [66.02%,69.95%]; Rényi: 68.63% [66.72%,70.66%]; Tsallis: 68.94% [67.03%,70.79%]).

sciforum-141001: Metabolomic Characterization of Portuguese *Ceratonia siliqua* Varieties

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The carob tree (*Ceratonia siliqua L.*) is a Mediterranean species known for its tolerance to salinity and drought stress, making it well suited to regions with unused soils and high exploitation, contributing to ecosystem recovery. Carob is also known for its resistance to biotic stress, requiring minimal inputs to control pests and diseases. To tolerate those conditions, carob synthesizes and accumulates protective compounds. Data from leaf extracts reveal the accumulation of metabolites with strong antioxidants and antimicrobial properties, highlighting the potential of these extracts. Despite the high diversity in the Portuguese carob germplasm, comprising nearly 30 varieties, few studies have characterized its metabolome. This study focused on the detailed metabolomic profiling of ten Portuguese varieties from the national carob field collection in Tavira (Portugal). Following exhaustive extraction with an ethanol/water solution, untargeted analysis was performed using chromatographic separation with a high-performance liquid chromatograph (HPLC) equipped with a diode-array detector (DAD). MassLynx software was used for data processing. A preliminary assay comparing three biological replicates per variety showed no significant differences. Consequently, a single sample per variety was considered. Preliminary results suggest the presence of over 20 compounds, primarily belonging to the phenolic class, particularly flavonoids and hydrolyzable tannins, as well as fatty acid derivatives. These metabolites are widely recognized for their central roles in plant defense mechanisms against biotic and abiotic stresses. Their potential applications will be further explored in relation to enhancing stress resistance in plants.

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sciforum-145778: Using gas chromatography and mass spectrometry (GC-MS) for the detection of drought stress effects on the metabolome of *Solanum tuberosum* varieties

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Background: Potato (*Solanum tuberosum* L.) is among the highest-yielding staple crops worldwide, making its resilience to abiotic stress a critical factor in global food security. Yet, the potato yield is strongly dependent on many abiotic factors. Pressuring environmental matters like climate change urge to understand plant stress response to abiotic factors, such as drought. This knowledge will be essential in breeding more adaptable crop varieties to maintain food security. Previous studies showed changes in plant stress-related metabolites [1,2]. **Methods:** Ten potato varieties were tested for their drought stress response in a field trial. Four biological replicates of each variety were collected from both irrigated (control) and non-irrigated (treatment) fields 2024 in two time points in July (at the onset of flowering). Leaf discs were grinded, and their weight normalized to ~22 mg (freshweight). Primary metabolites were targeted in the analysis by collecting the polar phase of the Methanol-Chloroform-Water extract. The metabolites were quantified and identified using Gas chromatography-mass spectrometry analysis. Further processing was done using MS-Dial and statistical analysis with R. **Results:** Preliminary results indicate separation between the control and the treatment group. Between the potato varieties and treatment significant metabolite changes (e. g. in proline) with p-value 0.05 could be measured. In total 39 primary metabolites (Sugar alcohols, amino acids and organic acids) were absolutely quantified and compared between the varieties. A correlation between the visual data (PolyPen RP 410 & ASD FieldSpec Handeld 2) obtained by AGES and a set of significantly changed metabolites was detected. **Discussion:** The differences could depend on varying conditions in the soil, the microclimate and pests in a field trial which were partially assessed and further analyzed by AGES. **Conclusion:** We were able to characterize metabolic changes in *Solanum tuberosum* that are likely related to the applied drought stress conditions.

Session 4. Metabolomics for Precision Nutrition in Humans and Animals

sciforum-134904: Anthocyanin-Enriched Fraction from *Callistemon citrinus* Modulates Leydig Cell Function: Insights into Cytotoxicity and Metabolic Impact

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The transition to a circular bioeconomy models promotes the exploration of natural, sustainable sources of bioactive compounds with potential therapeutic relevance. Polyphenols, especially anthocyanins, are known for their antioxidant and cytoprotective properties, making them promising agents in conditions associated with oxidative stress, such as male infertility. This study examines the effects of an anthocyanin-enriched fraction (EAF) from *Callistemon citrinus* (Lemon bottlebrush) flowers and its most rapresentative commercial anthocyanin, cyanidin 3-O-glucoside (cya-3-O), on Leydig cell viability, metabolic function, mitochondrial activity, and steroidogenic signaling. A green extraction protocol was employed, and the EAF was characterized via RP-HPLC-DAD-ESI-MS/MS, confirming an anthocyanin-rich profile (529.7 ± 5.40 mg equivalents of CyG/100 g DE composed of Cyanidin-3,5-O-diglucoside (56%), peonidin-3,5-O-diglucoside (34.2%), cyanidin-3-O-glucoside (7,2%), cyanidin-coumaroylglucoside-pyruvic acid (2,6%) . TM3 mouse Leydig cells were treated for 24 hours with EAF (0.01, 0.1, 1 μg/mL) and cya-3-O (0.5, 5, 50 μM). Cell viability, mitochondrial membrane potential, and intracellular ROS were assessed. EAF modulated cellular metabolic activity without inducing cytotoxicity, while cya-3-O led to a dose-dependent increase in LDH release, suggesting membrane damage at higher concentrations. No significant changes were detected in mitochondrial membrane potential or ROS levels. Western blot analysis was performed to assess mitochondrial respiratory complex activity, showing preliminary modulation in protein levels. In parallel, expression of key steroidogenic genes (STAR, CYP17A1, NRB02, SIRT1) was quantified via RT-qPCR, and protein activity of AC/PKA pathway was evaluated by Western blot. Results indicate that EAF and cya-3-O may modulate steroidogenesis through transcriptional and post-translational regulation. Ongoing analyses include ^{1}H-NMR-based exometabolomics and quantification of androstenedione hormone production. These findings suggest that EAF may influence Leydig cell function and bioenergetics, supporting their further investigation as potential modulators of male reproductive health. Additional studies are needed to fully elucidate the underlying molecular mechanisms and clinical potential.

