II SESMET CONFERENCE

ABSTRACT BOOK

3rd–5th JUNE

SEVILLE 2024

SESMET
SOCIEDAD ESPAÑOLA DE METABOLÓMICA

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ABSTRACT BOOK

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WELCOME

Dear Colleagues,

It is with immense pleasure that I, on behalf of the Scientific and Organizing Committee, extend a warm welcome to each of you to **the 2nd International Conference of the Spanish Society of Metabolomics** (SESMet 2024). We are delighted to host this year's edition at the **Universidad CEU Fernando III in Seville**, from June 3rd to June 5th. Seville is steeped in rich history, architecture, and culture, with the mesmerizing Guadalquivir River providing a marvelous setting for our gathering.

Reflecting on our journey, it was just two years ago that SESMet marked its inception with a one-day conference in Valencia. Today, we stand at the threshold of our second international conference, as evidence of SESMet's growth into a mature society and the establishment of a biannual tradition that unites distinguished national and international scientists at the forefront of metabolomics research.

This year is particularly momentous for us as we celebrate the 15th anniversary of CEMBIO at Universidad San Pablo CEU. In honor of this milestone, CEMBIO is proud to organize SESMet 2024 at our newly inaugurated university CEU. The unwavering support from our colleagues at CEU UF3 ensures that every aspect of the conference is poised for excellence.

The event's blueprint is the culmination of meticulous planning and a keen focus on detail. The Organizing Committee has curated a program that resonates with the current trends, pressing issues, and the diverse interests of our participants. With preliminary workshops fully booked, we have strategically arranged plenary lectures, sessions, meetings, lunches, and coffee breaks to foster maximum engagement and the exchange of knowledge. We are privileged to have an array of invited speakers—eminent academics and thought leaders whose contributions are pivotal to the conference's success. As we stand on the cusp of presentations and discussions, we eagerly anticipate the wealth of knowledge that will be shared. The dedication and hard work of our attendees will shine through in oral communications, poster sessions, and vibrant discussions, all contributing to the dissemination of invaluable insights.

A special focus of SESMet 2024 is the nurturing and empowerment of **young researchers**. We encourage them to present their findings, engage in dialogue, and take on active roles throughout the conference. In recognition of their efforts, Lilly and Jasco will award the best oral communication and poster presentations, respectively. Our gratitude extends to our sponsors, who are at the vanguard of technological innovation. Their generous support and enlightening scientific seminars are integral to our conference.

The conference delves into the challenges of metabolomics, bottlenecks in annotation, bioinformatics, technological advances, and applications across various domains, including health, disease, plant, food, environmental, and microbial metabolomics. We also explore the latest in metabolite identification, multiomics/data integration, databases, metabolic networks, and standardization.

Beyond the academic realm, we have arranged a social program brimming with opportunities for networking and camaraderie. Highlights include a welcome reception and a gala dinner complemented by a guided boat tour, promising memorable experiences for all.

Welcome to Seville! Your presence is the cornerstone of what promises to be an inspiring, and fruitful conference.

Warm regards,

Antonia García
Chair of the SESMet 2024 Conference

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PROGRAM

II SESMET CONFERENCE

PROGRAM

3 JUNE | MONDAY

10:00 - 13:30 WORKSHOPS

13:30 - 15:00 YOUNG RESEARCHER MEETING

15:00 - 15:30 OPENING CEREMONY

SESSION **METABOLITE ANNOTATION AND IDENTIFICATION**

15:30 - 16:15 PLENARY LECTURE

EVOLVING METABOLOMICS RESEARCH: THE POWER OF ANALYTICAL THINKING VS. AUTOMATED PROTOCOLS
Coral Barbas

16:15 - 18:00 ORAL PRESENTATIONS. SESSION I

A TAILORED LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY METHOD FOR THE SIMULTANEOUS QUANTITATION AND SCREENING OF HUMAN MILK OLIGOSACCHARIDES
Isabel Ten Doménech

SPECIFIC BIOMAKERS TO REWRITE THE HISTORY OF THE DOMESTICATION
Ane Gorostizu-Orkaiztegi Sanchez

NEXTFLOW WORKFLOW DEVELOPMENT. INTEGRATION OF TOOLS INTO GNPS2
Alberto Gil de la Fuente

COMPREHENSIVE METABOLOMIC CHARACTERIZATION OF WHOLE-BODY PIGR DELETION IN SERUM, LIVER AND VISCERAL WHITE ADIPOSE TISSUE BY GC-MS
Alessia Ferrarini

DRIED BLOOD MICRO-SAMPLING METHODS FOR DETERMINATION OF OXIDIZED LIPIDS
Abel Albiach Delgado

SPATIAL METABOLOMICS IDENTIFIES ACCUMULATION OF LPC(18:0) AND LPA(18:1) IN MURINE ADVANCED PLAQUES WITH TRANSLATION TO HUMAN CAROTIDS AND PLASMA FOR CV-RISK ESTIMATION
Marta Martín Lorenzo

ADVANCED SUSPECT SCREENING APPROACH TO UNVEIL CHILDREN'S PERSONAL CHEMICAL EXPOSOME USING SILICONE WRISTBANDS
Camilla Guerrini

18:00 - 18:40 SPONSOR PRESENTATION 1: LECO

18:40 - 19:30 POSTER SESSION - EXHIBITION AREA

19:30 - 20:00 LIVE MUSICAL DUO (MAIN ROOM)

20:00 - 21:00 WELCOME RECEPTION & COCKTAIL EVENT (EXHIBITION AREA)

4 JUNE | TUESDAY

SESSION

METABOLOMICS IN HEALTH AND DISEASE

9:00 - 9:45 PLENARY LECTURE

FROM HIGH TO LOW FIELD NMR-BASED METABOLOMICS: ROAD TO TRANSLATION?
Jesús Ruiz-Cabello Osuna

9:45 - 10:45 ORAL PRESENTATIONS. SESSION II-A

BENCHTOP NMR SPECTROSCOPY: A GAME-CHANGER IN CLINICAL METABOLOMICS
Jose Luis Izquierdo García

FECAL METABOLOMICS FOR THE STUDY OF THE EFFECT OF HYPOXIA AND SMOKING IN MICE WITH LUNG CANCER BY HIGH-RESOLUTION MASS SPECTROMETRY AND ION MOBILITY HPLC-QTOF-IMMS
Belén Callejón-Leblic

ADAPTATION OF LIVER METABOLISM TO EXERCISE WITH AND WITHOUT FOOD RESTRICTION – *IN VIVO* METABOLIC FLUX ANALYSIS USING MULTIPLE STABLE ISOTOPE TRACERS
Stanislaw Deja

INTEGRATIVE STUDY THROUGH FECAL METABOLOMICS AND METAGENOMICS UNVEILS COMPLEX HOST-MICROBIOME INTERACTIONS IN ANOREXIA NERVOSA
Laura Mayo Martínez

10:45 - 11:25 SPONSOR PRESENTATION 2: AGILENT

11:25 COFFEE

12:00 - 13:00 ORAL PRESENTATIONS. SESSION II-B

PROBING ERYTHROCYTES AS SENSITIVE AND RELIABLE SENSORS OF METABOLIC DISTURBANCES IN THE CROSSTALK BETWEEN CHILDHOOD OBESITY AND INSULIN RESISTANCE
Raúl González Domínguez

A CITIZEN-SCIENCE-ENABLED CORRELATION OF THE VAGINAL MICROBIOME, METABOLOME AND ASSOCIATED FACTORS
Denise Selegato

EXPLORING THE INDIVIDUAL VARIABILITY IN DRUG-INDUCED LIVER INJURY (DILI) RESPONSES THROUGH METABOLOMIC ANALYSIS
Marta Moreno Torres

MULTI-OMICS INTEGRATION REVEALS POTENTIAL BIOMARKERS FOR ASTHMA TREATMENT MONITORING WITH MEPOLIZUMAB
Alma Villaseñor Solís

13:00 - 14:00 POSTER SESSION II - EXHIBITION AREA

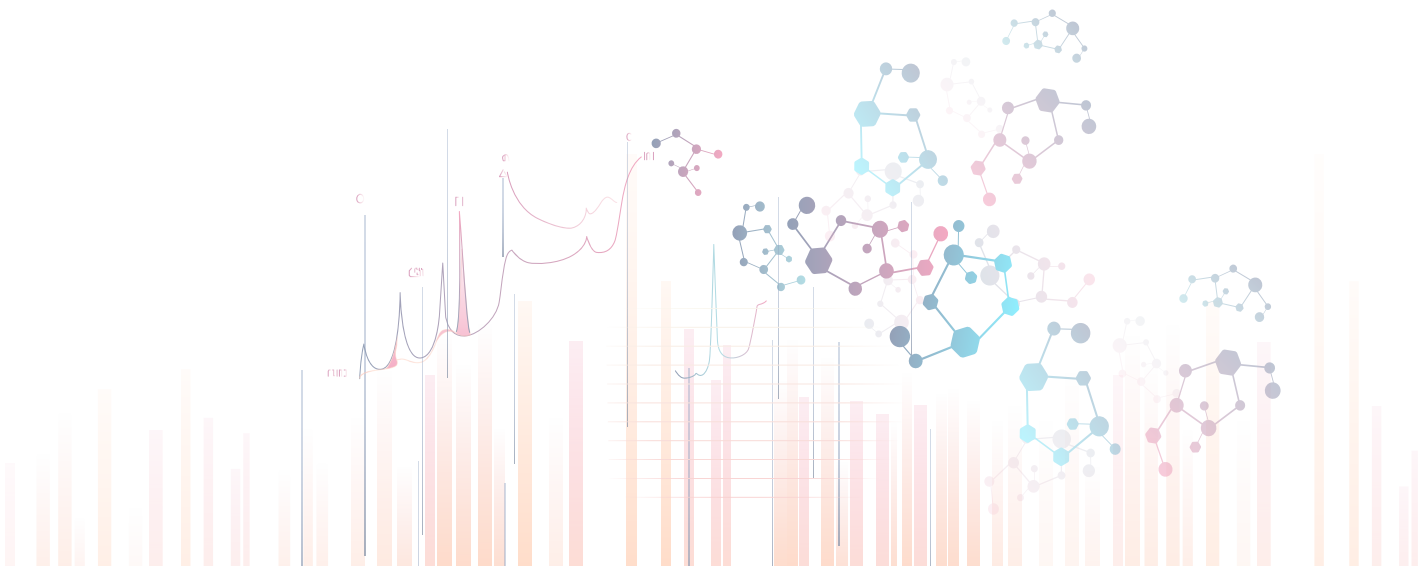
14:10 - 14:50 LUNCH SEMINAR / SPONSOR SESSION 3: WATERS

SESSION

TECHNOLOGY ADVANCEMENTS

15:00 - 15:45 PLENARY LECTURE

CHEMICAL METABOLOMICS - UNIQUE CHEMICAL BIOLOGY TOOLS FOR METABOLOMICS TO EXPLORE GUT MICROBIOME AND HUMAN METABOLISM
Daniel Globisch



15:45 - 17:00

ORAL PRESENTATIONS. SESSION III

DEVELOPMENT OF CHEMICAL BIOLOGY METHODOLOGIES FOR THE INVESTIGATION OF MAJOR PHASE II METABOLITE CLASSES IN HUMAN SAMPLES
Ioanna Tsiara

ASSESSING THE PRACTICAL UTILITY OF EXISTING MACHINE LEARNING-BASED COLLISION CROSS SECTION PREDICTION MODELS FOR SMALL MOLECULE ANALYSIS
Xavier Domingo Almenara

GCDUO: AN OPEN-SOURCE SOFTWARE FOR GC×GC-MS
Maria Llambrich Rodríguez

UNTARGETED METABOLOMIC ANALYSIS TO STUDY THE GUT MICROBIAL METABOLISM OF SAFFRON APOCAROTENOIDS FROM DIFFERENT SOURCES
Carlos Garcia Hernandez-Gil

INTEGRATING AN IN VITRO DYNAMIC MODEL OF INTESTINAL DIGESTION WITH METABOLOMICS, SHOTGUN METAGENOMICS AND METATRANSCRIPTOMICS: A COMPREHENSIVE FRAMEWORK FOR UNRAVELING GUT MICROBIOTA DYNAMICS
Maricruz Mamani Huanca

17:00 - 17:40 SPONSOR PRESENTATION 4: BRUKER

17:40 - 19:00 GENERAL ASSEMBLY OF MEMBERS

20:30 - 21:00 BOAT TOUR

21:00 - 23:30 GALA DINNER

5 JUNE | WEDNESDAY

SESSION **PLANTS, FOOD, ENVIRONMENT AND MICROBES**

9:00 - 9:45

PLENARY LECTURE
EXPOSOME & EXPOSURES IN PRECISION NUTRITION & HEALTHY AGING. MULTI-TARGETED QUANTITATIVE PLATFORM FOR EXPOSOME-BASED METABOLOMICS
Cristina Andrés-Lacueva

9:45 - 11:00

ORAL PRESENTATIONS. SESSION IV-A

PAPRIKA FINGERPRINTING BASED ON ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-HIGH-RESOLUTION MASS SPECTROMETRY (UHPLC-Q-ORBITRAP-HRMS) AS A PROMISING APPROACH TO REVEAL THE STERILIZATION IMPACT IN CONDIMENT'S METABOLOMIC COMPOSITION
Araceli Rivera Pérez

EVALUATION OF THE CULTIVAR EFFECT ON THE PHENOLIC VARIABILITY OF VIRGIN OLIVE OIL
Sonia Tomé Rodríguez

DIVERSITY OF PRIMARY AND SPECIALIZED METABOLITES IN TOMATO
Antonio Granell

DISTINCT METABOLITE SIGNATURES IN TOMATO WHEN SUBJECTED TO GENERALIST TETRANYCHUS URTICAE OR SPECIALIST TETRANYCHUS EVANSI FEEDING REVEALED BY METABOLOMICS
Vicent Arbona

UNTARGETED ANALYSIS TO EXPLORE CHANGES IN THE VOLATILE PROFILE OF TOMATO PLANTS UNDER COMBINED ABIOTIC STRESS
Enrique Serna Valverde

11:00 - 11:40 SPONSOR PRESENTATION 5: EURECAT

11:40 COFFEE

12:00 - 13:00

ORAL PRESENTATIONS. SESSION IV-B

INSIGHTS INTO ENVIRONMENTAL FACTORS AFFECTING STRAWBERRY FRUIT QUALITY: A COMBINED METABOLOMIC AND TRANSCRIPTOMIC ANALYSIS
José Gabriel Vallarino

METABOLOMICS IN QUERCUS ILEX: APPLICATION TO STUDIES OF VARIABILITY AND SELECTION OF ELITE GENOTYPES OF HIGH NUTRITIONAL VALUE AND RESILIENCE TO THE DECLINE SYNDROME AND CLIMATE CHANGE
María-Dolores Rey

TURBOOMICS: A WEB-BASED PLATFORM FOR THE ANALYSIS OF UNTARGETED METABOLOMICS DATA USING A MULTI-OMICS INTEGRATIVE APPROACH
Rafael Barrero Rodríguez

MULTI-OMICS DATA INTEGRATION INTO GENOME-SCALE METABOLIC MODELS REVEALS DISTINCTIVE METABOLIC VULNERABILITIES ASSOCIATED TO FOLFOX CHEMOTHERAPY RESISTANCE IN COLORECTAL CANCER
Marta Cascante

SESSION

COMPUTATIONAL METABOLOMICS, STATISTICS & BIOINFORMATICS

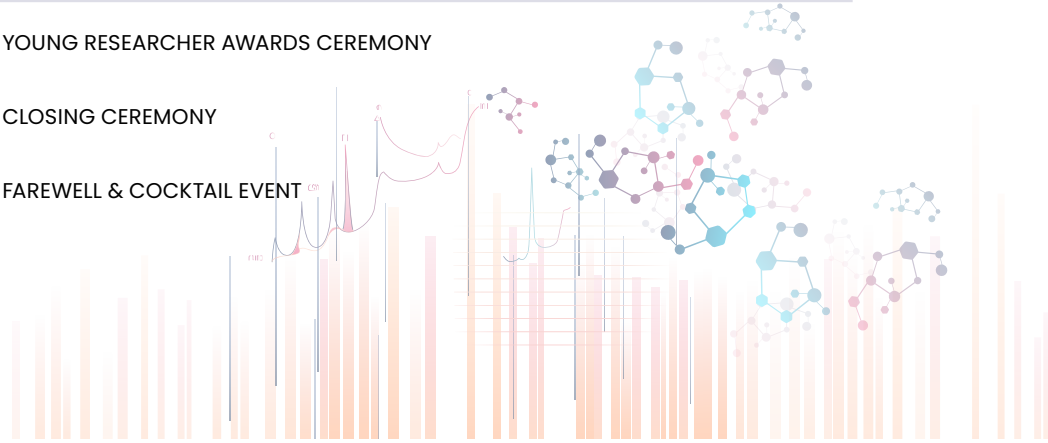
13:00 - 13:45

PLENARY LECTURE
NETWORK SCIENCE TO INTERPRET AND PREDICT METABOLIC PROFILES.
Fabien Jourdan

13:45 - 13:55 YOUNG RESEARCHER AWARDS CEREMONY

13:55 - 14:15 CLOSING CEREMONY

14:15 - 15:30 FAREWELL & COCKTAIL EVENT



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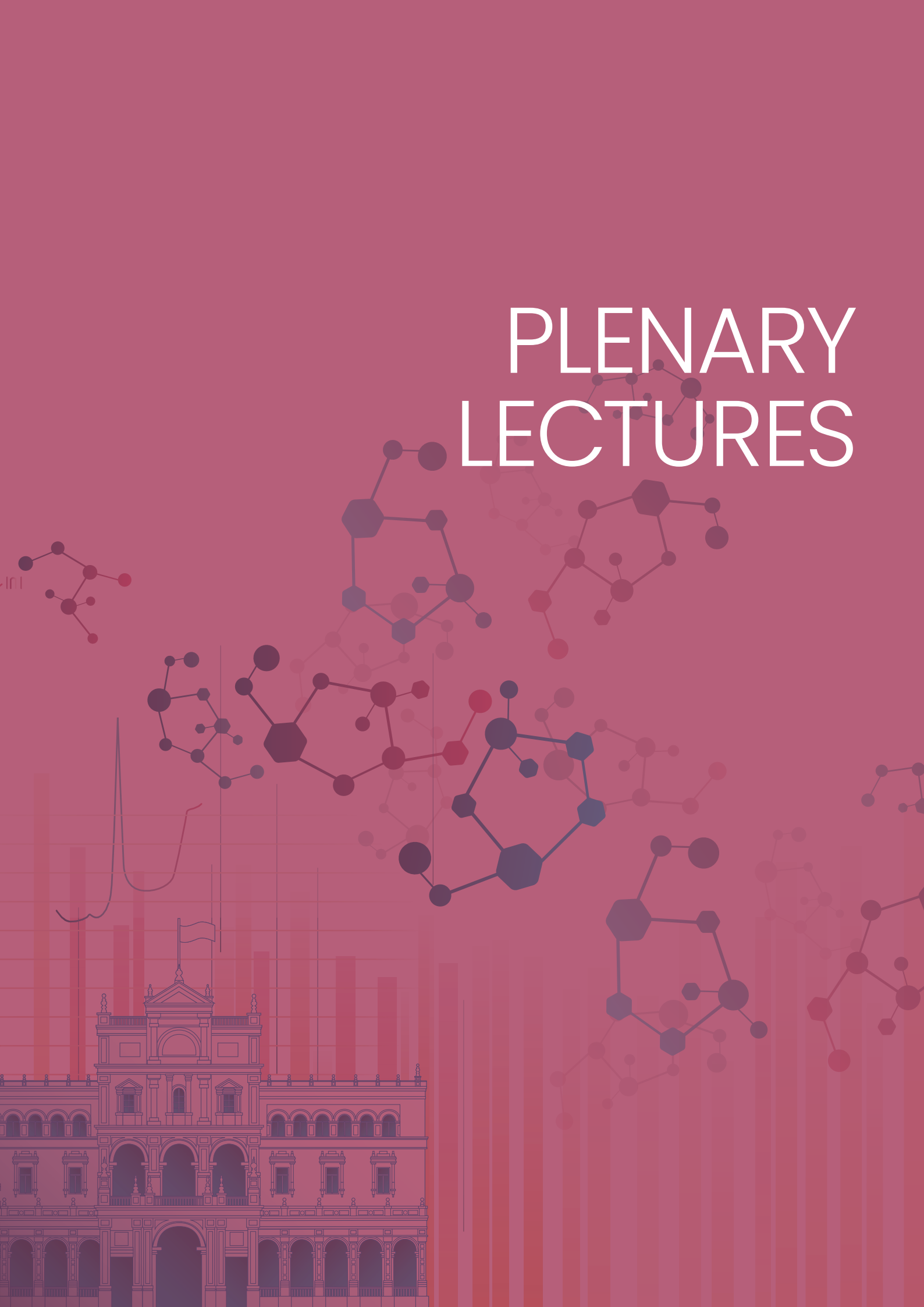


PL1
ADVANCING METABOLOMICS RESEARCH: ANALYTICAL THINKING VS. AUTOMATIC PROTOCOLS

Coral Barbas
Centre of Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia; Universidad San Pablo CEU, Madrid, Spain

Since its early stages metabolomics research has been dedicated to addressing a significant challenge: enhancing both the diversity and quantity of identified metabolites while simplifying and automating the process. However, even when utilizing advanced software tools, a profound understanding of analytical chemistry remains crucial in all the steps of the metabolomics workflow.