sciforum-145923: Breed-Dependent Metabolic Signatures in Bovine Milk Under Thermal Stress: Insights from Sahiwal, Tharparkar, and Karan Fries Cattle

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Heat and cold stress influence the physiology and productivity of dairy cows. Milk metabolite profiling may provide valuable insights into breed-specific adaptive responses. Understanding these metabolic adaptations is crucial for improving breed resilience, productivity, and nutritional quality of milk, particularly in the context of climate variability. This research investigates metabolic adaptations in cattle breeds under thermal stress conditions through milk metabolomic profiling using Gas chromatography. The individual milk samples of 12 mid-lactating animals of Sahiwal, Tharparkar and Karan Fries were collected from organized dairy farm. Samples were collected in three different seasons viz: winter (December-January), Thermo-neutral (March-April) and summer (May-June) seasons. The sample collection was done three times during each season (Total number of samples in each season for each breed was n=36). In Sahiwal, total 158, 135 and 153; in Tharparkar, total 152, 138 and 153 and in Karan Fries, total 147, 105 and 131 milk metabolites were identified during thermo-neutral, cold and heat stress, respectively. During heat stress, Sahiwal cows demonstrated significant increase in acetic and butyric acids,with reductions in alanine and fumaric acid concentrations, indicating a metabolic shift toward anaerobic processes.Tharparkar showed increases in 9,12-octadecadienoic acid and threonine that primarily impact the pentose phosphate pathway and unsaturated fatty acid biosynthesis. Karan Fries showed increase in dihydroxyacetone and valine but significant reductions in succinic acid, reflecting alterations in protein synthesis and energy metabolism.Under cold stress conditions, Sahiwal utilized branched-chain amino acid while Tharparkar cattle emphasized lipid metabolic pathways. Karan Fries cattle prioritized amino sugar metabolism for muscle maintenance. This study emphasizes breed-specific metabolic adaptations to thermal stress, providing insights into resilience mechanisms in indigenous breeds relative to crossbreds. Overall, these results emphasize the importance of breed-specific adaptations to thermal stress, which can inform management strategies aimed at enhancing productivity and welfare in dairy cattle.

sciforum-145827: Feeding Patterns with Circadian Misalignment and Their Relationship with Lipid and Glycemic Metabolic Responses in Healthy Adults

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Introduction

The circadian system regulates key metabolic functions through synchronization between the central clock and peripheral clocks in tissues such as the liver, muscle, and pancreas. Its misalignment—caused by behaviors such as night-shift work, late meals, or insufficient sleep—alters glucose and lipid metabolism, promoting metabolic dysfunction and increasing cardiometabolic risk. Time-restricted eating (TRE) has been shown to improve glycemic and lipid parameters by resynchronizing circadian rhythms and reducing the risk of metabolic diseases.

Objective

To evaluate the association between feeding patterns with circadian misalignment (disalignment between food intake timing and biological/sleep phase) and lipid and glycemic metabolic responses in healthy adults attending nutritional counseling.

Methods

Data were collected from 21 healthy adults aged 18–65 years, excluding pregnant women and individuals with chronic diseases. Lipid profile and glycemia were analyzed using *Stata* with Pearson correlation coefficients.

Results

Correlations between metabolic parameters (glycemic and lipid), sleep patterns, and eating habits were assessed. Later sleep onset was associated with higher glycemia (r = 0.63), total cholesterol (r = 0.43), and triglycerides (r = 0.31), as well as with lower HDL concentrations (r = -0.48). A greater number of sleep hours was associated with a healthier metabolic profile: lower glycemia (r = -0.47) and higher HDL (r = 0.33). Lower meal frequency per day was associated with higher total cholesterol (r = -0.44), triglycerides (r = -0.33), and glycemia (r = -0.32).

Conclusions

Sleep timing and duration, as well as regularity of food intake, are associated with glycemic and lipid profiles. Late bedtimes, short sleep duration, and skipped meals are linked to poorer metabolic control.

sciforum-145974: Impact of Nutrition on Blood Metabolic Biomarkers in Elderly Adults with Type 2 Diabetes: A Metabolomics Approach

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Introduction: Obesity in younger populations increases early manifestations of type 2 diabetes (T2DM), although the clinical and social relevance of this disease becomes more pronounced with age. Metabolomic studies have identified plasma biomarkers associated with T2DM, mainly in the context of obesity, but data in older adults remain limited. Understanding metabolic and dietary profiles in this group is crucial to guide interventions.

Methods: Dietary characteristics of individuals over 65 years were collected using standardized questionnaires. Plasma extracts were analyzed using Ultra-Performance Liquid Chromatography–Mass Spectrometry (UPLC-MS). Partial Least Squares Discriminant Analysis (PLS-DA) was applied to explore metabolic differences. Dietary variables included ratios of sugar-rich foods to vegetables, sugar-rich foods to fruits, starch to vegetables, and meat to fish. Statistical correlations were assessed using SPSS (IBM, v26.0.0.1).

Results: Among diabetic participants, 11.1% showed a sugar-to-vegetable ratio >1 and 18.5% a starch-to-vegetable ratio >1, compared to 9.9% in non-diabetics. Whole cereal consumption was low (22.2%), while processed meat intake was high (54.3%). PLS-DA rendered 12 metabolites as potential biomarkers, they including Lyso-PCs, gangliosides and the dipeptide Gly-His. Nonetheless, only LPC(14:0), one ganglioside, Gly-His, and LPC(20:4) showed significant differences between T2DM and controls. Gly-His showed a significant correlation with dairy consumption in non-diabetics ($r = 0.417$), with no correlations observed in diabetics, possibly due to altered amino acid oxidation. In T2DM, the starch-to-vegetable ratio correlated positively with LPC18:0 and LPC16:0, which was not observed in non-diabetics, indicating altered carbohydrate metabolism. Correlations between diet and metabolites differed between T2DM and non-T2DM individuals, reflecting disruptions in key amino acid, lipid, and carbohydrate pathways.

Conclusions: T2DM alters the associations between diet and plasma metabolites in older adults, evidencing specific metabolic disturbances. Gly-His and certain LPCs could serve as indicators of metabolic dysregulation. These findings highlight the potential of metabolomics to guide nutritional strategies and personalize dietary recommendations in this population.

sciforum-138503: Integrating NMR Spectroscopy and Machine Learning for Quality Assessment and Geographic Discrimination of Olive Oils

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Olive oil quality is shaped by genetic, environmental, and processing factors, demanding advanced analytical methods for precise characterization. This study introduces an innovative pipeline integrating ^{1}H NMR spectroscopy, GC-MS/HPLC, and machine learning to address three key challenges: authentication, quality control, and terroir profiling. We developed a rapid, sustainable NMR-based metabolic fingerprinting method that quantifies 50 critical parameters (e.g., fatty acids, polyphenols, tocopherols, sensory traits) in a single analysis while distinguishing olive cultivars and harvest years. Applying this approach to Tuscan EVOOs (monovarietal and polyvarietal), combined with GC-MS/HPLC and chemometrics, we found milling practices contributed to 13% of compositional variation. A two-stage machine learning strategy isolated geographic effects, identifying 33 robust markers (e.g., nonanal, hexyl acetate, oleuropein derivatives, cycloartenol) enabling 80% accuracy in origin classification. The same methodology distinguished filtered vs. non-filtered EVOOs with 90% accuracy. These results establish a dual-purpose framework: 1) a high-precision quality control system for industry, and 2) insights into how genetics, environment, and post-harvest practices collectively shape oil chemistry. The approach supports PDO certification by linking chemical fingerprints to terroir, guides genotype selection for climate adaptation, and optimizes processing to preserve raw material integrity. By decoding genotype-environment interactions, we enable targeted olive breeding for metabolic traits suited to specific growing conditions—a critical tool for climate resilience. Merging advanced analytics with AI, this research bridges analytical chemistry and agricultural innovation, offering transformative solutions for olive oil science and industry.

sciforum-144294: Metabolomics-Driven Ingredient Selection and Formulation for Targeted Health Outcomes

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Precision nutrition aims to tailor dietary strategies based on individual metabolic responses to improve health and sustainability. Metabolomics, the comprehensive analysis of small-molecule metabolites within biological systems, provides a powerful platform to elucidate the biochemical responses to dietary components, thereby informing ingredient selection and formulation with unprecedented precision. The SYLPLANT EU project focuses on the production of protein-rich ingredients via fermentation of diverse carbon substrates including lignocellulosic biomass and agricultural side streams. The PROMISEANG EU project develops alternative proteins derived from the microbial fermentation of underexploited marine resources, including marine invertebrate and macroalgae discards, as well as industrial biowastes. Applying metabolomic profiling enables the detailed characterization of how these novel protein sources modulate metabolic pathways, influence gut microbiota composition and function, and affect nutrient assimilation and utilization in both humans and animals. These insights facilitate the rational integration of these sustainable protein ingredients into precision nutrition frameworks, allowing for tailored dietary formulations that address individual metabolic needs. These solutions are not only personalized and metabolically targeted but also aligned with environmental sustainability objectives, thereby addressing the dual challenges of human and animal health and the global demand for sustainable food systems.

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Session 5. Applications of Metabolomics in Pharmacology and New Drug Development

sciforum-142009: In Silico Metabolomic Analysis of Saxitoxin and Its Analogs

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Toxins are used as biological weapons, one of which is marine toxins produced by dinoflagellates and cyanobacteria. Due to their potential toxicity, marine toxins exhibit a broad chemical and biological diversity, making them an exceptional reservoir for drug discovery. Saxitoxin and its analogs (STXs), about 60, are collectively known as paralytic shellfish toxins (PSTs). STX biosynthesis in dinoflagellates is known to be affected by environmental conditions, which demonstrate varying toxicities. The objective of study is to evaluate and understand the dynamics of STX and 14 its analogs activity through Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADME/Tox) profile to uncover insights to new therapeutic targets. For metabolite profile prediction, we will utilize the MetaTox online server. Swiss ADME, Deep-PK, and PASS Prediction of Activity Spectra for Substances (PASS) online servers were used to analyze physicochemical properties, pharmaceutically relevant descriptors based on violation of Lipinski's rule. As a result, all molecules present at least two violations of Lipinski's rule, such as >5 values in the hydrogen bond donor (HDB) and hydrogen bond acceptor (HDA) parameters. The STX analogs showed more affinity for the sodium channel than STX. However, STX, dcSTX, GTX5, and NeoSTX, showed more affinity for the HERG channel. Thus, with the predicted metabolites, as well as their ADME/Tox profile, it will be possible to predict new information and outline new drug development strategies.

sciforum-126432: Water fleas as “canaries in the coal mine” to monitor pharmaceutical pollution using metabolic perturbations as indices