For instance, in the realm of metabolomics, data normalization is often conducted 'by default' using protein content or the total useful signal. Yet, this approach can lead to misleading interpretations in specific contexts. In scenarios where cell growth is inhibited due to a treatment, or in the analysis of fibrotic tissue, normalization based on total protein content might inadvertently skew the results. Such instances underscore the importance of selecting normalization methods that align closely with the unique aspects of the biological question being investigated [1].

However, the most significant bottleneck in metabolomics lies in the identification of metabolites, a challenge that becomes particularly pronounced in lipidomics [2]. Here, the abundance of isobaric and isomeric species presents a formidable obstacle and the identification is becoming the "wild west". One of the profound consequences of misidentification is its propagation within scientific databases, creating a cycle of misinformation that can be challenging to correct. The development of atlases carefully curated for different tissues further refines the specificity and reliability of metabolite profiling, allowing for high-throughput screening across multiple samples and conditions [3].

Examples of these situations will be presented along the lecture.

References

1 Anal Bioanal Chem. 2020412(24):6391-6405.

2 J Chrom. Open, 2021, p. 100018

3 Commun Biol. 2024 Jan 57(1):45.

PL2
FROM HIGH-TO LOW-FIELD NMR-BASED METABOLOMICS, THE ROAD TO TRANSLATION?

Jesús Ruiz-Cabello Osuna
Basque Research and Technology Alliance (BRTA) at CIC biomaGUNE, CIBERES, Ikerbasque Foundation and Universidad Complutense Madrid (UCM)–San Sebastián/Donostia, Spain

The primary objective of this plenary lecture will be to explore the transformative potential of low-field NMR spectroscopy in metabolomics, particularly in clinical settings. Despite historical limitations in signal-to-noise ratio and spectral resolution, recent advancements in hardware and software have revitalized interest in low-field NMR technology, particularly with the emergence of benchtop magnets. The increased accessibility and affordability of these benchtop magnets have made low-field NMR technology more appealing to researchers and academics, enabling them to conduct experiments with greater ease and efficiency.

This presentation will explore the benefits of employing low-field NMR in conjunction with hyperpolarization techniques, with a focus on how the combination can enhance sensitivity and detection capabilities and create novel applications. Recent studies have shown that spectra acquired using low-field magnets can exhibit signal-to-noise ratios and spectral resolutions that are competitive with those obtained at high field strengths, representing a significant advancement in the field and attracting new ideas and actors to the NMR spectroscopy market. These findings have the potential to transform the way magnetic resonance spectroscopy is conducted because low-field magnets are significantly less expensive, have lower maintenance costs, occupy less space, and are more accessible than their high-field counterparts.

Finally, the lecture will illustrate the applications of low-field NMR in diagnosing tuberculosis, hihglithing its clinical usefulness and potential impact of this technology in disease detection and monitoring. By elucidating the benefits of low- field NMR and recent advancements, this lecture aims to shed light on the promising future of NMR spectroscopy in clinical metabolomics.

PL3
CHEMICAL METABOLOMICS–UNIQUE CHEMICAL BIOLOGY TOOLS FOR METABOLOMICS TO EXPLORE GUT MICROBIOME AND HUMAN METABOLISM

Daniel Globisch; Weifeng Lin; Alejandro Torregrosa Chinillach; Wawrzyniec Haberek
Uppsala University

One of the most exciting scientific developments in the past decade has been the realization that the gut microbiota profoundly impact human physiology. Due to the link to disease development from metagenomic analyses, the targeted investigation of their metabolism represents a tremendous potential for the discovery of biomarkers and bioactive metabolites. Mass spectrometry-based metabolomics is the method of choice for the analysis of known and discovery of unknown metabolites. Advanced Chemical Biology tools are still limited compared to other 'omics research fields. We have developed a series of new state-of-the-art Chemical Biology methodologies for an enhanced metabolite analysis using liquid chromatography-coupled with tandem mass spectrometry (UPLC-MS/MS).

Unique chemoselective probes immobilized to magnetic beads were designed and synthesized that allow for facile extraction of metabolites for an increased mass spectrometric sensitivity by a factor of up to one million.[1-4] An incorporated bioorthogonal cleavage site that is labile under mild, palladium-catalyzed conditions facilitates efficient release of captured metabolites without altering their chemical structure. These unique and multifunctional chemoselective probes with synthetic 13C/12C isotopically labelled analogues allow for comparative and quantitative analysis of metabolites in human samples at low concentrations. We have termed this new method Quantitative Sensitive CHEmoselective MetAbolomics (quant-SCHEMA) that facilitates the detection of metabolites at attomole quantities in human and bacterial samples.

Our analysis of carbonyls, thiols, amines, and short-chain fatty acids (SCFAs) in biological samples using newly applied chemoselective probe-heads for metabolomics analysis revealed previously unknown metabolites due to conjugation of the mass-spectrometric tag. We have also applied this methodology in dietary intervention study with 156 samples for nutritional biomarker discovery and identified four potential food biomarkers. The success of this large-scale application builds the foundation for combination of these Chemical Biology methods in combination with standard metabolomics analyses in neuroscience, microbiome analysis and biomarker discovery.

References

1 Lin, W. ... Globisch, D. Angew. Chem. Int. Ed. 2024, 63, e2023185.

2 Lin, W. ... Globisch, D. Angew. Chem. Int. Ed. 2021, 60, 23232.

3 Kaur, A. ... Globisch, D. Chem. Sci. 2023, 14, 5291.

4 Lin, W. ... Globisch, D. Food Chem. 2023, 425, 136481.

PL4
EXPOSOME & EXPOSURES IN PRECISION NUTRITION & HEALTHY AGING. MULTI-TARGETED QUANTITATIVE PLATFORM FOR EXPOSOME-BASED METABOLOMICS

María Cristina Andres Lacueva
University of Barcelona- CIBERFES-ISCIII, Barcelona, Spain

The concepts of metabolomics and nutritional metabolomics and their relationship with the human metabolome will be discussed. The latest laboratory techniques for the study of nutritional metabolomic biomarkers, the importance of quantitative techniques

and their suitability for the study of the Human Metabolome will be presented. We will also discuss applications of metabolomics in working with biomarkers of exposure and microbial activity in nutritional epidemiology studies. We will share examples of recent applications and publications in Personalized Nutrition and healthy aging. We will highlight the importance of open access databases and exchange information on the latest updates in this area.

PL5
NETWORK SCIENCE TO INTERPRET AND PREDICT METABOLIC PROFILES

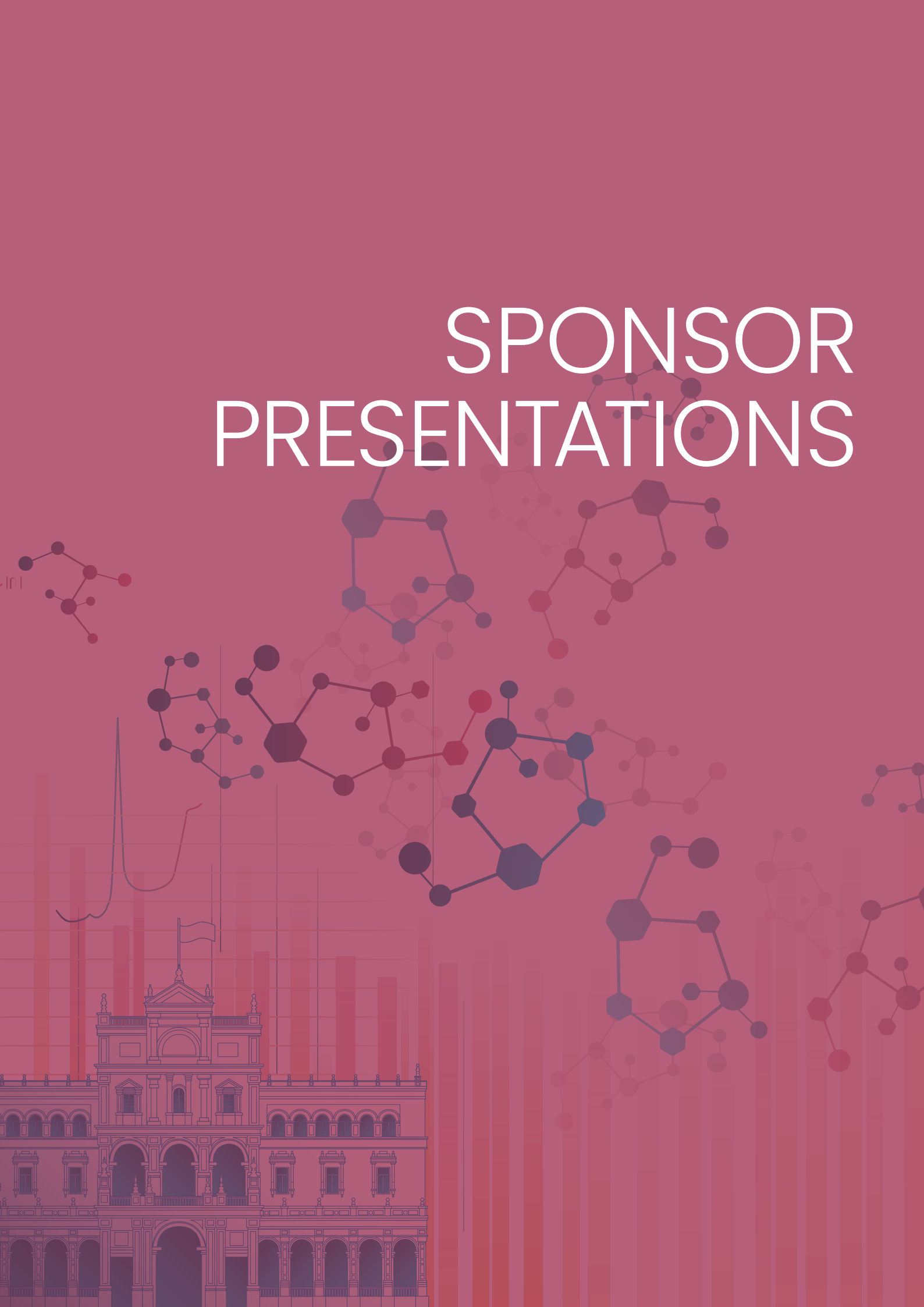
Fabien Jourdan
INRAE-MetaboHUB, Toulouse, France

Metabolic modulation is a cornerstone cellular response to genetic or environmental stresses. This plasticity is going beyond central metabolism and may involve complex processes spanning several metabolic pathways. Hence, it is a key challenge to be able to decipher metabolic modulations in a systemic and global perspective.

The aim of the computational methods and tools which will be presented is thus to consider the full complexity of metabolism. To do so, all metabolic reactions the cell is able to achieve are gathered in a single mathematical model call “genome scale metabolic network”. Based on this model it is then possible to identify metabolic specificities of different cell lines, predict metabolic behaviours, simulate metabolic responses to single or multiple knock-out and ultimately identify potential drug targets.

Finally, even if metabolic profiles and associated metabolic network modulations are very informative, their biological and physiological interpretation remain a challenge, requiring researchers to gather and connect various pieces of knowledge from a large range of resources. To aid in this task, we introduce FORUM: a Knowledge Graph (KG) providing a semantic representation of relations between chemicals and biomedical concepts, built from a large-scale federation of life science databases and scientific literature repositories. These associations allow to derivate new hypothesis from observed metabolites or anticipate which metabolites could be expected to be measured for a given disease.

SPONSOR PRESENTATIONS



SP1

AN INTER-LABORATORY STUDY TO INVESTIGATE THE USE OF 1D GC AND 2D GCXGC -TOF-MS AND -HRTOF-MS AS ACCURATE, ROBUST, AND ENRICHING TOOLS, FOR NON-TARGETED AND SEMI-TARGETED ANALYSIS OF MAMMALIAN BIOFLUIDS

Julio Lluch Bisbal (1); Nick Jones (2); David Alonso (2); Joe Binckley (2); Catherine L Winder (3); Warwick Dunn (3); Vanessa Nunes (3); James L. McRae (3)
(1) LECO Instrumentos S.L.; (2) University of Liverpool; (3) Francis Crick Institute

Over the pastcouples of decades, there has been an enormous evolution in GC-TOFMS technologyatributes including increasing in robustness, chromatographic resolution, detection sensitivity, rapid data acquisition speeds, and development of high-resolution accurate mass analyzers for rapid compound detection and caharacterization. Confident analyte identifications are seamless and facilitated through retention index filtering, and the avsilability of large, well-established databases, which includes accurate mass calculations for molecular and fragment formula determinations.

The objective of this investigation was to demonstrate the utility of 1D GC and GCxGC-TOFMS and HR-TOFMS for the analysis of mamalian biofluids. This was an inter-laboratory study conducted by researachers attempting to find and annotate unknown metabolites and in biofluids, asses method quality, and robustness, and evaluate non-target identification capability.

The samples consisted of unspiked plasma and urine to serve as QC standards. In addition, there were a series of spiked samples of plasma and urine used to test and compare the analytical technologies used at different laboratories. Data were analysed to asses analyte identifications and also use statistical approaches to ascertain alignment, accuracy, and robustness of the data sets. Additionally, HR-TOFMS instrumentation was use to compare and evaluate the ability to further improve semi-target analyte detection and identification capability.

Deconvolution of the rich data sets and statistical processing facilitated the differentiation of spiked and unspiked samples and validated robust and accurate results. The analytical methodology resulted in the high confidence identification of numerous metabolites including the spiked endogenous compounds. The GCxGC-MS demonstrated a significant increase in the total number of identified coumpounds as compared to GC-MS assays. Furthermore the use of GCxGC-HRTOFMS with complementary modes of ionization resulted in improved compound detectability and increase characterisation confidence.

SP2

AGILENT PROPOSALS TO HELP SCIENTISTS TO AFFORD TARGET AND UNTARGET METABOLOMICS

Jaume C. Morales
Agilent Technologies Iberia

Measuring the metabolome provides important information about biological phenotype and functional status of biochemical pathways. Its proximity to the phenotype of an organism provides complementary information to genomics and proteomics.

In parallel, metabolomics workflows have also been deployed in other scientific disciplines to profile and compare different kind of samples/products or monitoring biological processes like fermentation.

Innovative metabolomics solutions from Agilent provide a powerful portfolio of sample preparation, instruments, and informatics tools to help scientists to get faster and trusted answers.

Whatever your study or scenario needs to approach, using Targeted metabolomics assays or moving beyond a targeted approach with Discovery metabolomics, Agilent is providing innovative tools to make your research in a more straightforward way and boost your results in terms of reliability.

A common software platform combines the results from multiple analytical techniques, helping answer challenging biological questions faster. Agilent collaborates with leading metabolomics scientists to develop next-generation solutions and workflows to accelerate your research.

In this talk, we will overview what is Agilent proposal with our innovative hardware and software tools suit for most of the needs you can have.

SP3

METABOLOMIC PROFILING OF OSTEOCYTE EXTRACELLULAR VESICLES AND MATRIX BOUND VESICLES USING A PROTOTYPE BENCHTOP MULTI REFLECTING TIME-OF-FLIGHT (MRT) MASS SPECTROMETER

Pablo de La Iglesia González (1); Lisa Reid (1); Alicia Keenan (2); Lee A. Gethings (1); Liam M. Heaney (2); Owen G. Davies (2)
(1) Waters Corporation; (2) School of Sport, Exercise and Health Sciences, Loughborough University

Extracellular vesicles (EVs) are lipid delimited nanoparticles that function in development and intercellular signaling events throughout the body. Within bone there exists a subset of EVs, known as matrix binding vesicles (MBVs), which have been long proposed to associate with the underlying collagenous extracellular matrix to drive early mineralization events during bone development. However, the precise relationship between EVs and MBVs and their differential roles in bone development remains a point of contention. This study uses a prototype benchtop MRT to construct a comparative metabolite profile for EVs and MBVs obtained from MC3T3 pre-osteoblasts under osteogenic culture conditions.

MC3T3 osteoblast cells were cultured to confluence and differentiated towards a pro-mineralising phenotype. EVs were isolated from the media using a differential ultracentrifugation (UC) process and lysed using sonication. MBV's were liberated using collagenase digestion and subjected to UC isolation. EVs and MBV's were analysed by both HILIC and RP chromatographic methods on a prototype benchtop MRT mass spectrometer. Data were acquired using a data independent analysis (DIA) strategy, across the mass range 50-1200 Da, and the instrument consistently produced a mass spectral resolution in the region of 100,000 FWHM.

This feasibility dataset has putatively identified several significant biologically relevant polar metabolites, including amino acids, carboxylic acids, fatty acids, phenols, pyridines and indoles being present within both the EV and MBV extracts. LC-MS data were processed using MARS (Mass Analytica). Putative compound identifications were gained once data were confronted to the human metabolome (HMDB) database. Routine mass accuracies under the ppm allowed applying a mass tolerance of +/- 1ppm for compound matching. Unsupervised principal component analysis (PCA) revealed potential differences between the EV and MBV samples. A PLS-DA analysis was then performed which enabled the generation of an S- plot to highlight discriminating features, tentatively identified as fatty acids, amino acids and glycerophosphocholines.

SP4

THE JOURNEY OF NMR METABOLOMICS: FROM IN VIVO SPECTROSCOPY TO BIOFLUIDS PROFILING

María Luisa García Martín
Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina (IBIMA Plataforma BIONAND)

Metabolomics can be defined as the study of the metabolic profile (metabolome) of a biological system (cell, tissue, biofluid, organ, or organism) at a given moment. In this regard, NMR stands out as an unparalleled technique, being the only methodology capable of obtaining metabolomic profiles from a living organism non-invasively. Additionally, despite its inferior performance in terms of sensitivity compared to mass spectrometry, NMR metabolomics offers the advantages of being non-destructive, highly reproducible, and requiring minimal sample preparation.

The versatility of NMR provides us with tools to interrogate metabolism at different levels. While in vivo NMR spectroscopy and High-Resolution Magic Angle Spinning (HR-MAS) NMR provide information on the local metabolism of specific tissues, the analysis of biofluids offers a broader picture of the overall metabolic landscape. In vivo Spectroscopy allows for the real-time monitoring of metabolic processes within living organisms. It has been widely used both in preclinical and clinical settings, with a predominant application in tumor diagnosis and follow-up.

However, in vivo spectroscopy is limited by its intrinsic low sensitivity and spectral resolution, which restrict the number of detectable metabolites and, consequently, its diagnostic specificity. In contrast, HR-MAS spectroscopy, albeit losing the non- invasive nature of in vivo spectroscopy, offers the significant advantage of providing spectral resolution comparable to liquid spectroscopy. This

technique yields high-resolution NMR spectra from intact tissue samples, such as tumor biopsies, requiring only small amounts of sample (5-20 mg). HR-MAS is particularly useful for studying heterogeneous samples as no sample processing is required, thereby preserving the biological context of the metabolites. Within the applications of HR- MAS, it is worth highlighting the study of metabolic alterations in various types of tumors, such as brain tumors, prostate cancer, and breast cancer.

Finally, the use of high-resolution liquid NMR spectroscopy for analyzing complex biological fluids, such as blood, urine, or cerebrospinal fluid, has grown exponentially over the last couple of decades, becoming an essential tool for biomarker discovery, disease diagnosis, and understanding systemic metabolic responses, among other applications. This talk will provide an overview of the different NMR-based metabolomic approaches, with examples from both preclinical and clinical studies, mainly focused on cancer and neurodegenerative diseases.

SP5
GLYCOMICS UNVEILED: A MULTI-OMICS APPROACH TO DECIPHERING COVID-19 COMPLEXITY

Beatrix Patón Jiménez
EURECAT–Technology Centre of Catalonia

Glycosylation of circulating glycoproteins can reflect the inflammatory state of the organs during chronic diseases, offering a compelling dimension to the study of diseases like COVID-19. In this talk, we will describe the creation of a novel panel of biomarkers designed to differentiate mild from critical COVID-19 patients. Additionally, we will take a closer look at the highly glycosylated fetuin-A, using a site-specific glycan analysis approach to elucidate its glycosylation profile and shed light on its relevance in the context of COVID-19 outcomes. These findings underscore the significance of incorporating glycomics and glycoproteomics into a comprehensive multi-omics strategy, complementing proteomics, metabolomics and lipidomics in our quest for a more nuanced understanding of COVID-19 outcomes.

SP6
METABOLOMICS AND BEYOND: INTERPRETATION OF -OMICS DATA WITH COMPUTATIONAL PARADIGMS AT EURECAT

Xavier Domingo Almenara
EURECAT–Technology Centre of Catalonia

Since its establishment in 2012, the Centre for Omic Sciences (COS) a Rovira i Virigili University and the Technology Centre of Catalonia (EURECAT) joint unit, offers analytical and computational -omics analysis, as a cluster of the Unique Scientific and Technical Infrastructure (ICTS) OmicsTech platform. In this talk, we will introduce the center capabilities (both analytical and computational) for metabolomics in the context of different projects including the Glomicave H2020 project, aimed at designing a new cloud-based platform for multi-omics analysis using scientific literature mining and automatic interpretation.

ORAL COMMUNICATIONS

O1

A TAILORED LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY METHOD FOR THE SIMULTANEOUS QUANTITATION AND SCREENING OF HUMAN MILK OLIGOSACCHARIDES

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Human milk oligosaccharides (HMOs) are the third most abundant solid component in human milk (HM). They play a crucial role in shaping the gut-microbiota and modulate neonatal immunity. Despite the existence of up to 250 different HMOs, clinical research has primarily focused on a limited subset. We present a novel method for the simultaneous quantification of eight key HMOs, including 2'-fucosyllactose, 3-fucosyllactose, 3'-sialyllactose, 6'-sialyllactose, lacto-N-tetraose, lacto-N- fucopentaose I, lacto-N-difucohexaose I, disialyllacto-N-tetraose, as well as the screening of 241 HMOs. The method involves the isolation and analysis of HMOs using hydrophilic interaction liquid chromatography coupled to a Q-Exactive Orbitrap mass spectrometer in polarity switching mode, followed by automatic annotation of MS/MS spectra against the NIST Milk Oligosaccharide MS Library[1] using adjusted retention times. The extraction effciency of quantified HMOs was evaluated through recovery assays. Annotated entries were revised in terms of spectral match, diagnostic m/z ions, and adjusted retention time. Additionally, we showcase the utility of Feature-Based Molecular Networking (FBMN)[2] as a complementary tool to expand the annotation of HMOs and for the discovery of new HMO structures. Secretor and Lewis status of participants was established based on the abundance of specific HMOs. Results from the analysis of 40 donor human milk samples processed before and after pasteurization putatively identified 110 unique HMOs through the automatic annotation strategy and 17 additional HMOs with the FBMN approach. The thermal treatment did not significantly affect the levels of annotated HMOs. The developed methodology will be applied in future efforts to study differences in milk composition between mothers from term and preterm infants, as well as for a longitudinal survey of HMO concentrations along lactation.

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O2

SPECIFIC BIOMAKERS TO REWRITE THE HISTORY OF THE DOMESTICATION

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The Neolithic period marked the beginning of agricultural practices and animal domestication. The domestication of animals revolutionized human societies, enabling the transition from hunter-gatherer lifestyles to settle agricultural communities.

Sites for animal housing have been extensively studied by archaeologists with the objective of explaining the evolution of grazing. However, the identification of the animal species housed in the archaeological site is challenging. Nowadays, lipid proxies (sterols, phytosterols, and bile acids) are used for distinguishing among human, pig, and ruminant remains. Nevertheless, the inexistence of a specific biomarker for each animal species makes impossible to distinguish them in sites where different animal species were housed. Besides, in these proxies, the possible degradation of the compounds is not considered. Consequently, there is a necessity for searching specific biomarkers for each animal species that make possible to distinguish them unequivocally without using the proxies.

In this research work, a lipidomic profile of six animal species feces (goat, sheep, cow, horse, wild-pig, and pig) have been studied to identify possible specific animal biomarker. For that purpose, the best extraction conditions were selected previous to the analysis by liquid chromatography quadrupole Time of Flight method (LC-qTOF). Afterwards, a Partial least squares-discriminant analysis model (PLS-DA) has been created by Matlab to determine the most important features to distinguish between animal species. The minimum number of features were used to build the model and those biomarkers with the presence in a unique specie were selected as possible biomakers. Then, MS/MS spectra for those compounds were obtained and the identification of them was carried out using LipidMaps, Mefrag, and Massbank database. Finally, the archaeological samples from El Mirador cave in Sierra de Atapuerca (Burgos, Spain) were analyzed with the aim of finding the identified compounds and determine the animal species housed there.

O3
NEXTFLOW WORKFLOW DEVELOPMENT. INTEGRATION OF TOOLS INTO GNPS2

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GNPS2 is the next generation of the GNPS analysis platform for metabolomics data processing. Key features of GNPS2 are the ability to integrate user workflows. For example, GNPS2 includes support for classical and feature based molecular networking, visualization, massql and other external tools like MS2Query or ChemWalker. This work will describe how developers can integrate their own tools into GNPS2. We will highlight challenges and design principals in migrating MS2Query[3], MS2LDA [4], MSMS-choser, np-classifier, and ChemWalker[5].

GNPS2 permits remote execution in the UCR server as well as the deployment of the GNPS2 tool into dedicated servers, thus benifiting the laboratories resource-limited and resource-abundant laboratories and ensuring data privacy for those users who need it.

A ChemWalker use case will be presented to show the potential of integrating tools in Nextflow Workflows and in GNPS2 environment. The execution of ChemWalker reduced the execution time 5x by the use of parametrization and parallelization.

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O4
COMPREHENSIVE METABOLOMIC CHARACTERIZATION OF WHOLE-BODY PIGR DELETION IN SERUM, LIVER AND VISCERAL WHITE ADIPOSE TISSUE BY GC-MS

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Obesity represents the leading cause of several noncommunicable diseases, including type 2 diabetes mellitus (T2DM). In fact, the associated risk of developing T2DM in overweighed individuals is enormously high. Nevertheless, the mechanisms at the basis of this relationship are far from being understood and rely on complex interplays between glucose homeostasis, inflammation and autoimmunity.

Polymeric immunoglobulin receptor (pIgR) plays a central role in mucosal immunity by facilitating IgA translocation across epithelial barriers during the immune response. High fat diet (HFD) is well-known to trigger several immunogenic events and has been associated to altered IgA frequency and production in mice. Furthermore, HFD-fed-IgA-deficient mice show exacerbated adipose tissue inflammation and disrupted glucose and host-microbiome homeostasis, suggesting a possible involvement of pIgR in obesity-induced insulin resistance (IR).

In order to understand the role of pIgR and IgA during the onset of T2DM, we applied GC-MS-based untargeted metabolomics to characterized metabolites alterations in serum, liver and visceral white adipose tissue (vWAT) in the pIgR-/- murine model of obesity-induced IR.

Following metabolite extraction, supernatant was dried-out and submitted to a two-step derivatization process consisting of methoximation and sylilation. The amount of reagents and sample was optimized to achieved the best recovery. Samples were analyzed using an 8890 Agilent GC-system coupled to the LECO Pegasus BT-TOF-MS. Data processing, including deconvolution, alignment and metabolites identification were performed using LECO ChromaTOFSync. Low analytical variability was confirmed through quality assurance, with statistical analysis revealing significant differences in carbohydrates, fatty acids, nucleic acids and organic acids across serum, liver, and vWAT.

The identified alterations could serve as pivotal indicators, shedding light on the complex mechanisms underlying T2DM onset in an obesogenic environment. This study underscores the importance of PIgR and IgA in modulating metabolic processes and highlights the potential of metabolomics in elucidating disease pathogenesis and identifying therapeutic targets.

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O5
DRIED BLOOD MICRO-SAMPLING METHODS FOR DETERMINATION OF OXIDIZED LIPIDS

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Dried blood micro-sampling methods are designed for collecting minimally invasive small-volume blood samples (<100 µL) and their use may convey advantages in terms of stability, handling, and storage space. The aim of this work was to assess the performance of Pre-Cut Dried Blood Spots (PCDBS) and Volumetric Absorptive Micro-Sampling (VAMS®) devices for the

determination of oxidized lipids, using small-volume liquid whole blood (WB) sampling as reference. In this context, using 30 µL of spiked blood, both PCDBS and VAMS® were dried for two hours. Subsequently, a liquid-liquid extraction was conducted using 150 µL of methanol, followed by a sonication and centrifugation step. Further purification was achieved through solid- phase extraction (SPE) using Oasis HLB cartridges (30 mg, Waters). The extracted samples were then analyzed using Liquid Chromatography–tandem Mass Spectrometry (LC-MS/MS) to quantify the target compounds. The method was validated over a three-day period for a panel of 28 non-cyclic oxylipins. LOQs ranged between 0.44 and 0.88 nM. Recoveries ranged from 80% to 119% and precision remained consistently <20%. Additionally, the effects of different procedures for internal standard addition, antioxidant stabilization, and solvent elimination on the method performance were evaluated in spiked samples. All tested conditions and devices provided recoveries and precisions within acceptance limits (i.e., <20%), except when the IS was evaporated prior to the application of the sample in the case of PCDBS and liquid WB samples. This study demonstrates the applicability of different blood micro-sampling approaches for the analysis of a wide range of oxidized lipids. Future research is essential to assess long-term storage effects before integrating novel micro-sampling into clinical studies.

O6

SPATIAL METABOLOMICS IDENTIFIES ACCUMULATION OF LPC(18:0) AND LPA(18:1) IN MURINE ADVANCED PLAQUES WITH TRANSLATION TO HUMAN CAROTIDS AND PLASMA FOR CV-RISK ESTIMATION

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Background

Cardiovascular (CV) diseases are the leading cause of death globally being atherosclerosis the most common underlying cause. Atherosclerosis consists of profound arterial remodeling and plaque build-up. First, we aimed to identify and localize the metabolic changes occuring between control and atherosclerotic murine aortas. Second, we investigated the differences between early and advanced plaques and, third, the translation of the results to human carotid arteries and plasma for CV-risk estimation.

Methods

Atherosclerotic and control aortas (n=22) were obtained from low-density lipoprotein receptor-deficient mice. Mass spectrometry imaging (MSI) was applied to identify region-specific metabolic differences via histology-guided virtual microdissection. Early and advanced plaques were compared within the same atherosclerotic animals. Metabolites contributing to plaque progression were further analyzed by targeted MSI in 9 human atherosclerotic carotids and LC-MS/MS in human plasma from subjects with elective coronary surgery (CV-risk) and controls (n=54).

Results

MSI identified 362 local metabolic alterations in atherosclerotic mice (log2 fold-change≥1.5; p-value≤0.05). Cardiac tissue showed generalized accumulation of glycerophospholipids, except for lysolipids which were decreased in atherosclerotic animals. Lysolipids and other glycerophospholipids were found increased in all three arterial layers of atherosclerotic aortas. Murine advanced vs. early plaques had significantly higher levels of LPC(18:0) (lysophosphatidylcholine; p-value=0.024) and LPA(18:1) (lysophosphatidic acid; p-value=0.025). Both lysolipids were also found elevated in human advanced vs. early carotids in fibrosis-rich areas. LPA(18:1) (p-value=0.031) and LPC(18:0) (p- value<0.001) were found to be significantly reduced in human plasma from CV-risk subjects. LPC(18:0) showed CV-risk association (OR=0.479[0.225-0.883], p-value=0.0323) and CV-risk estimation potential (AUC=0.778[0.638-0.917]).

Conclusions

Spatially resolved metabolomics showed alterations in phospholipids in both aorta and adjacent cardiac tissue. LPC(18:0) and LPA(18:1) were identified in murine and human arteries implicated in plaque progression, with reflection in human plasma for CV-risk estimation.

O7

ADVANCED SUSPECT SCREENING APPROACH TO UNVEIL CHILDREN'S PERSONAL CHEMICAL EXPOSOME USING SILICONE WRISTBANDS

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Exposure to environmental chemicals is a global health threat, especially for children. Increasing evidence suggests that such exposure can potentially induce epigenetic and metabolic changes, leading to chronic and non-communicable diseases that may remain clinically undetected until later-in-life[1,2,3]. As the long-term effects of children's exposure are still underestimated, comprehensively characterizing the personal chemical exposome is crucial for understanding the link between early-life exposures to health outcomes. Traditional target approaches focus on determining specific classes of compounds, overlooking the combined effects of multiple exposures. In environmental biomonitoring, one of the main challenges is developing a wide-scope screening approach to access a broader characterization of the chemical exposome and environment-health associations[4]. Silicone wristbands (WBs) have recently emerged as low-cost, easy-to-use, child- friendly and effective personal passive sampling devices for volatile and semi-volatile organic compounds[5]. This study presents an advanced wide-scope suspect screening workflow to characterize the early-life chemical exposome using WBs of 218 children (8-11 years old) living in the area of Tarragona (Spain), which is home to the largest petrochemical site in Southern Europe. The workflow used liquid chromatography/high-resolution mass spectrometry and a suspect screening analysis based on HERMES (open-source R package)[6]. HERMES is a molecular formula-oriented and peak detection-free optimization method to generate sample-specific and non-redundant inclusion lists (ILs) for MS/MS analysis. NORMAN Substance Database and in-house thirdhand smoke database (38147 unique molecular formulas) were used as libraries. The analysis enabled the detection of 634 indoor and outdoor atmospheric pollutants in the WBs, including tobacco smoke toxicants, personal care products, insect repellents, and plastic additives, and 5 were identified by standards. Preliminary results confirm that optimized MS/MS acquisition by Hermes improved mass spectral similarity and identification rates to better characterize the early-life exposome.

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O8

BENCHTOP NMR SPECTROSCOPY: A GAME-CHANGER IN CLINICAL METABOLOMICS

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Building on our initial results presented at SESMET 2022 using a 60 MHz Magritek spectrometer (1,2,3), we have made significant strides in benchtop NMR (bNMR) metabolomics for clinical diagnosis. This presentation highlights three independent studies conducted over the past two years utilizing an 80 MHz Magritek Spinsolve spectrometer and optimized pulse sequences.

1. Unraveling COVID-19 Severity with bNMR:

   During the COVID-19 pandemic, accurate patient stratification was critical. Our pilot study employed bNMR analysis of serum samples to differentiate between COVID-19 patients based on disease severity (hospital discharge, hospitalization, and ICU admission). bNMR not only identified known respiratory failure biomarkers but also effectively separated patient groups using PCA. This pioneering work demonstrates bNMR's potential to aid clinical decision-making in COVID-19 management.

2. bNMR: An Ally in the Fight Against Zoonotic TB:

   Tuberculosis (TB) in domestic ruminants poses a zoonotic threat. This study validates bNMR's diagnostic accuracy in goats, differentiating bTB-infected from healthy animals and even distinguishing them from paratuberculosis (PTB), a TB mimic. This validate our approach as a multi-species tool and opens doors for bNMR as a complementary or alternative diagnostic tool for animal TB, potentially impacting public health.