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There is an ongoing increase in global population which is coupled with the excessive use of various resources from the human population. This significantly exacerbated environmental challenges and impacts and especially increased detection of pollutants in surface waters. While various analytical techniques are employed in water monitoring to identify contaminants, the analytical methods employed are often inadequate to provide early predictions and fail to capture the underlying mechanisms for observed effects in the ecosystem. This limitation highlights the need for more comprehensive approaches to pollution assessment. Consequently, there is a growing support for effect-based methods used to assess responses from key species, such as daphnids and others to pollutants. Daphnids are sentinel species often employed as indicators for the ecosystem. In the context of New Approach Methodologies (NAMs), these provide significant advantages in toxicity responses with extrapolations to more complex organisms. Our research focused on the impact of pharmaceuticals which are commonly encountered pollutants, on the physiology of daphnids. The integration of phenotypic endpoints such as feeding with key enzyme activities and metabolic perturbations points to the discovery of mechanisms in the actions of pharmaceuticals which serve as metrics for their detection in the environment, thus aiming to elevate the water flea as an equivalent to “a canary in the coal mine” to identify the presence of pharmaceuticals in the environment in actual river samples.

sciforum-146931: Antioxidant Potential of *Citrus limon* Essential Oil Against CCl_4-Induced Oxidative Stress in Wistar Rats

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This work aimed to extract essential oil from *Citrus limon* fruits (HEC) and to assess its biological activities, focusing on its in vitro antioxidant properties and its in vivo protective potential in rats subjected to carbon tetrachloride (CCl_4) toxicity.

The chemical analysis of the extracted oil revealed nine major compounds, representing 97.0% of the total composition. The dominant constituents were limonene (34.11%), Z-citral (32.10%), and γ-terpinene (29.90%). Fourier-transform infrared spectroscopy (FTIR) confirmed the presence of phenolic and aromatic structures, highlighting the abundance of limonene, Z-citral, and γ-terpinene. The oil contained approximately 3.6 mg gallic acid equivalents (GAE)/mL of total polyphenols and 1.9 mg catechin equivalents (CE)/mL of flavonoids. Antioxidant assays demonstrated a dose-dependent free-radical scavenging effect against DPPH and inhibitory activity against superoxide anions (O_2^-), though the potency was lower compared to vitamin C. The total antioxidant capacity was estimated at 23.20 mg vitamin C equivalents/mL.

To complement these in vitro findings, an in vivo study was conducted using a rat model of oxidative stress induced by CCl_4. The results showed that CCl_4 administration caused significant oxidative damage and hepatic injury. However, preventive treatment with *Citrus limon* essential oil (150 mg/kg body weight) markedly reduced these toxic effects, demonstrating its hepatoprotective and antioxidant potential.

sciforum-134644: Characterization of the bioactive profile of beach-cast seaweeds using a quantitative and large-scale metabolomics approach

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The proliferation and coastal accumulation of marine macroalgae (i.e., beach-cast seaweed) is a major environmental and socioeconomic issue, which requires new management and valorization strategies. In this vein, because of their rich content of compounds with health benefits, this natural resource could be exploited for elaborating nutraceuticals and food supplements. Herein, we aimed to compare the bioactive profile of various native brown (*Gongolaria barbata*, *Sargassum vulgare*), green (*Codium taylorii*, *Ulva linza*), and red (*Rhodymenia pseudopalmata*, *Laurencia hybrida*) species, as well as the invasive algae *Rugulopteryx okamurae*. To this end, samples were subjected to ultrasonic-assisted extraction and subsequent metabolomics analysis by liquid chromatography – mass spectrometry. Overall, higher levels of most phytochemicals under study were observed in brown algae, especially in *Rugulopteryx okamurae*, including polyphenols, carotenoids, and phytosterols. On the other hand, many essential amino acids and polyunsaturated fatty acids were found to be particularly abundant in the green algae Codium taylorii. Finally, although showing a poorer bioactive profile in general terms, red seaweeds stand out as an ideal source for specific compounds normally present at lower concentrations in the other aforementioned species, such as docosahexaenoic acid or vitamin B5. In summary, our findings demonstrate that the valorization of beach-cast seaweeds could be of great interest for the pharmaceutical and food industries, not only because of their high content of bioactive compounds with anti-inflammatory and antioxidant activity, but also in order to minimize and provide an added-value to these wastes within the framework of the circular economy and green chemistry.

sciforum-136803: HepG2 cells may not be reliable allies for the study of hepatic gluconeogenesis.

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Every cell in the human body needs energy to sustain its metabolic functions. To this respect, glucose regulation is essential, especially the gluconeogenesis pathway that converts non-hexose precursors, such as glycerol, lactate, pyruvate and glucogenic amino acids into glucose moieties. It is known that gluconeogenesis mainly occurs in the liver and, to a lesser extent, in kidneys. During fasting and under stress conditions, renal gluconeogenesis may account for 40% of systemic gluconeogenesis. To this respect, our lab is interested in liver-to-kidney crosstalk. To do so, we intend to build an *in vitro* model allowing the communication between hepatocytes and renal cells. Initially, we decided to use the HepG2 hepatocyte cell line mainly for its ease of implementation as well as for the numerous publications demonstrating their usefulness in various applications.

We started with the assessment of their metabolic skills by using a ^{1}H-NMR -based metabolomic approach. The gluconeogenesis pathway was stimulated by adding a cocktail of known precursors (lactate, pyruvate, cAMP and dexamethasone). However, the metabolomic findings revealed that HepG2 cells essentially displayed a Warburg-like profile, as expected for cancer cells, but with very limited or no gluconeogenesis activity even under stimulation.

In conclusion, the HepG2 cell line does not seem like a reliable ally to study gluconeogenesis and another model is needed. Primary hepatocytes could represent a valuable alternative. However, their use should be coupled with MS-based metabolomic instead of NMR due to a limitation in cell number.

sciforum-133857: May succination be involved in cardiotoxicity?