3. Early Detection of Tuberculosis in Children:

   Pediatric TB diagnosis faces hurdles due to insensitive tests, lengthy procedures, and the unsuitability of sputum collection methods for young children, hindering early detection and treatment. This study paves the way for a precise pediatric TB diagnostic tool. bNMR metabolic fingerprinting of serum samples, analyzed by PCA, successfully differentiated between tuberculosis, latent TB infection, and healthy controls. This validates the use of bNMR for TB identification in children, potentially leading to improved early detection and treatment.

Conclusion

These studies showcase the remarkable potential of bNMR technology for robust, cost-effective clinical metabolomics, offering valuable insights into disease diagnosis and management.

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O9

FECAL METABOLOMICS FOR THE STUDY OF THE EFFECT OF HYPOXIA AND SMOKING IN MICE WITH LUNG CANCER BY HIGH- RESOLUTION MASS SPECTROMETRY AND ION MOBILITY HPLC-QTOF-IMMS

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Lung cancer (LC) is the leading cause of cancer-related deaths globally. It is widely believed that smoking (S) accounts for nearly 90% of the risk of developing lung cancer [2]. Additionally, hypoxia (H) has been recognized as a significant factor in the progression and onset of cancer in various types of tumors, including LC [3]. Furthermore, the gut microbiome and metabolites are known to play a crucial role in the advancement of lung cancer. Changes in fecal metabolites can offer valuable insights into this disease, shedding light on the impact of smoking and hypoxia. In this study, 80 fecal samples from mice were divided into 8 groups: a group

of mice that were injected with a carcinogen for the development of lung cancer (PNN), a group of mice that were induced to hypoxia (NNP), a group of mice that were exposed to tobacco smoke (NPN), a control group without carcinogen, tobacco, or hypoxia (NNN), a group of mice exposed to carcinogenic and tobacco (PPN), a group of mice exposed to carcinogenic and hypoxia (PNP), and a group of mice exposed to carcinogenic, tobacco and hypoxia (PPP). We utilized a fecal metabolomic approach, employing reverse phase (RP) and Hydrophilic interaction chromatography (HILIC) high-performance liquid chromatography (HPLC) mass spectrometry (MS) with a quadrupole and time-of-flight analyzer (QTOF) and ion mobility (IM). The results of the Partial Least Square Discriminant Analysis (PLS-DA) indicated distinct classifications of the groups, with several fecal altered metabolites identified, including steroids, prenol lipids, and fatty acyls. These metabolites were predominantly found in the PPP group compared to the control group, suggesting that smoking and hypoxia can lead to disturbances in lung cancer

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O10

THE INTERACTION OF SELENIUM AND XENOBIOTICS WITH THE GUT MICROBIOTA BY COMBINING METALLOMICS AND (META)-OMIC METHODOLOGIES

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Metals and bioelements present in the environment or food, essential or toxic, can shape the microbiota diversity, richness, composition and the microbial-produced metabolites in mammals and thus, they may affect the gut-brain and gut-gonad crosstalks [1]. Among bioelements, selenium (Se) is very relevant to health, especially due to the antioxidant character of selenoproteins [2] and for this reason, it has been widely used in nutraceuticals and functional foods. Herein, we investigated the potential role of Se and xenobiotics in the gut-gonad and gut-brain axes using BALB/c mice (Mus musculus) fed Se-supplemented or standard rodent diets. Gut, brain and testicular metabolomes were determined by ultra-high performance liquid chromatography coupled to quadrupole time of flight (UHPLC-QTOF-MS) and gas chromatography coupled to mass spectrometry (GC-MS), while gut microbiota taxonomy was obtained by 16S rRNA gene sequencing. A metallomic approach based on 2D-chromatography with inductively coupled plasma mass spectrometry (ICP-MS) was combined with transcriptomics for the determination of mice selenoproteome. Pollutants and Se modulated gut microbiota and brain, testicular and gut metabolomes with specific associations between them. Moreover, testicular and brain selenoproteins were also associated with specific gut microbes 2,3]. Thus, there is a potential key interaction between Se intake–microbiota–metabolites with effects on the host health at multiple levels including gut-brain axis and reproductive health. Several metabolic impairments caused by environmental pollutants were antagonized by Se. Another axis is the entero-mammary gland route for the transference of maternal microbiota to the newborn through the human milk (HM), skin and parturition. Our results suggested that selenoproteins present in HM are associated with HM microbiota and with the gut microbiota of the newborn, and that maternal health shape HM composition. Our results open further research related with metals, metabolites and microbiota due the potential implications for the maternal-infant health that should be explored.

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O11

ADAPTATION OF LIVER METABOLISM TO EXERCISE WITH AND WITHOUT FOOD RESTRICTION – IN VIVO METABOLIC FLUX ANALYSIS USING MULTIPLE STABLE ISOTOPE TRACERS

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Exercise is an important lifestyle intervention for the prevention and treatment of obesity and can lower hepatic steatosis without weight loss. This effect may stem from increased oxidative demand in liver. Increased hepatic gluconeogenesis and TCA cycle flux have been observed during exercise, but it is unclear whether this is sustained chronically and if the adaptation is also altered by nutritional status. Liver metabolism is altered by nutritional status: fasting promotes hepatic fat oxidation, gluconeogenesis and ketogenesis, while feeding promotes glycogen storage and fat synthesis. Consumption of food during and between bouts of wheel running likely diminishes the role of liver in maintaining euglycemia via gluconeogenesis which requires oxidative metabolism and thus may prevent hepatic adaptations to exercise. Here we tested the hypothesis that daily wheel running exercise when food is restricted will induce upregulated gluconeogenesis and oxidative flux, an effect that will be blunted with ad libitum feeding. Sprague-Dawley rats were kept sedentary or provided voluntary wheel running (VWR) for 4 weeks in the presence or absence of food restriction during the dark phase when rats spontaneously run. Experiments were conducted one day following the last exercise bout. In vivo metabolic flux was examined by stable isotope infusions of [U-13C3]propionate, [3,4-13C2]glucose and 2H2O in rats using mass spectrometry and computational analysis. Chronic VWR in the fed state had opposite effect comparing to food restriction with respect to glucose metabolism. Exercise in fed state resulted in lower anaplerotic fluxes, but not in food restricted group. Notably, food restricted VWR rats had chronically induced mitochondrial fat oxidation which coincided with substantially lower ketone and non-esterified fatty acid levels. Thus, VWR during food restriction triggered an increase in hepatic energy demand that was sustained for at least 24 hours, while the effects of exercise in the fed state were opposite.

O12

INTEGRATIVE STUDY THROUGH FECAL METABOLOMICS AND METAGENOMICS UNVEILS COMPLEX HOST-MICROBIOME INTERACTIONS IN ANOREXIA NERVOSA

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Anorexia nervosa (AN) is a complex psychiatric disorder marked by extreme weight loss and body image distortion. Despite extensive research, the pathogenesis of AN remains enigmatic. However, emerging evidence suggests a significant role of host-microbiome interactions in its development1,2. This study presents an integrative analysis of fecal metabolomic and metagenomic profiles to elucidate host-microbiome interactions and their potential in assessing disease severity.

We analyzed 60 fecal samples from AN patients and 20 from healthy individuals (Hospital Universitario Niño Jesús, Madrid, Spain). Untargeted metabolomics of AN subjects by high-resolution GC-QTOF-MS post-methyl chloroformate derivatization and quantitation of microbial metabolites by LC-QqQ-MS was performed, including short-chain fatty acids (SCFAs) and D-/L- amino acids (AAs), for the first time in this cohort. Additionally, shotgun metagenomic sequencing was conducted using the Illumina HiSeq platform.

Over 200 compounds were annotated in AN fecal samples based on retention times and EI-MS spectra matched against exact mass libraries. Statistical analyses discerned metabolic variability, leading to the identification of three distinct metabotypes (M1, M2, and M3). Notably, microbial metabolites varied significantly between patients and controls. Metabotype M3 closely

resembled the control group in bacterial and metabolome composition, exhibiting higher commensal abundance and SCFA concentrations. In contrast, M2 and M1 displayed pro-inflammatory microbial profiles. Pathway analysis indicated an enrichment of xenobiotic metabolism in patients, alongside altered starch and amino acid metabolism.

The observed metabolomic dysbiosis, corroborated by metagenomic sequencing, suggests profound gut microbiome alterations in AN. Metabolic profiling identified unique AN metabotypes with varying fecal metabolites and microbiome compositions, implicating their potential role in disease severity. Elevated commensal and SCFA levels, associated with anti- inflammatory and neuroprotective effects, underscore the therapeutic potential of modulating the gut-brain axis. This study highlights the criticality of multi-omic integration to decode the intricate host-microbiome dynamics in AN, paving the way for novel gut-centric therapeutic strategies.

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O13

PROBING ERYTHROCYTES AS SENSITIVE AND RELIABLE SENSORS OF METABOLIC DISTURBANCES IN THE CROSSTALK BETWEEN CHILDHOOD OBESITY AND INSULIN RESISTANCE

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Although insulin resistance (IR) is among the most frequent and pathogenically relevant complications accompanying childhood obesity, its role in modulating and exacerbating obesity pathophysiology has not yet been completely clarified. To get deeper insights into the interplay between childhood obesity and IR, we leveraged a comprehensive experimental design based on a combination of observational data, challenge tests (i.e., oral glucose tolerance test), and ex vivo assays (i.e., incubation of erythrocytes with insulin) using a population comprising children with obesity and IR, children with obesity without IR, and healthy controls, from whom plasma and erythrocyte samples were collected for subsequent metabolomics analysis. Children with concomitant IR showed exacerbated metabolic disturbances in the crosstalk between endogenous, microbial, and environmental determinants, including failures in energy homeostasis, amino acid metabolism, oxidative stress, synthesis of steroid hormones and bile acids, membrane lipid composition, as well as differences in exposome-related metabolites associated with diet, exposure to endocrine disruptors, and gut microbiota. Furthermore, challenge tests and ex vivo assays revealed a deleterious impact of IR on individuals' metabolic flexibility, as reflected in blunted capacity to regulate homeostasis in response to hyperinsulinemia, at both systemic and erythroid levels. Thus, we have demonstrated for the first time that metabolite alterations in erythrocytes represent reliable and sensitive biomarkers to disentangle the metabolic complexity of IR and childhood obesity. This study emphasizes the crucial need of addressing inter-individual variability factors, such as the presence of comorbidities, to obtain a more accurate understanding of obesity-related molecular mechanisms.

O14

A CITIZEN-SCIENCE-ENABLED CORRELATION OF THE VAGINAL MICROBIOME, METABOLOME AND ASSOCIATED FACTORS

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A vaginal microbiome dominated by Lactobacillus taxa is considered as 'healthy' or 'optimal' especially when L. crispatus is represented in high numbers. Lactobacilli can prevent preterm birth and vaginal infections, but the mechanisms are not well understood, in part because the functionality of the vaginal microbiome is only studied at a limited level with omics and related approaches. To better understand the composition and activity of the vaginal microbiome in healthy women, we have organized a large-scale citizen's science project, named Isala. in Flanders. Women provided self-taken vaginal swabs that are analyzed

by high-throughput genomics and metabolomics combined with dedicated functional experiments, such as cell culture models and synthetic microbial communities. A subpopulation of 257 Isala participants was selected based on infection history, age (19-53 years old), and contraceptive method. The vaginal metabolic profiles were shown to cluster based on their microbiome composition. Indeed, most of the variance in the untargeted metabolomics data, generated through Hydrophilic Interaction Liquid Chromatography (HILIC) LC-MS QToF, could be explained by the dominant taxon, and not by lifestyle factors such as age, having had children, or hormonal use. For instance, the less optimal Prevotella and Gardnerella-dominated profiles showed distinct metabolic signatures compared to Lactobacillaceae-dominated samples. Moreover, a targeted metabolomic strategy was used to search for cross-feeding and immunomodulatory molecules, which showed elevated levels of indolelactic acid and RL-6,7-diMe (intermediate of vitamin B2) in L. crispatus dominance, and fumarate with L. jensenii dominance. Altogether, these results suggest species-specific biosynthesis roles for lactobacilli versus pathobionts in the vagina. These functionalities are currently being validated with synthetic communities (mono, di- and tri-cultures) of members of the L. crispatus module and molecular networking analysis.

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O15
EXPLORING THE INDIVIDUAL VARIABILITY IN DRUG-INDUCED LIVER INJURY (DILI) RESPONSES THROUGH METABOLOMIC ANALYSIS

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Background and Objectives

Drug-induced liver injury (DILI) is an adverse hepatic event presenting diagnostic and prognostic challenges. Clinical DILI categorization into hepatocellular, cholestatic, or mixed phenotype is based on the ratio of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) over normal values, however, this classification may not capture the full spectrum of DILI subtypes. In addition, there are differences in patient's response exposed to the same drug. For assessing this individual variability, we examined metabolomic changes in plasma of affected patients.

Material and Methods

79 DILI patients assessed by the RUCAM diagnosis scale were recruited. 278 plasma samples were collected and subjected to LC/MS for metabolomic analysis. Metabolite annotation was performed using MS/MS data and databases like the HMDB and METLIN. For multivariate supervised analysis, PLS-DA was performed.

Results

This study involved the analysis of 278 plasma samples from a cohort of 79 DILI patients exposed to 31 different drugs, using PLS-DA to discriminate between cholestatic, hepatocellular, and recovered DILI patients. Results were integrated into a ternary diagram to display the disease phenotype, the severity of the liver damage, and its progression. Through the identification of various metabolites (including free and conjugated bile acids and glycerophospholipids), and the integration of this information into predictive models, we evaluated the extent of the hepatocellular or cholestatic phenotype and assigned a numeric value with the contribution of each phenotype into the patient's general condition. While some compounds exhibited consistent responses across patients, others displayed significant variability. Additionally, our analysis unveiled the potential transition from one sub-phenotype to another during disease progression, highlighting a spectrum of residual DILI metabolic features often overlooked in current clinical diagnosis and patient follow-up protocols.

Conclusions

Monitoring the metabolome might be an additional informative and helpful procedure to assess DILI events in clinical practice.

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O16

MULTI-OMICS INTEGRATION REVEALS POTENTIAL BIOMARKERS FOR ASTHMA TREATMENT MONITORING WITH MEPOLIZUMAB

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Asthma presents as a multifactorial, inflammatory condition marked by airway hyper-responsiveness, obstruction, and remodeling. Severe cases suffering from recurrent exacerbations may require add-on biological treatment, such as the humanized monoclonal antibody mepolizumab, which targets IL-5. Previous results from our group suggest that metabolites such as sphingolipids, lysophospholipids, along with proteins such as Th2 cytokines and growth factors, are correlated with allergic inflammation and asthma severity. Thus, we aim to identify potential biomarkers for monitoring treatment evolution by integrating targeted metabolomics and proteomics data from severe asthmatic patients treated with Mepolizumab.

Serum samples from severe asthmatic patients treated with mepolizumab (n=36) were collected before treatment initiation (T0), and 6 (T1) and 18 (T2) months after starting the treatment. Samples were analyzed liquid chromatography coupled to triple quadrupole mass spectrometry (LC-QqQ-MS) from Agilent Technologies, for the analysis of previously identified metabolites. Additionally, targeted proteomics using Olink® Target 48 Cytokine panel was employed. Metabolomics and proteomics were integrated using OmicsAnalyst tool and Receiver Operating Characteristics (ROC) analyses; moreover, a correlation analysis between clinical variables and omics data was performed.

Mepolizumab induced changes in the metabolomic (e.g., sphingolipids, fatty acids, carnitines) and proteomic (e.g., chemokines, cytokines, growth factors) profiles of severe asthmatic patients. Metabolic changes were predominantly found at T1, while the protein profile showed a continuous change until T2. Interestingly, omics integration and ROC analyses showed that arachidonic acid, oleic acid, palmitoleic acid, propionylcarnitine, CCL11 and TNFSF10 were the more useful biomolecules for monitoring Mepolizumab treatment. Furthermore, these changes showed a significant correlation with the measured clinical variables.

Mepolizumab treatment altered the metabolomic and proteomic profiles in severe asthmatic patients. Omics integration results provide new potential biomarkers for treatment monitoring. Nonetheless, more analyses are needed to further confirm and understand these results.

O17

DEVELOPMENT OF CHEMICAL BIOLOGY METHODOLOGIES FOR THE INVESTIGATION OF MAJOR PHASE II METABOLITE CLASSES IN HUMAN SAMPLES

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The clearance of xenobiotics in the human body is a multistep process consisting of phase I and phase II modifications to alter the metabolite structure. These biotransformations are important across diverse fields such as biomarker discovery, toxicology, nutrition, and microbiome metabolism. The major phase II modifications are sulfation and glucuronidation facilitated by specific enzymes that have also been associated with microbiota-human host co-metabolism. Furthermore, the importance of the microbiome metabolism on the conversion of dietary compounds has been revealed to impact human physiology, influencing

the production of toxins. We have previously developed selective enzymatic treatment methods of metabolites in human samples for improved analysis of sulfated and glucuronidated metabolites. This approach led to the detection of 206 sulfates and 191 glucuronides, including more than 70 microbiota-derived compounds.

As a next step, we recently introduced a new chemical biology tool to overcome the challenge of parallel investigation of these two phase II modifications and their corresponding unconjugated aglycons in a single sample. In this new methodology, we utilized immobilized recombinant enzymes for treatment of human urine samples obtained from a dietary intervention study. After the selective enzymatic conversion of sulfated and glucuronidated metabolites, the samples were subjected to UHPLC-MS/MS analysis. The obtained raw data were processed with the XCMS metabolomics framework to selectively identify phase II metabolites and the metabolite structure was validated via authentic standards or MS/MS fragmentation. This separate MS investigation of each metabolite class in a single sample was successfully applied to obtain the dietary glucuronidation and sulfation profile of about 100 compounds. We have now upscaled the investigation of the altered sulfatome and glucuronide metabolome in human samples, and also included N-acetylated compounds. Our advanced metabolomic strategies offer valuable insights into elucidating metabolic biomarkers associated with interactions between the gut microbiota and the human host.

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O18

ASSESSING THE PRACTICAL UTILITY OF EXISTING MACHINE LEARNING-BASED COLLISION CROSS SECTION PREDICTION MODELS FOR SMALL MOLECULE ANALYSIS

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Due to the advent of ion mobility, new machine learning (ML)-based models to predict collision cross section (CCS) values are continuously being reported. Often, these models are trained over different datasets, which hampers their comparison. This further raises questions pertaining to benchmarking and unbiased evaluation of existing and new models. We compared four representative ML-based CCS prediction models using the recently introduced and large-scale METLIN-CCS dataset, which is composed of 61,000 CCS values from 27,000 molecules. We analyzed how the training and test set molecular similarity impacts the model accuracy to assessed whether this similarity can be used as a metric for the reliability of the prediction. We also assessed the generalization capability of the existing models, i.e., the model's capability to predict new data that has not been seen by the model before. Finally, we tested the models' practical utility for metabolite annotation.