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Cardiovascular diseases (CVDs) are one of the leading causes of morbidity and mortality worldwide. CVDs can be caused by different conditions including heart failure, atherosclerosis, and ischemic cardiomyopathy. The impact of CVD on patients' quality of life is significant, highlighting the urgent need for better management, including the development of new therapies that request deeper molecular understanding. One possible target could be SUMOylation, a post-translational modification (PTM) in which Small Ubiquitin-like Modifier (SUMO) proteins are covalently attached to lysine residues on target proteins, has emerged as a key regulatory process in cardiovascular development and function. In certain pathological cases, the SUMOylation of proteins that are critical for cardiac function can be significantly modified. It is particularly the case for SUMO1, one of the members of the SUMO family, which is essential for regulating SERCA2a, a calcium pump that plays an essential role in cardiac muscle relaxation by regulating calcium reuptake after contraction. In this study we investigated how succination, a PTM caused by the reaction of fumarate with cysteine residues following its accumulation in cells, impacts SUMO1 function of SUMOylating SERCA2a. Using AC16 cardiomyocytes treated with sodium fumaric acid, we detected some metabolic shifts following the elevation of fumarate concentration via ^{1}H-NMR spectroscopy. Moreover, Western blot analysis suggests that succinated SUMO1 has a lower capacity to SUMOylate SERCA2a. This finding indicates that succination could potentially impair calcium fluxes, thus contributing to cardiotoxic effects. By exploring the link between both PTMS, succination and SUMOylation, this research reveals a novel regulatory mechanism in cardiac physiopathology, offering potential new avenues for therapeutic intervention in cardiovascular diseases.

sciforum-146471: Metabolomics-Guided Profiling of *Azadirachta indica* Reveals Novel Bioactive Signatures for Anticancer Drug Development

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Background: Natural products remain a cornerstone of drug discovery, yet their pharmacological translation is often hindered by incomplete understanding of bioactive metabolites. *Azadirachta indica* (Neem) is traditionally recognized for its anticancer potential, primarily attributed to nimbolide and gedunin. However, a comprehensive omics-driven approach is required to systematically map its pharmacological relevance.

Methods: Fresh Neem leaves were extracted in 80% methanol and analyzed using an untargeted metabolomics workflow integrating UPLC-QTOF-MS and ^1H-NMR. Metabolite annotation was achieved through HMDB, METLIN, and GNPS databases, followed by multivariate statistical analysis (PCA, PLS-DA) to distinguish bioactive metabolite clusters. In silico network pharmacology (STRING, STITCH) was applied to link candidate metabolites with cancer-related signaling pathways. Cytotoxic validation was performed on breast cancer (MCF-7, 4T1) and lung cancer (A549) cell lines.

Results: A total of 246 unique metabolites were identified, including triterpenoids, flavonoids, limonoids, and alkaloids. Metabolomics revealed distinct enrichment of nimbolide, salannin, and quercetin derivatives associated with apoptosis and redox modulation. Pathway mapping highlighted PI3K/AKT/mTOR, NF-κB, and p53 signaling as major molecular targets. In vitro validation showed potent cytotoxicity of nimbolide-rich fractions (IC50: 5.2 μM in MCF-7), correlating with elevated caspase-3 activity and ROS generation. Integrative analysis indicated strong synergy between limonoids and flavonoids, suggesting metabolite-metabolite interactions as drivers of efficacy.

Conclusion: Metabolomics provides a powerful systems-level framework to deconvolute the chemical complexity of Neem and accelerate the identification of pharmacologically relevant leads. By integrating omics profiling with functional assays, this study establishes Neem as a rich reservoir of candidate molecules for anticancer drug development. Future directions include clinical metabolomics and precision formulation of Neem-derived nanomedicines.

sciforum-142006: NMR-based Metabolomic Investigation Of The Effects Of Alzheimer's Molecular Stressors On Neuroblastoma Cells

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Alzheimer's disease (AD) is an irreversible neurodegenerative disorder that slowly destroys memory, thinking skills, and eventually the ability to accomplish even the simplest daily tasks. The amyloid-beta (Aβ42) plays a central role in Alzheimer's disease; indeed, soluble oligomers of Aβ42 (ADDLs) accumulate and cause functional deficits prior to overt neuronal cell death or plaque deposition. Moreover, recent results have shown that glutamate pathway may play a substantial role in the AD pathogenesis since its early stages.

We have studied via NMR-based metabolomics the effects of ADDLs and glutamate addition (below toxicity levels) on neuroblastoma SH-SY5Y human derived cell cultures as compared to untreated cultures. Six independent replicates for each group (ADDLs, glutamate, untreated) were prepared and cell lysates and growth media were analyzed after incubation. All the 1H NMR spectra were recorded with a Bruker 600 MHz spectrometer using the Carr–Purcell–Meiboom–Gill (CPMG) one-dimensional sequence with water presaturation.

Our results demonstrated that glutamate treatment led to pronounced metabolic alterations, affecting both intra- and extracellular metabolite levels. Specifically, intracellular concentrations of 15 amino acids increased significantly, while 5 amino acids decreased in the extracellular compartment. Additionally, glutamate-treated cells exhibited lower intracellular levels of 2-oxoisocaproate, 3-methyl-2-oxovalerate, ATP, AMP, glutathione, and fumarate. Alanine and α-ketoglutarate levels were elevated both intra- and extracellularly. Extracellular levels of citrate, fumarate, lactate, fructose, choline, and myo-inositol were reduced, whereas intracellular sn-glycero-3-phosphocholine, taurine, and phosphocreatine levels were increased. In contrast, ADDLs treatment did not induce significant metabolic changes.