Results showed that existing models share a similar performance, that they suffer from a significant lack of generalization capability, and that simpler linear models can be used as an alternative. Results also showed the limited practical use of current prediction models, and their lack of suffcient accuracy to be adopted in routine small molecule analysis. Our results also reiterate the dependence of the prediction reliability on the structural similarity between training and predicted data. This implies that the diversity of the training datasets is the most important factor in the prediction performance, and any new model will likely perform comparatively similar to previous ones if trained on the same data. Altogether, our study highlights the potential hype around the use of complex ML algorithms to solve basic problems, and it provides a rational framework for testing and validating new ML-based CCS prediction models which can be extrapolated to other prediction problems in metabolomics.

O19
GCDUO: AN OPEN-SOURCE SOFTWARE FOR GC×GC-MS

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Comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS) has emerged as a powerful tool for the analysis of metabolites in complex matrices. The adoption of GC×GC-MS has been hindered by the inherent challenges associated with data analysis. In conjunction with new commercial systems that amplify resolution capabilities, the volume of data generated has continued to grow and their manipulation has become an issue. Furthermore, the gold-standard analysis tool, ChromaTOF (Leco corporation, USA) can be considered a black-box, as no information on how its software functions is detailed, making customizations difficult to incorporate. Other alternatives exist, like Chromspace (SepSolve Analytical Ltd, UK) or Gc Image (Zoex Corp., USA), but none have developed a truly open-source workflow. To solve this issue, we introduce GcDuo, a complete open-source tool tailored for GC×GC-MS data analysis. GcDuo leverages a unique approach by utilizing four-dimensional (4D) raw data applied to a parallel factor analysis (PARAFAC) algorithm. GcDuo software is implemented as an R statistics package, with friendly usage, making it accessible to both beginners and experienced researchers. Key features include data preprocessing, peak detection, identification, and visualization tools. The usefulness of GcDuo was tested with two mixtures (C8-C20 alkanes, and 12 reference standard representative of breath metabolites), at different concentrations. In both datasets, GcDuo successfully analyzed and detected the compounds in an untargeted analysis. Performance of GcDuo was evaluated comparing results to ChromaTOF. Notably, the areas exhibited strong correlations, with Spearman's rho coefficients of 0.856 for the alkane's dataset and 0.909 for the breath mixture dataset. GcDuo represents a new valuable asset in metabolomics toolbox, empowering researchers to create more controllable, automatic, transparent, and scalable workflows. We will present the new GcDuo workflow for GC×GC-MS data, showing these results and the validation with complex datasets.

O20
UNTARGETED METABOLOMIC ANALYSIS TO STUDY THE GUT MICROBIAL METABOLISM OF SAFFRON APOCAROTENOIDS FROM DIFFERENT SOURCES

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Crocin and crocetin are natural apocarotenoids found in the stigmas of saffron flowers (Crocus sativus L.). These compounds have been attributed numerous biomedical and pharmacological properties, mainly related to effects on the central nervous system. This makes saffron extracts highly attractive to consumers and economically beneficial for the nutraceutical industry. Recent studies highlight the key role of intestinal microbiota in the metabolism and neurological effects of these compounds; however, their bioactive metabolites remain unknown. This study evaluated the metabolism of crocins by the human gut microbiota in vitro using an untargeted metabolomics approach. Fecal samples of two volunteers with different metaboypes, presupposing different microbiota, were incubated with standards of crocins and crocetin and also with an extract of genetically modified tomato able to produce crocins as a new source of these apocarotenoids. The metabolomics analysis revealed a cluster of microbial metabolites produced after incubation of both the commercial standards and the tomato extract. The study of the MS/MS fragmentation pattern allowed us to identify these metabolites as a new family of of dihydrocrocetins and tetrahydrocrocetins obtained after rapid transformation of crocin into crocetin by deglycosylation and the further metabolism of crocetin by reduction of double bonds and demethylations as the main reactions produced by intestinal bacteria. These results show for the first time the ability of human microbiota to produce these new metabolites and suggest differences between individuals that may lead to new crocin metabotypes. Furthermore, this work shows tomato extract enriched in crocins as a new source of these apocarotenoids also accesible and metabolized by intestinal bacteria.

O21

INTEGRATING AN IN VITRO DYNAMIC MODEL OF INTESTINAL DIGESTION WITH METABOLOMICS, SHOTGUN METAGENOMICS AND METATRANSCRIPTOMICS: A COMPREHENSIVE FRAMEWORK FOR UNRAVELING GUT MICROBIOTA DYNAMICS

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Deciphering the complex interactions between dietary components, host physiology, and the gut microbiota necessitates sophisticated experimental models capable of recapitulating the dynamic processes occurring within the gastrointestinal environment. In this regard, *in vitro* models of gastrointestinal digestion have emerged as indispensable tools for elucidating the multifaceted interactions between dietary substrates, digestive processes, and microbial communities inhabiting the gut.

In this work, we explore the applicability of a comprehensive framework that integrates *in vitro* model of intestinal digestion with targeted metabolomics, shotgun metagenomics and metatranscriptomics to study gut microbiota dynamics and dietary-microbiota interactions. LC-MS/MS-based targeted analysis of metabolites involved in relevant microbial metabolic pathways was performed to study the evolution of colonic microbiota in the dynamic gastrointestinal simulator SIMGI® fed with L-carnitine, a dietary precursor of microbial trimethylamine (TMA). Gene and genome centric approaches based on state of the art bioinformatic tools were applied for the analysis of MinION sequencing data in DNA and RNA samples obtained from the simulator at different time points.

The results of this study revealed a succession in microbial taxonomy diversity over time. In addition, metagenomic analysis provided a detailed view of the changes and taxonomic distribution of microbial traits that are expected to drive TMA-related metabolism and SCFA generation. Metatranscriptome data pointed to *Colibacter* and *Acidaminococcus* as the most active genus in the butyrate production from acetyl-CoA and L-glutamate, respectively. Furthermore, 168 metagenome-assembled genomes were screened for carbohydrate gene clusters, which were then analyzed to infer their glycan substrates. Obtained substrate predictions for carbohydrate utilization revealed the increase over time in the abundance of genomes belonging to some members *Bacteroides* and *Parabacteroides*, most of them harboring a large number of polysaccharide utilization loci for a great variety of substrates.

O22

PAPRIKA FINGERPRINTING BASED ON ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-HIGH-RESOLUTION MASS SPECTROMETRY (UHPLC-Q-ORBITRAP-HRMS) AS A PROMISING APPROACH TO REVEAL THE STERILIZATION IMPACT IN CONDIMENT'S METABOLOMIC COMPOSITION

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Paprika (*Capsicum annuum*) is one of the most distinctive and worldwide used spice due to its characteristic organoleptic properties [1]. During the production of spices and culinary herbs, they may be contaminated with pathogenic microorganisms of concern. Thus, heat sterilization of condiments, which is free of chemicals and radiation, is consequently becoming a must to ensure microbiologically safe condiments in the European market [2]. In this context, this study presents an innovative untargeted metabolomics approach based on ultra-high performance liquid chromatography (UHPLC) coupled to quadrupole-Orbitrap-high-resolution mass spectrometry (HRMS) fingerprinting to assess for the first time the sterilization impact on paprika metabolomic composition. Paprika fingerprints were obtained applying a simple solid-liquid extraction assisted by sonication using MeOH:H_2O (80:20 *v/v*). Then, a supervised orthogonal partial least squares discriminant analysis (OPLS-DA) model was built based on UHPLC-HRMS paprika fingerprints to discriminate sterilized vs. untreated samples, providing successful sample clustering, high-quality model parameters (R^2Y = 0.988 and Q^2 = 0.904), and excellent predictability for further samples (full correct classification rate of 100%). As a further step, this methodology allowed the putative identification of more than 15 significant metabolites (so-called markers) with high discriminant potential to differentiate between sterilized and non-sterilized paprika, noticing fatty acids and derivatives, amino acids, and organic acids, among other chemical markers. The findings highlighted not only the overall decrease of the fatty acid signature (e.g. linoleic acid, stearidonic acid, etc.) which was markedly affected by the sterilization,

but also the enhancement of other metabolites that may be used as specific markers of sterilized paprika (e.g. DL-malic acid). This study opens the path to novel metabolomics strategies to support the integrity of paprika and other high-valued spices, especially focused on sterilization processes scarcely studied so far. This research was funded by "PPITUAL, Junta de Andalucía-FEDER 2021-2027. Programa: 54.A".

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O23
EVALUATION OF THE CULTIVAR EFFECT ON THE PHENOLIC VARIABILITY OF VIRGIN OLIVE OIL

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Phenols of virgin olive oil (VOO) belong to one of the main families of compounds that are associated with its health benefits and sensory features. In fact, they are responsible for the only health claim specific of VOO included in the European Regulation EU 432/2012. However, its composition is influenced by several factors, with special emphasis on the cultivar, which involves the effect of the enzymatic activity, but also the fatty acid (FA) profile and fruit moisture that determine the polarity of the extraction medium.

In this research, we evaluated the phenolic variability explained by cultivar, fruit moisture, fatty acid, expressed as MUFAs/(SFAs+PUFAs) ratio, and interactions between them, in VOO and pomace. Also, we evaluated the polarity in the transfer of phenols between both phases based on moisture content and fatty acid, in a set of 40 cultivars monitored for three crop seasons. We considered two contributions on the phenolic composition variability: enzimatic, the activity of methylesterase was a critical variable to differences among cultivars by oleacein/oleocanthal content and the aglycone isomers of oleuropein and ligstroside; and polarity of the involved phases in the oil extraction process. Fruit moisture favoured the conversion of oleuropein and ligstroside aglycone form to oleacein/oleocanthal, and VOOs with a high oleic acid were less enriched in oleacein as compared to VOOs with moderate oleic acid content. Considering results obtained, the polarity of involved phases should play an important role in the enzymatic activity and in the transfer of phenols between pomace and oil. Thus, through metabolomics it is possible to know the possible changes in the chemical composition of the VOO, which is affected by several external or internal factors that influence both its nutritional and sensory properties.

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O24
DIVERSITY OF PRIMARY AND SPECIALIZED METABOLITES IN TOMATO

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A Granell in representation of the TRADITOM and HARNESSTOM consortia.

Up to 2 million metabolites are predicted to be present in the plant kingdom, this complexity representing the diversity of metabolism as it results from the evolutionary adaptation of plants as sessile organisms to changing environmental conditions. In tomato small molecules produced in the vegetative parts are key to protect the plant against pathogens and essential in the interaction with insects. At the fruit level small molecules such as carotenoids are important for the aspect of the tomato, for its nutritional value and at least 20 volatile compounds define the flavor of tomato. We have analyzed the composition of a large collection of tomato varieties representing the genetic diversity present in Europe. Metabolomcis analysis included, primary (GC-MS), semipolar and non-polar (UPLC-MS) and volatile compounds (GC-MS). Analyses covered most of the compounds that are believed to impact fruit characteristics but also many others whose functionality remains to be revealed. We have applied this metabolomics platform covering over 500 metabolites to a collection of close to 300 different tomato varieties representing the genetic diversity of traditional tomato present in Europe. Metabolomics analysis revealed main classes of varieties based on global metabolomics analysis and on targeted compounds that are associated to flavor and revealed that occasionally they map against geography. Combination the metabolomics analysis with genotyping data through GWAS allowed the identification of regions of the genome that are important for the accumulation of specific compounds and how specific alleles they were selected in different locations. Metabolomics combined with network and correlation analysis allow us to identify the interactions between different metabolic pathways in the fruit.

O25
DISTINCT METABOLITE SIGNATURES IN TOMATO WHEN SUBJECTED TO GENERALIST TETRANYCHUS URTICAE OR SPECIALIST TETRANYCHUS EVANSI FEEDING REVEALED BY METABOLOMICS

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Spider mites of the Tetranychidae family are phytophagous arthropods feeding on different plant species by piercing cell membranes and sucking the content generating extensive leaf damage and triggering specific hormonal and metabolic responses. Among others, Tetranychus urticae (Tur) is the most widespread species able to feed on more than 1000 plant species through the induction of P450 monooxygenases which act either as pesticide or toxic plant metabolites detoxification enzymes (Dermauw et al. 2013). Non-adapted Tur individuals induce jasmonic acid (JA)-dependent responses which include the synthesis of toxic metabolites and the induction of inhibitors of spider mite gut peptidases (Alba et al. 2015). Conversely, T. evansi (Tev) is a specialist which easily feeds and establishes on tomato. The specialization mechanism is thought to act through the injection of effectors in plant cells that act dampening jasmonic acid (JA)- dependent defenses (Arbona et al. 2020). In recent years, abscisic acid (ABA) has drawn attention (Rosa-Díaz et al. 2024) as a mediator of spider mite-plant interaction. The metabolic response of tomato Solanum lycopersicum cv. Lukullus challenged with Tur or Tev compared to the ABA-deficient cv. notabilis has been studied. ABA deficiency had a different effect on the feeding ability of Tur and Tev, whereas it exacerbated Tur damage and significantly reduced Tev damage, indicating an essential role of this plant hormone in the infestation mechanism of these two spider mites. At the metabolic level, the increased leaf damage induced by Tur in notabilis was accompanied by a reduction or no-accumulation of defense metabolites (α-tomatin, flavonoids, phenylpropanoids, etc...) and an accumulation of nutritional metabolites (sucrose and citric acid). On the contrary, Tev induced in cv. Lukullus an elevated number of metabolites associated to lipid metabolism and signaling which were absent in notabilis, indicating a more orchestrated feeding mechanism of Tev compared to Tur.

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O26

UNTARGETED ANALYSIS TO EXPLORE CHANGES IN THE VOLATILE PROFILE OF TOMATO PLANTS UNDER COMBINED ABIOTIC STRESS

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Plants are often subjected to a combination of multiple stresses, and how they respond to them may have a direct impact on crop production. The main objective of this work was to identify differences in volatile compounds produced by tomato plants under combined oxidative stress and salinity. The effect of stressor concentration and application timing on the metabolic profile was also addressed. Untargeted analysis was employed to identify distinct compounds.

Plants at two different stages of development (5 and 7 weeks old) were subjected to two different stressing levels of combined salinity and oxidative stress (imposed by the herbicide Paraquat): a mild stress (75 mM NaCl and 1 µM Paraquat) and a strong stress (150 mM NaCl and 2 µM Paraquat). Young leaves were sampled at 1, 3, 7 and 14 days after the onset of stress treatment and flash frozen in liquid nitrogen.

The determination of volatile compounds was carried out by gas chromatography coupled to single quadrupole mass spectrometry. MetAlign software (PRI, WUR) was used for the untargeted analysis. Significant differences were identified based both on stress application timing and stressor concentration. The most dramatic effect was observed on younger plants under strong stress. Over 40 volatile compounds were significantly altered, including an increase in several phenylpropanoid volatiles. This study underscores the relevance of carefully establishing an appropriate experimental system, taking into account parameters such as stressor dose and application timing, before systematically studying a particular metabolic process.

Currently, a system is being fine-tuned to study volatile emission and to establish potential correlations between the observed differences in leaf volatile content and the compounds actually emitted in vivo by the plants. Ultimately, this work aims to investigate interplant communication in response to combined abiotic stress conditions in the context of climate change.

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O27

INSIGHTS INTO ENVIRONMENTAL FACTORS AFFECTING STRAWBERRY FRUIT QUALITY: A COMBINED METABOLOMIC AND TRANSCRIPTOMIC ANALYSIS

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Strawberry (*Fragaria × ananassa*) holds a significant status as a globally important crop, both economically and nutritionally. Over the past few decades, there has been a noticeable increase in customer dissatisfaction regarding the declining quality of this crop. The environmental alterations caused by climate change have affected this situation, presenting new challenges in achieving high-quality fruits. Moreover, the composition of strawberry fruit is subject to significant genotype × environment interaction (G×E). Current challenges facing strawberry breeding strategies involve enhancing quality-related parameters alongside the development of resilient cultivars to promote food security and sustainable production. To address this issue, we performed an integrated analysis involving transcriptomics, metabolomics, and machine learning on four june- bearing strawberry cultivars ('Clery' (IT), 'Frida' (NO), 'Gariguette' (FR), and 'Sonata' (NL)) renowned for their genetic diversity and adaptability to different geographical environments across five European locations (Norway, Poland, Germany, Italy and France) spanning from EU-North to EU-South. Recent advances in high throughput metabolomic and transcriptomic approaches have proved to be great tools to address GxE interactions, enabling simultaneous detection and quantification of metabolites under specific conditions and insights into the genetic variants and external factors influencing plant composition. Through this approach, we dissect the plasticity of strawberry genotypes and their potential as breeding material. Additionally, we compared flavor- and nutritional-related metabolites together with transcript profiles, revealing a predominant influence of environment over genotype. Temperature and radiation were determined as primary influential factors on strawberry fruit composition, with highly variable influence across the cultivars, especially on volatile compounds. This underscores the anticipated significant impact of climate change on the composition and fruit quality, emphasizing the need for further resilience studies to capitalize on the cultivars' genetic diversity. In conclusion, our study emphasizes the imperative for continued research aimed at unravelling the underlying mechanisms governing fruit quality traits.

O28

METABOLOMICS IN QUERCUS ILEX: APPLICATION TO STUDIES OF VARIABILITY AND SELECTION OF ELITE GENOTYPES OF HIGH NUTRITIONAL VALUE AND RESILIENCE TO THE DECLINE SYNDROME AND CLIMATE CHANGE

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Quercus ilex is the emblematic and most representative tree species of the Mediterranean forest and the agrosilvopastoral ecosystem "*dehesa*". Since 2004, our research group has been developing a research program aimed at characterizing this species from a molecular biology perspective [1]. Two main objectives are pursued: i) the selection of elite genotypes resilient to stresses and ii) the phytochemical characterization of the acorn. Both objectives are related to the demands of the productive sector, linked to the establishment of breeding programs aimed at addressing two critical issues: firstly, combating tree mortality linked to the decline syndrome, and predicted under a climatic change scenario, and secondly, validating the nutritional and nutraceutical value of acorns for dietary purposes. By following the Central Dogma, a multiomics approach (genomics, transcriptomics, proteomics, and metabolomics) is being employed in the direction of Systems Biology, complemented by physiological, classic biochemistry,

and DNA-based marker techniques. Our goal is to understand the high phenotypic variability of the species, and how the genetic structure, epigenetic marks, allelic variants, gene expression, and gene products interactions determine specific phenotypes (resistance and tolerance to stresses, productivity, fruit size, and organoleptic properties) and contribute to the basic equation of genetics: Phenotype = genotype- epigenetics + environment. We will present data on the metabolomic analysis of: i) leaf tissue of seedlings from different provenances, located in declining and non-declining areas, and subjected to combined stress (drought and *Phytophthora cinnamomi*); and, ii) acorns from different trees grouped by morphotypes and flavours (sweet, neutral, and bitter). Metabolomics data will be integrated with those obtained by other -omics approaches, with particular emphasis on phenolic biosynthetic pathways. The methodology employed and some of the data have been reported in previous publications [2-6].

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O29

TURBOOMICS: A WEB-BASED PLATFORM FOR THE ANALYSIS OF UNTARGETED METABOLOMICS DATA USING A MULTI-OMICS INTEGRATIVE APPROACH

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Multi-omics integrative analysis is an emerging field that allows for a deeper and more comprehensive profiling of interactions among molecular entities across various functional levels. Untargeted metabolomics holds particular promise in this context due to its unbiased approach and its ability to capture the dynamic interactions of the metabolome with the proteome and the genome. However, the integration of these omics datasets is often hindered by the lack of tools that combine putative annotations of metabolomic features with multivariate statistical techniques.

To address this issue, we present TurboOmics (https://proteomics.cnic.es/TurboPutative/TurboOmicsApp.html), a web-based platform that facilitates the integration of proteomics and transcriptomics data with untargeted metabolomics. TurboOmics accepts quantitative data from these three omic fields along with metadata describing the experimental design and sample characteristics. The metabolomics features are annotated based on their experimental mass/charge ratio using Ceu Mass Mediator (1), and the resulting annotations are simplified using TurboPutative (2). TurboOmics provides basic functionalities for preprocessing and exploration of quantitative data (e.g. density plots and PCA). Multi-omics integration is performed using Multi-Omics Factor Analysis (3) coupled with linear regressions between the projections and the metadata of the observations. This approach enables the fast and intuitive detection of statistically significant latent factors and, consequently, the identification of highly correlated molecular entities (transcripts, proteins and metabolites) exhibiting differential behavior among sample groups. Furthermore, TurboOmics performs functional enrichment analysis of relevant proteins and transcripts using g:Profiler

(4). In addition to the putative annotations of the metabolomics features, this novel approach allows a comprehensive analysis that facilitates an integral understanding of the biological system under investigation.

The platform will aid the metabolomics community in performing simple, fast, and intuitive integrative analyses of metabolomics, proteomics and transcriptomics data, thereby gathering valuable information about the associations between these biomolecules and with disease phenotypes.

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O30

MULTI-OMICS DATA INTEGRATION INTO GENOME-SCALE METABOLIC MODELS REVEALS DISTINCTIVE METABOLIC VULNERABILITIES ASSOCIATED TO FOLFOX CHEMOTHERAPY RESISTANCE IN COLORECTAL CANCER

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Colorectal cancer (CRC) is among the most frequent cancers worldwide and the second leading cause of cancer-related deaths in Western countries. In the last few decades, significant progress in survival has occurred with the use of chemotherapy and target-agents. But despite of high initial responses, practically all CRC patients succumb due to disease progression and metastatic CRC (mCRC). Platinum-based therapies, such as FOLFOX chemotherapy, are the most widely used for the treatment of CRC, but its effectiveness is limited by chemoresistance. Metabolic reprogramming is a sensitive target for cancer therapy, but there is a need of more precise understanding of individual patient tumor metabolic reprogramming in order to predict the best metabolic targets in the design of new combined therapies.

Here, we have used multi-omics data integration into Genome-Scale Metabolic Models (GSMMs) to identify that cell lines from different CRC metabolic subtypes present a distinctive metabolic reprogramming associated to FOLFOX treatment. Combining GSMM and the GIMME algorithm, we have identified for each CRC metabolic subtype new metabolic targets to combine with FOLFOX, that would increase the efficacy of FOLFOX as chemotherapy. Altogether, the results show that adaptation to the standard pharmacological treatments entails a metabolic reprogramming at the level of the central metabolism and that GSMMs are a useful tool for the identification of druggable metabolic vulnerabilities towards the design of combined therapies tailored to the patient-specific metabolic subtype.

POSTER COMMUNICATIONS

P1
RECOGNITION AND AVOIDANCE OF ION SOURCE-GENERATED ARTIFACTS IN LIPIDOMICS ANALYSIS

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Background

Mass spectrometry (MS)-based analysis has recently become the gold standard in lipidomics as an effective analytical tool with high sensitivity, specificity, and the power of structural elucidation. However, the artifacts generated using electrospray ionization (ESI) source -and the well-known generation of adduct and clusters- impact the complexity of datasets and spectra and may cause many problems during the identification step.

The aim of this work is to present selected examples for a comprehensive understanding of artifacts coming from in-source fragmentation (ISF), as well as the generation of adducts and clusters in representative lipid subclasses. This was achieved using reverse-phase ultra-high performance liquid chromatography (RP-UHPLC), coupled to a Quadrupole Time-of-Flight Mass Spectrometer (QTOF-MS).

Methodology

For this work, data collected from plasma and serum samples were used. Protocol analysis for plasma samples used mobile phases containing: (A) CH_3COONH_4, NH_4F in H_2O/MeOH; (B) CH_3COONH_4, NH_4F in ACN/MeOH/IPA, and protocol analysis for serum samples used mobile phases containing: (A) HCOOH in H_2O; (B) HCOOH in ACN.

Results

We have selected examples from ESI(+/-) for the recognition of ISF, adducts and clusters in lipidomic analyses that often lead to false-positive annotation: (1) Artifacts resulting from ISF and unexpected heterodimers: examples of cholestadiene ion from cholesteryl esters, and examples from glycerophosphocholines-(PC). (2) Generation of unexpected adducts: when methanol from mobile phases can be oxidized to formic acid during ESI, we identified formate adducts [M+HCOO]- formation in PCs when only [M+CH_3COO]- are expected with our protocol for plasma samples. (3) Generation of clusters from non- volatile salts giving repeating units contaminating MS1 spectra. (4) Generation of convoluted spectra using automated data- dependent acquisition of MS/MS spectra.

Conclusion

Our findings demonstrate that the examples described in this work limit the unambiguous lipid identification. Therefore, a more comprehensive understanding is crucial for an accurate lipid annotation.

P2
RETENTION TIME MODELLING IMPROVES LIPID ANNOTATION IN LC-MS-BASED LIPIDOMICS

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Liquid chromatography (LC) coupled with high-resolution mass spectrometry (HRMS) is the most prominent analytical technique used for untargeted lipidomics. Lipid annotation relies on the acquisition of MS/MS information coupled to spectral matching or the use of fragmentation rules. However, the number of false annotations remains too high for most current lipid annotation tools, most likely due to inappropriate annotation of adducts or identification of in source fragments as intact species, among other reasons. We hypothesize, that the use of the retention time (RT) variable during the annotation process might contribute

to improve lipid annotation, particularly for those species where MS/MS information does not provide sufficient level of evidence to assign an identification.

First, lipid classes capable of being ion source fragments of other classes are analyzed combining clustering analysis based on RT, carbon number (CN) and double bounds (DB) with RT overlapping [e.g. lysophospholipids (LPLs) overlapping with the distribution of phospholipids (PLs)]. Only the non-overlapping clusters are retained. Then, for each lipid class, species that do not follow the expected RT trend based on their CN and DB are removed. Once a subset of highly confident annotations is obtained, RT is modeled for each lipid class based on CN and DB using ordinary least squares (OLS) regression. If the distribution does not follow a linear model, additional models are tested. Finally, new lipid annotations based on MS and RT information are provided. The algorithm will be implemented as a new function within the next release of LipidMS software but it also can be used as a stand-alone tool using as input lipid annotations and features generated with any lipid annotation tool. By using this strategy, the number of incorrect annotations is significantly reduced and new lipids annotations based on MS and RT are proposed.

P3

COMPREHENSIVE CHARACTERIZATION OF THE BRUCELLA ABORTUS LIPIDOME BY LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY REVEALS UNREPORTED LIPID SUBCLASSES AND LOW-LEVEL FATTY ACYL CHAINS

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Brucella are gram-negative, facultative intracellular bacteria that causes worldwide extended zoonosis. One of the pathogenicity mechanisms of these bacteria is their ability to avoid rapid recognition by innate immunity because of a reduction in lipopolysaccharide, free-lipids and other envelope molecules. The composition of Brucella abortus cell-envelope lipidome has been explored by techniques with limited coverage or structural elucidation capabilities, such as thin-layer chromatography[1-4] and gas-chromatography coupled to mass spectrometry[5,6] or flame ionization detection[2]. Thus, a molecular species level picture of the B. abortus lipidome represents a gap in our knowledge. Here, we performed a comprehensive characterization of the B. abortus lipidome applying a lipidomics workflow using reversed-phase liquid chromatography coupled to quadrupole-time-of-flight high resolution mass spectrometry (LC-QTOF-HRMS) followed by a combination of lipid annotation using Agilent MassHunter LipidAnnotator (1.0) with ad-hoc search of free fatty acids, ornithine and lysoornithine lipids (FA, OL and LOL, respectively), subsequent annotation assessment (evaluating m/z tolerance, MS/MS data, adduct profile, and retention time) and compound integration. We determined >200 lipid molecular species encompassed into 8 lipid subclasses known to be present in B. abortus (phosphatidylethanolamines or PE, lysophosphatidylethanolamines or LPE, phosphatidylglycerols or PG, OL, LOL, phosphatidylcholines or PC, cardiolipins or CL, FA), and 3 unreported subclasses (acyl-phosphatidylglycerols or Acyl-PG, lysophosphatidylcholines or LPC, lysophosphatidylglycerols or LPG). In consistency with previous analyses[1,2], FA, PE, PG, PC, and OL represented the gross lipid content, while lyso-, CL and unreported species were minor components. We also expanded our knowledge on the qualitative and semiquantitative composition of FA and lipid-bound fatty acyl chains (FAC) within several subclasses. These ranged from C10 to C20, primarily being these mostly saturated or monounsaturated, and exceptionally diunsaturated. In sum, our study represents the first draft of a comprehensive lipidome characterization of B. abortus and advances our knowledge on its envelope composition.

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P4

IN VITRO AND IN VIVO COMPARISON OF PHYTIC ACID DEPHOSPHORYLATION MECHANISMS BY 3- AND 6-PHYTASES IN BROILER DIETS

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Phytases are supplemented in monogastric animals to increase the dietary bioavailability of phosphorus. Generally, 6-phytases have been preferentially administered over 3-phytases due to the diversity of isolation sources, operational pH, and principally by kinetics, which theoretically is faster for 6-phytases because the axial carbon in phytate is in position number 2; therefore, 6-phytases must have two dephosphorylation mechanisms from positions 6 to 1 and 6 to 5; instead, 3- phytases have only one mechanism from positions 3 to 4. To better understand the activity of these enzymes, we developed in vitro kinetics and in vivo model studies of the dephosphorylation of phytate by 3- and 6-phytases. The results of these studies were analyzed using a direct infusion high-resolution mass spectrometry (LC-QTOF-MS/MS) method to determine phytic acid, all inositol phosphate intermediates, and free inositol. Our results reveal that although 6-phytases begin to dephosphorylation faster than 3-phytases, the conversion rates at the end of the reaction (average digestion time for broilers) were similar. In the same way, differences in reaction mechanisms between the two enzyme types were observed in vivo. For example, broilers supplemented with 6-phytase had higher levels of inositol in the crop and gizzard than those supplemented with 3-phytase. However, no significant differences were found in the ileum of animals supplemented with both phytases. 3-Phytases proved to be an efficient choice for releasing phytic phosphorus in broiler diets as an alternative to the more widely used 6-phytases.

P6

THOROUGH CHARACTERIZATION AND ANALYSIS OF POLYOLS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY IN DIFFERENT BIOLOGICAL SAMPLES

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Polyols are ubiquitous sugar alcohols. Myo-Inositol is involved in signal transduction, and sorbitol is an intermediate in glucose conversion to fructose (polyol pathway). However, there is a lack of knowledge of their sources as well as their functions in diseases such as diabetes, renal dysfunction, cancer or cardiovascular diseases. Therefore, the efficient separation and reliable quantification of the polyol´s epimers could help to achieve these aims. However, their poor ionization yield in electrospray ionization sources and poor resolution of their isomers hinder the capability of LC-MS for a sensitive quantification. GC coupled to high resolution MS is a good alternative overcoming these drawbacks. Here, we present the comprehensive characterization and quantitation of 12 polyols (glycerol (C3 series), erythritol and threitol (C4 series), arabitol, xylitol and ribitol (C5 series), mannitol, sorbitol, galactitol, iditol, talitol, myo-inositol (C6 series)) in multiple biological matrices.

After methoximation-silylation derivatization of pure standards and samples, polyols were separated by high-resolution GC- QTOF/MS, with a DB5-MS column (30 m length, 0.250 mm i.d., 0.25 mm film thickness) and a pre-column (10 m). Different split ratios were tested (1:10 and 1:3). The derivatives were characterized by their corresponding retention times, fragmentation patterns, isotopic profiles, and accurate masses. Quantitation was based on internal standard method.

With the proposed methodology, threitol and erythritol eluted sequentially around 12 min. Xylitol, arabitol and ribitol elute sequentially , and then, mannitol, sorbitol, iditol, galactitol and talitol, elute around 17 min. LOQ were 20 ng.mL-1 and linear range from 20 ng.mL-1 to 750 mg.mL-1. Polyol profiles were studied in plasma/serum, placenta, spleen, macrophages, monocytes, stem

cells, and the similarities and differences among them were reported for identification and quantification. This information could open new perspectives for the study of the function of polyols in health and disease.

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P7

LIQUID CHROMATOGRAPHY-ION MOBILITY-MASS SPECTROMETRY IN METABOLOMICS: BUILDING A LIBRARY FOR OXYLIPIN ANALYSIS

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Oxylipins, key lipid mediators of physiological processes such as inflammation and its resolution, are challenging targets in metabolomics analyses due to their diversity and isomeric structure. Conventional LC-MS techniques often fail to distinguish these isomers effectively[1]. Given their role in different diseases like Niemann-Pick or multiple sclerosis, accurate identification of these compounds could lead to a better understanding of their pathology and to the discovery of reliable diagnostic markers.

Ion mobility spectrometry (IMS) separates ions based on their spatial configuration and provides the collision cross section (CCS) as an additional molecular descriptor. When combined with liquid chromatography (LC) and mass spectrometry (MS),

LC-IMS-MS stands out as a powerful approach for analyzing and characterizing isomeric compounds[2].

In this study, seventy-two oxylipin commercial standards were analyzed using LC-IMS-MS, obtaining each standard's RT, m/z, and CCS in both positive and negative ionization modes. This information was combined into a library which was applied to rat brain samples, more specifically on astrocytes, neurons, and synaptosomes.

Oxylipin measurements were conducted over three different days and showed a relative standard deviation (RSD) of 1% or lower, highlighting the method's reproducibility. Results further demonstrated that positive ionization facilitated the separation of coeluting isomeric oxylipins pairs in the drift time dimension, such as 9-HODE and 13-HODE, and 8-HETE and 12-HETE. In samples, drift time separation permitted the discovery of new oxylipins species.

The coupling of IMS to LC-MS and the measurement of CCS for different oxylipin adducts have facilitated the resolution of isomers previously indistinguishable by LC-MS alone. Moreover, this technique permits the reliable identification of oxylipins in neural samples, providing insights into their differential distribution and role.

This technological advancement offers unprecedented detail in oxylipin characterization, providing new opportunities to explore oxylipin dynamics across various conditions and processes.

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P8
SPECTRAL ANNOTATION ROBUSTNESS IN METABOLOMICS: ADDRESSING VARIATIONS IN ACQUISITION CONDITIONS

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Tandem mass spectrometry (MS/MS) is a powerful tool widely used in metabolomics to identify and quantify metabolites. Spectral annotation, a key step in MS/MS data analysis, involves comparing acquired spectra with spectral databases to assign putative identifications with confidence scores. This process requires automation to efficiently handle the large amounts of data generated in metabolomics experiments.

The reliability of spectral comparison is critically affected by the mismatch between the acquisition conditions of acquired spectra and those established on the spectral databases, often vendor libraries such as NIST20 (1). The use of open-source libraries, such as MonaEU (2), can magnify even more the variability of acquisition workflows, instrumentation and settings, resulting in a low reliable and heterogeneous spectral library.

The collision energy (CE) used for spectral fragmentation plays a crucial role in determining the fragmentation patterns observed in MS/MS spectra. Different collision energies in CID dissociation of the same precursor ion produce spectra with different precursor / product intensity ratios and fragment heterogeneity (3), resulting in poor metric scores. A standard practice in MS/MS data acquisition is the use of stepped collision energy spectra, where the precursor ion is fragmented at multiple collision energies and compared to conventional (single CE) spectral databases, which poses a challenge for accurate annotation of stepped spectra.

This study aims to evaluate different annotation methods to assess their performance when the acquisition conditions of acquired spectra differ from those used to build the spectral database. By systematically comparing different annotation strategies under different conditions, this research aims to identify the most robust and reliable approaches for spectral annotation in metabolomics.

The results of this study are expected to contribute to the development of improved spectral annotation methods that are more resilient to variations in acquisition conditions, improving the reproducibility of metabolomics analyses.

P9

HARNESSTOMDB A DATABASE FOR TOMATO METABOLOMICS INTEGRATED WITH OTHER OMICS AND TOOLS

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Small molecules play vital roles in plant and fruit development, defense, and environmental interactions. Over past decades, the scientific community has made significant advancements in characterizing tomato metabolome across diverse accessions. However, this valuable metabolomic information, dispersed across various and labs, often lacks integration with essential datasets like plant passport, environmental conditions, other phenotypes, and genetic data. These limited the use of metabolites and their source of origin for breeding initiatives focused on sustainable agriculture and food quality.

HarnesstomDB (accessible at https://gateway.harnesstom.eu/) integrates tomato genetic resources information and pre-breeding bioinformatics tools. It is provided by a BrAPI-compliant JSON-based REST API, allowing integrate tools and services from other databases web services and data exchange with other systems. HarnesstomDB serve as a centralized hub, enabling efficient storage, integration and exploration of experimental metadata, accession details, phenotypic, metabolic, images, genetic, genotyping, QTL, and GWAS variant data. Currently, HarnesstomDB houses metadata for 872 metabolites found in tomatoes, with the capability to create or submit new ones as needed. Each metabolite consists of a unique combination of a metabolic trait, an analyte, and a scale. Metabolic traits are based on CHEBI ontology. Analytes are identified by the lab-specific code or ID and annotated following Minimum Reporting Standards for Metabolomics Experiments (MSI), with a strategy that accommodates variations in the analyte name assigned or the level of identification stringency. One invariable part composed by the analyte code uniquely and invariably linked to the Mass to charge ratio or the retention time and the detection method, while name linked to annotation and identification confidence Levels. Additionally, metabolites are categorized into biochemical groups and classified based on health benefits and flavor profile associations.