These findings underscore the sensitivity of cellular metabolism to glutamate-induced stress and highlight the potential of metabolomics in characterizing early biochemical responses in AD pathology. Importantly, the observed alterations may suggest that glutamate-induced metabolic shifts could represent early hallmarks of AD, paving the way for the development of novel therapeutic strategies.

sciforum-143655: Omics-Based Insights into the Hypocholesterolemic Effects of *Fucus vesiculosus* Extract on Intestinal Cells

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Cardiovascular diseases remain the leading cause of mortality worldwide, with hypercholesterolemia being a major contributing factor. Marine-derived compounds, particularly from brown algae, have emerged as promising candidates for functional food development targeting lipid disorders. The main objective of this work was study the molecular effects of a purified aqueous extract of *Fucus vesiculosus*, rich in phlorotannins and peptides, on differentiated Caco-2 cells, simulating the human intestinal barrier. Using untargeted metabolomics (LC-HRMS/MS) and gel-based proteomics, it was explored the extract's impact on cellular metabolism and protein expression. Metabolomic analysis revealed significant alterations in lipid-related metabolites, including increased levels of fatty acid amides and C16 phytosphingosine, compounds associated with anti-inflammatory and cholesterol absorption-modulating properties. Notably, a marked depletion of glutathione was observed, suggesting oxidative stress induction, which may have therapeutic implications in cancer contexts. Proteomic profiling identified several proteins exclusively expressed in extract-treated cells, including Niemann–Pick C1 (NPC1) and fatty acid-binding protein 1 (FABP1), both key regulators of cholesterol transport and homeostasis. Gene ontology enrichment highlighted biological processes related to intestinal absorption and lipid metabolism, reinforcing the extract's potential role in modulating cholesterol uptake. This integrative omics approach provides novel insights into the intestinal-level mechanisms of *F. vesiculosus* bioactive compounds. The findings support its hypocholesterolemic potential described in traditional medicine and suggest additional anti-inflammatory and antitumor properties, positioning this seaweed extract as a promising candidate for nutraceutical development and cardiovascular disease prevention.

sciforum-146007: Seasonal Metabolomic Variability of Osteogenic Phytoconstituents in Cissus quadrangularis (Vitaceae)

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Background: Cissus quadrangularis (family Vitaceae), traditionally known for its osteogenic potential, contains bioactive phytoconstituents that contribute to bone health. However, the seasonal dynamics of these metabolites remain poorly understood.

Methods: Over a three-year period, we investigated seasonal variations in osteogenic metabolites of C. quadrangularis through systematic extraction and fractionation. Pulverized dried plant material was sequentially extracted with ethanol, followed by liquid–liquid partitioning into hexane, chloroform, methanol, water, and finally n-butanol fractions. The n-butanol fraction, known to be enriched in osteogenic phytoconstituents, was prioritized for metabolomic profiling. Extracts were obtained under uniform experimental conditions, with replicate sampling, and analyzed using TLC and comparative metabolite quantification.

Results: Seasonal variation significantly influenced metabolite abundance. Maximum concentrations were observed in the cool and dry months (January–March, November–December), with February showing the highest metabolite yield. A gradual decline was recorded from March to July, followed by a slight increase in August, and subsequent reduction until October. Both ethanolic and n-butanol fractions exhibited comparable seasonal trends, indicating consistent partitioning of osteogenic metabolites. The ratio of ethanol-to-n-butanol extractability further supported differential biosynthesis of metabolites across climatic conditions.

Conclusion: Our findings demonstrate that the osteogenic phytoconstituents of C. quadrangularis exhibit clear seasonal fluctuations, with optimal accumulation during cooler, drier months. This highlights the importance of harvest timing for maximizing bioactive yield and provides new insights into metabolomic variability of osteogenic phytoconstituents in medicinal plants.

Session 6. Microbial Metabolites with Novel Functions in Diseases and Health

sciforum-144429: Bacillus coagulans improves late laying hen performance through bile acid modulation that suppresses hepatic lipid accumulation and alleviates ovarian oxidative stress

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In production, laying hens often accumulate fat in their bodies due to high-fat diets, which in turn affects their production performance. Bacillus coagulans has good tolerance in the digestive system and is beneficial to intestinal health. The objective of this study was to evaluate the effect of Bacillus coagulans on production performance, lipid metabolism, and ovarian function in late-phase laying hens. Research has found that adding Bacillus coagulans to feed can reduce fat accumulation in the liver. Bacillus coagulans supplementation increased the abundance of Firmicutes and decreased the abundance of Actinobacteriota, whereas promoting the proliferation of Lactobacillus and Bacillus, taxa favorable for reducing fat deposition. Additionally, In addition, Bacillus coagulans significantly increased ileal bile acid levels of glycochenodeoxycholic acid (GCDCA), tauroursodeoxycholic acid (TUDCA), taurohyodeoxycholic acid (THDCA), lithocholic acid (LCA), and hyodeoxycholic acid (HDCA), and significantly reduced levels of tauro-α-muricholic acid (Tα-MCA). Serum levels of estradiol (E_2), progesterone (P_4), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), as well as ovarian expression of estrogen receptor alpha (*ER-α*), FSH receptor (*FSHR*), and LH receptor (*LHR*) genes, were significantly increased in the BC group($P<0.05$). Regarding ovarian antioxidant capacity, *Bacillus coagulans* supplementation significantly up-regulated the expression of nuclear factor erythroid 2-related factor 2 (*Nrf2*) signaling pathway genes, increased the activities of total superoxide dismutase (T-SOD) and catalase (CAT), and decreased malondialdehyde (MDA) content($P<0.05$). In conclusion, dietary supplementation with Bacillus coagulans improved production performance by regulating hepatic lipid metabolism and enhancing ovarian function in aged laying hens.

sciforum-145862: Aromatic Microbial Metabolites and Biomarkers in Cerebrospinal Fluid for the Diagnosis of Secondary Bacterial Meningitis

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The urgent need for sensitive and specific diagnostic techniques for secondary bacterial meningitis remains a significant challenge in neurosurgical and intensive care units. Combining various clinical indicators, biochemical parameters, as well as biomarkers and metabolites found in cerebrospinal fluid (CSF), offers a promising approach for developing multivariate diagnostic models. In this research, 96 CSF samples from 53 patients suspected of secondary meningitis were examined. The first group included patients with residual effects of severe brain damage and chronic critical illness: 7 patients with secondary bacterial meningitis and 29 patients without it. The second group consisted of individuals following neurosurgical procedures: 10 patients with secondary bacterial meningitis and 7 patients without this complication. Combining data, one group of 33 CSF samples represented patients with secondary bacterial meningitis, while another group of 63 CSF samples included those without the infection. These groups showed significant differences in cellular and biochemical composition, with higher levels of biomarkers such as interleukin-6, S100 protein, and some aromatic metabolites in the meningitis group. Univariate prognostic models based on three aromatic microbial metabolites of phenylalanine, tyrosine, and tryptophan achieved excellent discrimination with AUC-ROC more than 0.9. A comprehensive multivariate model incorporating all measured biomarkers and metabolites yielded an AUC-ROC of 0.94, with a sensitivity of 94% and specificity of 86%, representing the most accurate method currently available for diagnosing secondary bacterial meningitis in patients with both acute and chronic conditions.

sciforum-145953: Chemical Ecology and Biotechnological Potential of the *Pseudodiploria* sp. Microbiome

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Coral reef ecosystems in tropical zones maintain very high and unique biological diversity; this diversity is essential for maintaining marine homeostasis. The coral reef is composed of countless species, including fish, marine algae, and invertebrates (cnidarians, marine worms, mollusks, arthropods, and echinoderms, among others). Additionally, coral reefs harbor an even greater diversity of microorganisms. Various studies indicate that the coral microbiome provides resistance and resilience to corals to face pathogens and warmer oceanic conditions. The unique temperature and pressure conditions of oceanic waters also stimulate the biosynthetic machinery of these microbes to produce novel metabolites with a plethora of biological activities, ranging from antimicrobial, anticancer, and antiparasitic, among others.

Our study examines the chemical ecology and biological activities of the microorganisms associated with the brain coral *Pseudodiploria* sp. from the Caribbean coast of Panama, aiming to prioritize the bioprospection of metabolites expressed by its microbiome. Using isolation techniques, genetic sequencing, and untargeted GC-MS metabolomics, we have identified and characterized microorganisms associated with this brain coral. Additionally, biological activity screening of cultivable bacteria and fungi isolated has been tested against marine and clinical pathogens.

Preliminary results show a rich diversity of associated bacteria and a low prevalence of fungi in the brain coral. We have identified specific bacterial strains that exhibit significant biological activities against the multidrug-resistant strain *Staphylococcus aureus*, as well as the marine pathogens *Vibrio coralliilyticus* through antagonistic interactions and direct inhibition tests. Preliminary metabolomic profiles, based on GC-MS and processed using the GNPS molecular networking bioinformatics platform, reveal various chemical compounds produced by the coral microbiome. These compounds are potentially linked to the observed clinically and ecologically relevant biological activities. Our results offer potential avenues for coral conservation and novel drug discovery strategies.

sciforum-141948: Emerging Roles of GC-MS-Detected Aromatic Metabolites in Clinical Diagnostics

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Microbial metabolites, particularly those derived from gut microbiota, are increasingly recognized as crucial mediators between host and microbial communities. These metabolites, such as phenolic compounds, play significant role in inflammation and infection development, influencing clinical outcomes in various patient populations such as those with sepsis, post-surgical complications, and post-COVID-19 syndrome. Understanding their profiles and functions is essential for advancing personalized medicine and improving diagnostic and prognostic strategies in clinical practice.

Modern studies utilize gas chromatography–mass spectrometry (GC-MS) as a primary tool to quantitatively assess microbial metabolites within blood serum and fecal samples. This technique is prized for its sensitivity, specificity, and reproducibility, allowing for comprehensive detection of both phenolic compounds and dicarboxylic acids. Enhanced analytical power is achieved through careful sample processing, including extraction and derivatization, while advanced multivariate statistical approaches relate metabolite data to patient outcomes.

Notably, distinct serum elevations in phenyllactic, 4-hydroxyphenyllactic, and 4-hydroxyphenylacetic acids have been observed among sepsis patients, far exceeding levels seen in healthy controls. These findings lend support to the idea that sepsis is accompanied by gut microbial metabolic disturbances, as substantiated by experimental models.

In cardiac surgery patients, marked deviations in both microbial and mitochondrial metabolites have been recorded. Combining metabolic profiles with clinical information in multivariate models enables robust prediction of postoperative complications, achieving high accuracy and reliability.

Furthermore, individuals with post-COVID-19 syndrome display persistent abnormalities in microbial metabolite spectra—particularly elevated levels of 4-hydroxybenzoic, succinic, and fumaric acids—that do not normalize even after extended rehabilitation.

In summary, serum metabolite profiling via GC-MS represents a promising avenue for diagnosing, monitoring, and prognosticating various disease states, enhancing patient-specific clinical care. Integrating microbial metabolite analysis into clinical practice can enhance personalized medicine, improve patient outcomes, and guide the development of targeted therapies for a range of acute and chronic conditions.

sciforum-141570: Fermented and Unfermented Whole-Grain Rye Modulate and Methylated Quaternary Ammonium Compounds Systemically in High-Fat Diet-Fed Mice

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Whole-grain rye (WGR), a key component of the healthy Nordic diet, is linked to reduced metabolic disease risk. Gut-microbial metabolites and methylated-quaternary ammonium compounds (mQACs), such as betaines and acylcarnitines, are associated with WGR consumption and may drive these health benefits. These metabolites influence tissue-specific metabolic pathways (*i.e.,* each tissue responds distinctively), with fermentation potentially enhancing their effects. Therefore, a comprehensive understanding of WGR's role in whole-body metabolic response and how it modulates metabolic homeostasis is needed.