In summary, the integration provided by HarnesstomDB facilitates the understanding of the metabolomics networks its regulation and access to genetic materials for breeding for metabolites

P10

VALIDATION OF NMR-BASED URINE METABOLOMICS OF PEDIATRIC TUBERCULOSIS DIAGNOSIS

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Introduction

Pediatric tuberculosis (TB) diagnosis is complex due to the gold standard method is required a sputum sample. The sputum collections in children are challenging, therefore other non-invasive samples are considered. Urine Nuclear Magnetic Resonance (NMR)-based metabolomics provides unique fingerprinting of the disease's metabolic status, making it a promising tool to TB

diagnosis. This was demonstrated in our previous study where we found metabolic patterns to differentiate between healthy children and those with tuberculosis in a Haitian cohort with an accuracy of 0.7.

Aims

Validate the use of urine samples to identify metabolomic fingerprinting associated with TB and distinguish them from latent TB.

Methods

The study included 145 patients under 18 years from 11 hospitals of the Spanish Pediatric TB Network. The cohort included children with active TB, cases of latent TB infection (TBI), and healthy contacts (HC). Samples acquired post- initiation of anti-TB treatment were excluded from analysis (TB= 62; TBI= 17; HC= 22). Urine samples were mixed with 0.2M phosphate buffer solution (2:1) to adjust the internal pH to 7.4 and were analysed by a High-Resolution NMR spectrometer (Bruker AVIII 500 MHz). Mnova software (Mestrelab, Santiago de Compostela) was used to process the resulting NMR spectra and Metaboanalyst 6.0 software was employed for multivariate statistical analyses.

Results

Partial Least Squares Discriminant Analysis (PLS-DA) models were trained in three scenarios: 1) TB+TBI vs HC, 2) TB vs HC, 3) TB vs TBI, and their performance was evaluated using cross validation. Accuracy values of 0.76, 0.7 and 0.77 was obtained, respectively.

Conclusions

This study highlights the potential of NMR-based metabolic fingerprinting as a feasible non-sputum option for diagnosing pediatric tuberculosis.

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P11
DECIPHERING FRAILTY IN CKD: A METABOLOMIC AND LIPIDOMIC APPROACH

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Introduction

Frailty is a clinical syndrome marked by decreased physical activity, strength, and physiological reserve, leading to increased vulnerability. Chronic kidney disease (CKD) patients often exhibit frailty, which correlates with adverse outcomes. Despite this, routine frailty screening in nephrology is hindered by the absence of specific biomarkers and a comprehensive understanding of its molecular basis and its association with CKD progression.

Objective

The intricate nature of CKD and frailty necessitates the identification of precise biomarkers for personalized medical approaches. Untargeted metabolomics and lipidomics analyses serve as powerful methodologies to dissect metabolic interactions in CKD, potentially unveiling novel biomarkers through extensive plasma metabolite and lipid profiling.

Methods

A cross-sectional analytical study was conducted using untargeted metabolomics and lipidomics analyses on plasma samples from 48 CKD patients, categorized by frailty status. Each group comprised 24 patients, matched for sex, age, and diabetes presence. Analyses were performed using CE-TOF-MS and LC-QTOF-MS, employing both positive and negative ESI modes.

Results

Lipidomics enabled frailty classification in patients, revealing notable alterations in lipid profiles. Frail individuals showed increased medium-chain acylcarnitines and lysophospholipids, alongside reduced alkyl/alkenyl ether-linked phospholipids, diglycerides, ceramides, and cholesteryl esters levels. Metabolomics findings, while not fully discriminative, highlighted significant metabolite level changes, including reduced tryptophan and 2-aminoadipic acid in frail subjects, and elevated dimethyl-guanosine, 2-oxoglutaramate, and uremic toxins. Potential biomarker identification was further explored through multivariate ROC curves analysis.

Conclusions

Our findings open the door for the search and discovery of potential biomarkers in CKD and frailty, showing the utility of metabolomics strategies in clinical applications.

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P12
THE SHARK FIN: SERUM METABOLOME IDENTITY IN A GBM MODEL

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Glioblastoma multiforme (GBM) is the most frequent malignant brain tumor and the one with the worst prognosis[1 2]. Important efforts have been made to understand the molecular basis surrounding this pathology. To this aim, animal models have been developed that consistently emulate GBM features. Among them, the Fischer rat with F98 cells has been widely used as a reproducible and weakly immunogenic model generating heterogenic and infiltrative tumors with surrounding oedema[3], which, in fact, highly resembles the human GBM[4].To further unveil tumor physiology, nuclear magnetic resonance spectroscopy (NMR) is becoming an essential tool in the study of metabolism in vitro and in vivo due to its intrinsic features[5]. Based on NMR, we performed a multiple approach to the metabolomics characterization of the Fischer-F98 model with High- Resolution Magic Angle Spinning (HR-MAS) NMR of serum and tumor tissue samples. Results revealed a relation between serum and tumor metabolic profiles. Some of those metabolites identified in both samples showed opposite tendencies compared to healthy controls. In tumors, increased levels of alanine, betaine, glycine, leucine, threonine and valine, and a reduced concentration of creatine were detected, whereas in serum, increased levels were observed for creatine, glycine and phenylalanine, and decreased concentrations of acetate, alanine, betaine

and lactate, compared to healthy controls. Interestingly, an increase in ketone bodies (acetoacetate and 3-hydroxybutyrate) in the serum of tumor-developed animals and a reduction of pyruvate and citrate were also identified, suggesting an unbalance to high energetic demand. On the other hand, tumor samples showed common features of GBM, such as reduced levels of N-acetyl aspartate, in addition to the aforementioned increase of glycine and the reduction in creatine concentration. These results provide a hopeful insight into the characterization of GBM that could serve for the identification of metabolic biomarkers in serum that reflect GBM pathology.

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P13
METABOLOMIC PROFILES FOR DIAGNOSING SEPSIS IN VERY LOW BIRTH WEIGHT NEWBORNS

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The lack of reliable markers of neonatal sepsis leads to the use of unnecessary antibiotics. The objective was to characterize the metabolomic profiles of serum and urine of very low birth weight infants (VLBWI) with sepsis in comparison to those with similar clinical and demographic characteristics but without sepsis. Furthermore, metabolomic profiles of VLBWI with sepsis caused by Gram-positive and Gram-negative bacteria were compared. A double cohort, single-center, prospective, and observational study was designed. During a period of 36 months, VLBWI admitted to a tertiary referral hospital were included. Serum and urine metabolomic fingerprints of 123 samples were acquired using Ultra-Performance Liquid Chromatography-quadrupole Time-of-Flight Mass Spectrometry. Significant differences were obtained between neonatal sepsis compared to a control group in serum samples. Performing functional analysis, four metabolic pathways including tryptophan metabolism, lysine degradation, porphyrin and chlorophyll metabolism, and aminoacyl-tRNA biosynthesis were consistently impaired (p-value<0.05) in neonates with neonatal sepsis. Furthermore, significant differences were found between newborns with sepsis caused by Gram-positive vs. Gram-negative germs (tryptophan, caffeine, taurine, and hypotaurine, and D-glutamine and D-glutamate metabolism). Regarding urine samples, significant differences were found in the steroid hormone biosynthesis, and tryptophan and tyrosine metabolism between patients with confirmed sepsis and controls. Concerning differences among causative agents, statistically significant differences were found for tryptophan metabolism, phenylalanine metabolism, metabolism of xenobiotics by cytochrome P450, and phenylalanine, tyrosine, and tryptophan biosynthesis in serum and urine. The analysis of metabolomic profiles in serum and urine seems to be useful as a diagnostic tool for sepsis in VLBWI. Specifically, the tryptophan metabolism pathway appears to be a promising way to diagnose sepsis in VLBWI. According to our results obtained from urine, it may provide a particularly useful option as a non- invasive marker in this particularly vulnerable population.

P14
DISTINCTIVE METABOLIC COLORECTAL CANCER SUBTYPES IDENTIFIED BY MULTI-OMICS DATA INTEGRATION

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Colorectal cancer (CRC) is among the most frequent cancers worldwide and the second leading cause of cancer-related deaths in Western countries. In the last few decades, significant progress in survival has occurred with the use of chemotherapy and target-agents. But despite of high initial responses, practically all CRC patients succumb due to disease progression and metastatic CRC (mCRC). Metabolic reprogramming is a sensitive target for cancer therapy, but the wide landscape of metabolic phenotypes among CRC patients requires a more precise understanding of individual patient tumor metabolic reprogramming to predict the best metabolic targets in the design of new combined therapies.

Here, we have used transcriptomics data from biopsies of CRC patients to generate their Genome-Scale Metabolic Models (GSMMs), and this analysis revealed that CRC patients grouped in two distinctive metabolic subtypes: broadly speaking, one more glycolytic, and the other with enhanced oxidative phosphorylation (OXPHOS). We also applied this approach to classify transcriptomics data from different CRC cell lines, and we selected eight different cell lines, 4 closer to the glycolytic phenotype and 4 closer to the OXPHOS phenotype. All cell lines were subjected to different metabolic and transcriptomics assays, and multi-omics integration of all data into GSMM was used to better define their metabolic traits. Finally, cells were incubated with drugs targeting different metabolic pathways to demonstrate their different sensitivity according to their belonging to one or other CRC metabolic subtype.

Altogether, our results demonstrate the existence of different metabolic phenotypes in CRC, that allow to predict their different sensitivity to metabolic drugs, thus opening new avenues to new CRC treatments tailored to the patient-specific metabolic subtype.

P15
PLASMA GLYCEROPHOSPHOLIPIDS AND ACYLCARNITINES AS HALLMARKS OF THORACIC AORTIC ANEURYSM ASSOCIATED WITH BICUSPID OR TRICUSPID VALVES

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Background

Thoracic aortic aneurysm (TAA) is a progressive dilatation of the aorta. It develops asymptomatic with life- threatening consequences and its diagnosis is usually fortuitous. Although TAA is mostly idiopathic, its prevalence is significantly higher in subjects with bicuspid aortic valve (BAV) for unknown reasons. We hypothesize that TAA development courses differently if associated to BAV or tricuspid aortic valve (TAV), so its reflection in plasma could be a screening tool for specific monitoring and treatment of BAV-TAAs and TAV-TAAs.

Aim

We aimed to identify a metabolomic fingerprint for aortic dilatation in idiopathic TAA and decipher its pathophysiological mechanisms, differentiating between patients with BAV or TAV.

Methods

Plasma samples from TAA patients and subjects without aortic dilatation (control, C) were collected and classified according to aortic valve type (BAV or TAV). Untargeted LC-MS/MS was carried out by reverse phase and hydrophilic interaction chromatography in both positive and negative electrospray ionization modes (BAV-C: n=8, BAV-TAA: n=7, TAV-C: n=6, TAV-TAA: n=6). Identification was achieved by NIST and HMDB databases and MS/MS spectra. Metabolites of interest (FDR-adjusted p-value<0.05) were validated using commercially available standards by LC-MS/MS in selected reaction monitoring mode (SRM) in a different cohort (BAV-C: n=34, BAV-TAA: n=30, TAV-C: n=22, TAV-TAA: n=12) (t-test/Mann Whitney, p-value<0.05).

Results

BAV patients presented alteration in glycerophospholipid metabolism. Particularly, lysophosphatidylcholines and lysophosphatidylethanolamines were increased in BAV-TAA vs BAV-C patients, in agreement with the stimulation of glucose utilization through glycolysis. In contrast, TAV subjects showed alterations in carnitine-related metabolism evidenced by an increment in short and large carnitines in TAV-TAA, suggesting an altered β-oxidation of fatty acids and mitochondrial overload.

Conclusion(s)

Our data supports an independent diagnosis and treatment for BAV and TAV idiopathic TAA patients, while showing new therapeutic targets and diagnostic panels valve-associated for a personalized medicine.

P16
METSCORE: A MOLECULAR METRIC TO EVALUATE THE RISK OF METABOLIC SYNDROME BASED ON NMR METABOLOMICS

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Metabolic Syndrome (MetS) comprises a cluster of medical conditions and risk factors associated with insulin resistance, dyslipidemia and hypertension, collectively increasing the likelihood of developing serious cardiometabolic health issues.

While urine analysis is sensitive to glucose levels and potentially hypertension markers (1), it may not fully capture the complexities of MetS, particularly in relation to obesity and dyslipidemia, which are closely tied to blood metabolites and lipoproteins.

To assess the diagnostic potential of combining serum and urine in evaluating MetS risk, we examined a diverse range of metabolites and lipoproteins in serum samples, integrating them with urine-derived metabolic profiles.

Nuclear Magnetic Resonance (NMR) metabolomics of serum allows for the characterization of metabolites and enables profiling of lipoproteins. Bruker´s IVDr 1H-NMR serum measurements offers detailed information by characterizing lipoproteins based on their size, density and composition. Serum samples from 20,662 donors, along with urine data from 14,111 donors were analyzed by NMR, all representing the general population of the Basque Country.

Information on risk factors as diabetes, obesity, dyslipidemia and hypertension was coded using binary notation (1 for presence, 0 for absence), with a profile of all zeros indicating an asymptomatic state. An ad hoc cohort was constructed by merging sub-cohorts to ensure adequate representation of the 16 intermediate conditions resulting from the four risk factors.

We developed MetSCORE, a metabolic model based on serum lipoprotein and metabolite data, which effectively distinguishes MetS patients from the general population, achieving an AUROC of 0.94. This model facilitates quantitative risk factor stratification based on their contribution to MetS progression offering potential utility in identifying individuals at risk of MetS through urine and blood analysis.

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P17
RBCS METABOLOMICS FOR THE STUDY OF COVID OUTCOME

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Affectation of the red blood cells (RBCs) in COVID-19 has been associated with severe disease evolution, including changes in hemoglobin levels and RBC shape and renovation.

We aim to identify a specific association between altered metabolic pathways in RBCs and unfavorable outcomes in hospitalized COVID-19 patients.

Patients were stratified into moderate or severe outcomes (acute respiratory failure, acute myocardial injury, acute kidney failure, ICU admission or death). We analyzed the metabolic profile of RBCs using Nuclear Magnetic Resonance spectroscopy. The resulting profiles were then compared, performing multivariate PLS-DA analysis between groups.

We identified several metabolites specifically altered in patients with severe outcomes related to different pathways, including an affectation of glutathione redox system and the pentose phosphate pathway (necessary for NADPH production). Based on the RBCs profile, we identified two subgroups of severe patients according complications and serverity, in whom the Luebering-Rapoport pathway, which regulates oxygen release from hemoglobin and delivery to tissues, seemed to be altered, as well as glycolysis.

Our study allowed the identification of specific alterations in RBC metabolism related to pathways that regulate the response to oxidative stress and the release of oxygen to the tissues that are associated with a worse evolution in COVID-19 patients. Our results open new avenues in the identification of novel prognostic factors and potential therapeutic targets based on the metabolic profile of RBCs in these patients.

P18
PLASMA METABOLIC PROFILE AS A POTENTIAL PREDICTIVE BIOMARKER OF RESPONSE TO NEOADJUVANT TREATMENT WITH ICIS IN TRIPLE-NEGATIVE BREAST CANCER (TNBC) PATIENTS

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Immune checkpoint inhibitors (ICIs) are showing promising results in TNBC, but there is a percentage of patients who do not respond to therapy. The aim of this study is to investigate cellular and metabolic immune profiles that allow us to predict a complete (CR) or partial (PR) response.

Twenty-four patients with TNBC treated with ICIs and chemotherapy (CT+ IT) in neoadjuvant setting were included, and samples were taken before treatment (baseline), after 3 weeks (3W), and prior to surgery (Preop). Immunophenotype panels were analyzed using flow cytometry, cytokine production using ELISA technique. Metabolic profiling was analyzed using UPLC-MS/MS and a DataBase-guided dynamic Data Dependent Acquisition (dynamic-DDA) to improve the annotation ratio.

A significant increase was observed in the percentage of total lymphocytes, NKT, and CD8+at the end of treatment (24-30 weeks). A positive correlation was detected between NKT cells and perforin A levels. The increase in NKT cells was significant only in CR. PR patients presented higher levels of all subpopulations of MDSCs before treatment compared to CR. This difference was statistically significant in total MDSCs at 3W and Preop, and granulocytic MDSCs mainly increased at 3W. The analysis of the associations between these biomarkers and the metabolic profiles using functional analysis identified significant alterations in the Purine, Pyrimidine, and Tryptophan metabolism pathways at baseline. At 3W, another set of altered pathways was observed, including Lysine degradation and Inositol phosphate metabolism.

Treatment with immunotherapy can trigger activation of the immune system. This effect was most evident in CR patients.Differences in the metabolomic profile between CR and PR may allow to predict the effectiveness of ICI therapy in TNBC and aid in clinical decision-making. This study is still in the recruitment and metabolomic analysis phase, and we hope to continue and reinforce the significance of these trends.

P19
BILE ACID AND PROTEOMIC PROFILE OF PECTIN-BASED DIETARY INTERVENTION IN NON-SPECIFIC LIPID TRANSFER PROTEINS (NSLTPS) FOOD ALLERGY MODEL

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Food allergy (FA) caused by the non-specific lipid transfer proteins (nsLTPs) is the main cause of FA in the Mediterranean region. Additionally, alterations in gut microbiome composition have been related to FA development, thus, administration of prebiotics such as pectins could be a potential intervention strategy for these patients. Omic technologies, such as metabolomics and proteomics, can provide a better view of the complex mechanism underlying an intervention, especially the analysis of bile acids which

are metabolites of the host-gut microbiota crosstalk. Considering this, the aim of this study was to investigate the effects of a pectin intervention in a nsLTPs allergy model.

In the study, 34 patients with nsLTPs-FA were divided into three groups for treatment using Placebo, or two types of pectin (Active 1 and Active 2). Serum samples were obtained before (T0) and after two months (T2) of intervention. For the analysis, a targeted metabolomic approach using Liquid Chromatography coupled to Triple Quadrupole Mass Spectrometer (LC-QqQ-MS) to study 26 bile acids was performed. Additionally, targeted proteomics using a panel of 92 inflammation- related proteins through Olink® platform was also measured.

All patients after two months of pectin treatment tolerated a bigger amount of the allergen. In serum, 26 bile acids including primary and secondary bile acids were found. From these, after two months of treatments, we found a significant increase of isolithocholic acid (iLCA) in Active 1 compared to Placebo. Interestingly, decreased levels of IL2, IL4, and IL13 were found in both pectins-treated groups compared to the placebo group, indicating a downregulation of the Th2 response. STRING analysis showed a significant alteration of the inflammatory response.

Our findings suggest that dietary intervention with pectin leads to distinct bile acid and proteomic profiles in nsLTP-allergic patients, offering insights into potential immunomodulatory mechanisms and therapeutic targets for FA.