We conducted a dietary intervention in high-fat-diet (HF)-fed mice, comparing fermented (FRB) and unfermented rye bread (URB) using LC-MS-based metabolomics across key metabolically active tissues (heart, muscle, ileum, cecum, colon), along with plasma and stool.

Both WGR diets significantly (p0.05) increased gut-microbial metabolites and mQACs-TMAO, 5-AVAB, trigonelline, and homostachydrine, confirming them as rye-associated biomarkers. FRB elevated TMAO—a cardiometabolic regulator—across plasma, muscle, and gut. FBR also reduced its precursor, trimethyllysine, linked to increased cardiovascular risk, suggesting microbial modulation may counterbalance TMAO production. FRB further increased 3-phenyllactate, indoles, and kynurenate (plasma, stool), hippurate (muscle, cecum), trigonelline (muscle), and long-chain acylcarnitines (gut), all associated with enhanced energy metabolism and immune function. Despite these beneficial shifts, FRB increased plasma insulin, glucose, and cholesterol; and correlated positively with plasma mQACs and gut-microbial metabolites (Ex:hippurate, indolelactate), suggesting an early-stage adaptive response to dietary shifts or potential metabolic stress. Conversely, URB increased antioxidant ergothioneine and reduced pro-inflammatory p-cresol sulfate, potentially reducing oxidative-stress and chronic inflammation commonly associated with metabolic diseases.

These findings highlight the role of WGR in shaping diet-microbe-host interactions that support metabolic health and gut function, with fermentation enhancing beneficial metabolic shifts, while URB reduces the levels of potentially harmful metabolites. The interplay between tissue-specific metabolic responses and gut-microbiota interactions highlights the need for integrative, whole-body approaches to improve dietary recommendations for managing metabolic diseases.

sciforum-146093: Profiling developmental dynamics of aromatic amino acid fermentation in infants

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Objectives:

Aromatic amino acids (AAA) are indispensable building blocks of protein and essential precursors of metabolic cofactors, hormones, and neurotransmitters. Owing to their stable aromatic rings, AAA form a group of distinctive metabolites with diverse biological activities after microbial fermentation in the large intestine. The profile of microbial AAA metabolites is strongly correlated with the composition of the gut microbiome. This study aims to define the developmental dynamics of AAA fermentation in infants *and* its correlation with the developing gut microbiome.

Methods:

A total of 250 fecal samples were collected from 138 full-term and healthy infants at 1, 4, 12, and 24 weeks of age. Concentrations of AAA and their microbial metabolites were quantified using dansyl chloride and 2-hydrazinequinoline derivatizations coupled with liquid chromatography–mass spectrometry-based metabolomic analysis. Shotgun metagenomic sequencing was conducted to define the microbiome, especially the genes contributing to AAA fermentation.

Results:

Compared to steady increases of many free amino acids in feces from week 1 to 24, the nadirs of free AAA were at week 4 in newborns. AAA-derived intermediates, such as 4-hydroxyphenylacetic acid (HPAA), phenylacetic acid, and 4-hydroxyphenyllactic acid, and end products, such as *p*-cresol, 4-hydroxyphenylpropionic acid, and indole-3-propionic acid, were present in feces in sequential, progressive, and selective patterns, showing gradualness and variations in the establishment of AAA microbial fermentation across human newborns. Preliminary examination of the correlations between AAA fecal metabolome and metagenomes revealed a strong association of *p*-cresol with the large subunit of 4-hydroxyphenylacetate decarboxylase (4-Hpd), but not with the more prevalent presence of 4-Hpd activating enzyme in newborns.

Conclusion:

Newborns undergo gradual development of microbial AAA fermentation, particularly the conversion of HPAA to *p*-cresol. Characterizing the microbes responsible for this development will provide useful insights for nutritional and therapeutic interventions of AAA fermentation in humans and animals.

sciforum-144224: Sepsis-associated microbial metabolites as an indicator of complications during extracorporeal therapy in cases of sepsis and septic shock in children

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Introduction

Modern blood purification technologies are widely used in intensive care to treat patients with sepsis and septic shock. Mortality was significant and increased as organ failure progressed, therefore, further evaluation of the effectiveness of extracorporeal therapy for severe sepsis and septic shock in children is necessary.

Aim

To evaluate the change in the concentration of sepsis-associated microbial metabolites when using the method of extracorporeal therapy of severe sepsis and septic shock in children.

Materials and methods

The hemosorption was performed using a device for extracorporeal blood purification, Efferon LPS NEO (Efferon Company, Moscow, Russia) for children with sepsis and septic shock (n=30). The study protocol ClinicalTrials.gov ID NCT05707494 is available at Study Details | Lipopolysaccharide Adsorption (Efferon LPS NEO) in Children With Sepsis | ClinicalTrials.gov. The level of sepsis-associated microbial metabolites in the blood serum of children with sepsis or septic shock was measured using the method of gas chromatography-mass spectrometry.

Results

The concentration of phenyllactic acid before hemosorption was 0.8 (0.6; 1.3) μmol/l and after hemosorption it was 0.6 (0.3; 1.2) μmol/l (p-value=0.034). The concentration of p-hydroxyphenylacetic acid before hemosorption was 1.1 (0.7; 2.7) μmol/l and after hemosorption it was 0.8 (0.2; 1.2) μmol/l (p-value=0.023). The concentration of p-hydroxyphenyllactic acid before hemosorption was 2.8 (1.4; 4.6) μmol/l and after hemosorption it was 0.8 (0.4; 3.7) μmol/l (p-value=0.041).

Conclusion

Blood metabolite analysis in sepsis has important clinical implications: elevated and persistently elevated metabolite levels indicate irreversible changes in the body and an unfavorable prognosis. This emphasizes the central role of metabolic monitoring and the importance of microbial metabolism in both understanding and treating sepsis and septic shock in children.

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