P20
IN VITRO ASSESSMENT OF A HIGH-PHENOLIC EXTRA VIRGIN OLIVE OIL ON THE MODULATION OF GUT MICROBIOTA AND THEIR DERIVED METABOLITES IN DIABETICS

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Recent studies indicate that individuals with Type 2 Diabetes Mellitus (T2DM) often experience changes in the composition and diversity of their gut microbiota, a condition known as dysbiosis, in comparison to those without diabetes. The metabolites produced by the microbiota significantly impact T2DM by influencing various aspects of host metabolism and participating in numerous pathways. Consequently, a bidirectional relationship exists between microbiota and diabetes, whereby T2DM alters microbiota composition, while the altered microbiota influences the pathophysiology of the disease.

The Mediterranean Diet (MedD) emerges as a significant factor in shaping gut microbiota composition. Extra virgin olive oil (EVOO), a key component of the MedD, not only serves as the primary source of dietary fat but also functions as a recognized functional food rich in various healthy compounds, particularly phenolic compounds. These compounds exert multiple beneficial effects, partially through their modulation of the gut microbiota.

In this study, a high-phenolic Extra Virgin Olive Oil (EVOO) derived from co-crushing two ancient autochthonous varieties from north-western Spain, was selected for simulating gastrointestinal digestion following the standardized INFOGEST protocol. The extract containing non-absorbable phenolic compounds underwent immediate faecal fermentation (from 0 h to 48 h) under simulated colonic conditions. Fresh faecal samples were collected from two diabetic volunteers, one of whom was also obese. This in vitro model, mimicking the human gastrointestinal tract, enabled the evaluation of the effect of the oily substrate on:

1. Diabetic gut microbiota composition using 16S rDNA sequencing, distinguishing between obese and non-obese profiles: significant increases in Shannon and Simpson alpha diversity were observed at 48 h compared to baseline (0 h) only in the obese subject.
2. Colonic microbiota metabolism, assessing the production of short-chain and medium-chain fatty acids (SCFAs and MCFAs) and elucidating the principal pathways of EVOO phenolic microbial metabolites generated over time by metabolomic approaches.

P21
METABOLOMICS RESEARCH IN HYPERTENSION: IMPACT OF A BRASSICA-RICH DIET ON CARDIOVASCULAR HEALTH

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Introduction

Phytochemicals from Brassica vegetables are well known for their multiple bioactivities, however, to deepen into the biological mechanisms affecting cardiovascular diseases (CVD) it is necessary to delve the pathophysiological metabolic processes. Metabolomics helps studying non-invasive biological samples, and using this information to search for markers with potential theragnostic value.

Aim

To investigate the impact of a brassica-rich diet on the hypertensive phenotype using classical and metabolomics parameters to establish the molecular basis and metabolic processes that influence CVD.

Methodology: A diet containing 5% w/w of brassica microgreens (MGB) was administered to adult male SHR and WKY rats for 1 day or 4 weeks (N=6 per group). Systolic blood pressure (SBP) and classic oxidative-inflammatory markers were also evaluated. For the metabolomics study, plasma and urine samples were analyzed by UHPLC-ESI-QTOF-MS using an untargeted approach. Masses were detected in both negative and positive ionization modes. With the obtained signals, univariate (fold change) and multivariate (PCA, PLS-DA, cluster analysis) analyses were performed to identify significant signals in each study group, which were then annotated based on their exact mass, MS/MS fragmentation pattern, and isotopic distribution.

Results

A reduction of 30 mmHg in SBP was detected, along with significant changes ($p<0.01$) in oxidative and inflammatory markers when comparing chronic vs control SHR group. Plasma metabolomes were robustly classified according to the treated groups, with over 50 discriminant signals between each one (VIP score > 2.5). Exogenous metabolites such as N-acetyl-cysteine conjugated isothiocyanates, as well as glycerophospholipids and several lysophospholipids were identified as endogenous metabolites in MGB-treated samples, implying metabolic pathways of anti- inflammatory processes and lipid metabolism.

Conclusions

Adopting healthy habits such as chronic consumption of MGB promotes benefits on CVD. Metabolomics proved to be an innovative theragnostic tool in arterial hypertension

P23
DYNAMIC CHANGES IN METABOLIC PROFILE OF PATIENTS ALLERGIC TO HYMENOPTERA VENOM SUBJECTED TO ALLERGEN SPECIFIC IMMUNOTHERAPY

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Venom allergen immunotherapy (VIT) represents the only causative treatment for hymenopteran insect venom allergy (HVA), showing 77-96% efficacy in bee and wasp venom allergies. Despite its proven clinical efficacy, limited data exist on the systemic

effects of VIT and its impact on homeostasis. In fact, the vast majority of published papers to date refer to changes in systemic adaptive immune cell repertoires. Metabolomics offers a novel approach capable of identifying molecular signatures and elucidating VIT-induced dynamic changes in the metabolic profiles, which allows a better understanding of VIT mechanisms. Here, we aimed to conduct targeted plasma metabolomics in patients with HVA undergoing VIT and correlate these data with changes in the systemic immune responses.

The study was conducted on 22 and 16 patients with HVA to bee and wasp venom, respectively, and 16 healthy, nonatopic and nonallergic individuals. Plasma samples were collected at baseline after 24 hours, 3 months, 1 year, 2 years, and 4-5 years of VIT treatment. Single-time plasma collection was performed in the case of the control group. The collected samples underwent complete blood count with differential and targeted metabolomics analysis using a commercial kit, allowing the quantification of over 180 metabolites. Nonparametric Mann-Whitney U-test and Spearman correlations were used for statistical analysis.

Changes in eosinophils, basophils, monocytes, and concentrations of amino acids, lipids, and biogenic amines were unveiled. Patients allergic to bee or wasp venom exhibited distinct serum metabolite profiles compared to controls, and both groups had VIT-induced changes in serum metabolite concentrations. Metabolomic analysis unveiled novel VIT-induced biochemical pathways in HVA patients, holding a promise for fostering precision medicine paradigms tailored to the individualized management of HVA.

P24
METABOLOMICS REVEALS THE MECHANISM OF ACTION OF (R,R′)-4′-METHOXY-1 NAPHTHYLFENOTEROL ON HUMAN-DERIVED U87MG GLIOBLASTOMA XENOGRAFT MODEL

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Glioblastoma (GBM) is the most common and aggressive malignant brain tumor. Despite advances in treatment like surgical resection, radiation, and chemotherapy, the life expectancy is still less than one year. Significant improvements in the understanding of the molecular basis of GBM and associated metabolic and signalling pathways have opened opportunities for new therapeutic options. Metabolomics holds the promise to unravel novel targets and enable more precise disease treatment.

We aim to study mechanisms of antitumor activity of (R,R')-4'-methoxy-1-naphthylfenoterol (MNF), b2-adrenergic receptor agonist and antagonist of GPR55-mediated pro-oncogenic responses (1), in a xenograft model of human-derived glioblastoma cells in mice.

Female Balb/c nude mice were inoculated subcutaneously with 5×106 U87MG cells for tumor development. Mice were administered a single i.p. injection of either vehicle (1% hydroxypropyl-β-cyclodextrin) or 40 mg kg-1 (R,R')-MNF for 5 days per week for 3 treatment cycles. Plasma samples were collected and analysed by liquid chromatography-mass spectrometry (LC-QTOF/MS) operated in positive and negative ionization modes. Acquired data were evaluated by examination of the reproducibility of sample treatment procedure and analytical performance by raw data inspection. Principal component analysis (PCA-X), a projection method was used to check for signal drift, variation in QC samples and outlying observations. Univariate and multivariate analyses were applied to perform statistical evaluation of acquired data. The annotation of compounds was performed by available metabolite databases through CEU Mass Mediator tool (2).

The analysis revealed alterations in the metabolic pattern under MNF treatment. The most interesting changes were associated with conjugated bile acids, carnitine derivatives and phospholipids. A decrease in the concentration of L-lactic acid, a product in the main energy pathway of cancer cells was also observed. The data indicate promising results for future new targets for GBM treatment.

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P25

THE APPLICATION OF GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY FOR UNTARGETED METABOLOMICS ANALYSIS OF PLASMA SAMPLES FROM PATIENTS WITH SEPSIS AND URINARY TRACT INFECTION

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Urosepsis is a fatal condition that developes from urinary tract infection (UTI). Despite the medicine achievement, sepsis remains a serious medical problem. Nonspecific symptoms in onset, makes it difficult to differentiate sepsis and UTI and increase patients mortality.

The sepsis pathomechanism is also not sufficiently understood therefore predicting in which patients will sepsis develope. There are no specific biomarkers signaling the early onset of sepsis.

Metabolomics may support our understanding of sepsis. The untargeted metabolomic approach enables to study the unique chemical signature, reflecting the body processes. We provide the results designed to explore the molecular signature and disturbed metabolic pathways associated with sepsis. Gas chromatography-mass spectrometry analysis was performed on plasma samples from UTI and sepsis patients. 50 patients was enrolled into the study. Standard operating procedures were applied for the metabolite extraction samples preparation followed chemical conversion and analysis in DB5–MS column, GC system coupled to a mass spectrometer with triple-Axis detector (Agilent Technologies). Quality control samples and quality assurance procedure were applied in order to control any source of unwanted variation. Spectral deconvolution with Agilent Unknown Analysis software was performed. Assignment of the target ion and the qualifiers, entire batch pre-processing and manual data inspection including peak area and RT integration was performed with Agilent MassHunter Quantitative Analysis. For compound identification the target metabolite Fiehn GC-MS Metabolomics (Retention Time Locked) and the NIST (National Institute of Standards and Technology) mass spectra library were used. Data matrix was subjected to statistical analyses, providing a list of significant metabolites. Data obtained indicate the metabolic pathways altered in patients with sepsis and UTI patients and could provide deeper insights into the mechanisms of the diseases. This, may support searching for specific indicators of early onset of sepsis and improve patient stratification.

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P26

PARENTAL OBESITY PREDISPOSES THE OFFSPRING TO EXACERBATED METABOLIC DISTURBANCES LATER IN CHILDHOOD

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Family history of obesity is known to increase the risk of developing childhood obesity in the offspring, but molecular mechanisms underlying this greater predisposition remain to be elucidated. Herein, we investigated a population-based cohort comprising children with obesity and parental obesity (when at least one of the parents had obesity), children with obesity without parental obesity (when none of the parents had obesity), and lean healthy children as controls, from whom plasma and erythrocyte samples were collected for metabolomics analysis using ultra-high performance liquid chromatography coupled to high resolution mass spectrometry. Interestingly, we found parental obesity to associate with unhealthier outcomes in children with obesity, as reflected in higher fasting insulin levels and impaired insulin sensitivity. This was in turn accompanied by alterations in multiple obesity-related metabolic pathways, such as energy homeostasis, amino acid metabolism, oxidative stress, synthesis of steroid hormones and bile acids, membrane lipid composition, and exposome-related factors. Notably, some differential metabolites showed more pronounced changes in children from parents with obesity, especially at erythroid level, when compared to counterparts not suffering from this parental predisposition. Therefore, we hypothesize that family history of obesity could be an important risk factor in modulating and exacerbating the characteristic metabolic impairments that typically underly childhood obesity, with

erythrocytes serving as sensitive sensors to decipher the impact that parental conditioning may have on metabolic health in the offspring.

P27
EXPLORING MULTI-OMICS INTERACTIONS: MICROBIOTA, METABOLITES, AND HOST RESPONSES IN A LIFESTYLE INTERVENTION MODEL

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The microbiota plays a crucial role in maintaining host health by contributing to immune system development, synthesizing essential metabolites like vitamins and amino acids, and supporting gut structure[1]. Shifts in microbiota composition have been linked to conditions such as obesity[2], with interventions aimed at microbiota restoration showing promising but incompletely understood outcomes3. We investigated how epigenetically relevant microbiota-derived metabolites, including short-chain fatty acids, vitamins, and folate[4,5], may influence host gene expression, particularly in response to dietary and lifestyle changes. To explore this, we employed a multi-omic approach in a C57BL/6J mice model undergoing a lifestyle intervention. Mice were divided into three groups (n = 10 each): standard diet, ad-libitum high-fat diet, and high-fat diet followed by a lifestyle intervention involving healthier diet and exercise. We analyzed liver gene expression (RNA- Sequencing), epigenetic modifications (Whole Genome Bisulphite Sequencing and histone proteomics), and metabolites (targeted and untargeted); along with microbiome data from the cecum and colon (16S sequencing, shotgun sequencing, and functional analysis), metabolites (targeted), metals, and serum metabolites. Integration of these data using Multi-Omics Factor Analysis (MOFA2) revealed two primary trends: the first reflected features restored by the lifestyle intervention, while the second highlighted persistent alterations. Factor 1 enrichment implicated immune system pathways; Factor 2 indicated shifts in fatty acid, amino acid, and retinol metabolism; and arachidonic acid metabolism in both. Multi-omic correlation networks revealed highly interconnected clusters associated with arachidonic acid, fatty acids, and sugar metabolic pathways, linking them to microbiota composition and function.

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P28
METABOLOMICS INVESTIGATION OF THE BENEFICIAL EFFECT OF ACUTE SEAWEED CONSUMPTION IN MODULATING THE METABOLIC STRESS INDUCED BY A MIXED-MEAL CHALLENGE

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The consumption of seaweed may have beneficial repercussions on health because of their rich nutritional profile, comprising fibers, essential minerals (e.g., iodine) and lipids (e.g., PUFAs), and other bioactive compounds (e.g., polyphenols, carotenoids). In this respect, metabolomics has shown great potential to unravel the complex molecular mechanisms through which food components influence health status and protect against disease development.

Herein, we have conducted a randomized crossover trial in 11 healthy subjects who were asked to consume a mixed meal accompanied by a seaweed smoothie containing 75 g of red ogonori (intervention group) or water (control group). At different time points along the challenge test, plasma (0, 30, 60, 120, 180 min) and urine (0, 3, 6, 12, 24 h) samples were collected for further metabolomics analysis using liquid chromatography coupled to high-resolution mass spectrometry.

The intervention with seaweed was primarily mirrored in the urinary excretion of several bioactives, including vitamins, phenolic compounds, and furan fatty acids, which are well-known to participate in endocrine control and antioxidant systems. Furthermore, we found that acute seaweed intake contributes to maintain a tighter control of metabolic adaptations occurring in response to the mixed-meal challenge. On the one hand, many differential metabolites suggested that seaweed supplementation improves individuals' metabolic flexibility in energy homeostasis, as reflected in lower postprandial peak of carbohydrates, more effective blocking of alternative energy sources (e.g., ketogenesis, lipolysis, β- oxidation, proteolysis), and faster return to basal metabolic conditions. Other beneficial effects of the dietary intervention included ameliorations in oxidative stress, production of uremic toxins, and hormonal changes. Altogether, this study pinpoints that dietary seaweed might strongly influence health status by modulating a myriad of central metabolic processes, which opens the door to their use as nutraceuticals for preventing and treating a variety of disorders.

P29
IN VIVO METABOLIC FLUX ANALYSIS–EFFECT OF EXERCISE AND AEROBIC CAPACITY ON LIVER METABOLISM

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Exercise is an important lifestyle intervention for obesity and liver disease. However, studying the mechanisms by which aerobic capacity affects liver metabolism independent of exercise is challenging. Here we used rats selectively bred for intrinsically high (high-capacity runner (HCR)) vs. low aerobic fitness (low-capacity runner (LCR)) in sedentary conditions over multiple generations to understand how aerobic capacity impacts metabolic disease. LCR and HCR rats were additionally subjected to high fat diet (HFD) feeding. We compared these animals to voluntary wheel running (VWR) rats in order to evaluate the contribution of active exercise to genetic adaptation independent of exercise. Finally, acute effects of exercise were studied in mice actively running on the treadmill. All experiments except acute studies were conducted one day following the last exercise bout. Blood glucose and liver organic acid were analyzed using mass spectrometry following in vivo stable isotope infusions of [U-13C3]propionate, [3,4-13C2] glucose and 2H2O in animals. Isotopologue data were regressed to a metabolic network model to estimate metabolic fluxes in liver as well as whole-body turnover rates. Endogenous glucose production, glycogenolysis, and gluconeogenesis were similar between LCR and HCR rats on either diet. However, in response to HFD, LCR rats increased anaplerosis, cataplerosis, and pyruvate cycling, while these fluxes remained unchanged in HCR rats. Hepatic TCA cycle turnover was elevated in HCR rats on chow diet due to elevated fat oxidation. Ketones were increased by HFD but not different between LCR and HCR rats. Notably, the effects of inherent aerobic capacity were not equivalent to the effects of chronic VWR exercise in rats. Furthermore, acute exercise in mice evaluated by paired comparison between sedentary phase and active running phase were different from chronic adaptations. These data suggest that long-term programming of liver metabolism by exercise is not equivalent to pure breeding for aerobic capacity.

P30
EXAMINING THE ADDITIVE EFFECT OF METFORMIN AND HIGH-INTENSITY EXERCISE ON LIPID METABOLISM

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Maintaining adequate levels of physical exercise is an optimal way to keep good health. Prolonged and intense physical exercise is known to produce large changes in multiple different lipid changes. However, these changes over multiple time points are not fully understood. It has been suggested that the effects of exercise together with the consumption of the antihyperglycemic drug metformin have an additive effect on the metabolism and function of different organs.

Thus, this study aims to find differences between plasma lipidomic profiles of 9 healthy male individuals after performing high-intensity interval exercise under metformin treatment.

Individual participates in two sessions lasting 24 h where they received 1000 mg of metformin. In one of the sessions, they performed a high-intensity interval exercise test with an average intensity of 67% HRmax and a total duration of 76 min (Session A). In the other session, they rested (Session B). Plasma samples were collected before taking metformin and during each session up to 13-time points spanning 24 h. Samples were analyzed using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6545 QTOF mass spectrometer. Data were acquired in both ionization modes in separate runs. In addition, 10 iterative-MS/MS runs were performed to assist in the annotation process. Paired Wilcoxon tests between each session at each time point were performed for the statistical analysis.

The observed variations in the lipid profiles -particularly in fatty acids, acylcarnitines, glycerophospholipids, sphingolipids, and triacylglycerols-, underscore the dynamic and complex physiological adaptations to high-intensity interval exercise.

Altogether, these changes provide crucial insights into understanding the complex physiological response of humans to intensive exercise under an antidiabetic drug and pinpoint the main biological processes implicated in maintaining a healthy status.

P31
EFFECT OF SALIVA STIMULATION ON THE SALIVARY METABOLOMIC PROFILE IN PEDIATRIC CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) involves a gradual loss of kidney causing severe morbidity and high mortality. Pediatric patients are more prone to suffering from end-stage renal disease, the most severe stage of CKD. Previous metabolomics studies revealed new plasma metabolites that might improve early CKD diagnosis in children[1]. However, despite being a suitable alternative to blood collection, saliva metabolomics in pediatric CKD has been underexplored compared to blood or urine[2].

This study aimed to identify potential saliva biomarkers that could improve early CKD diagnosis in pediatric patients. Fifteen children diagnosed with CKD and twenty-five healthy children were recruited for the study. Unstimulated saliva was collected by the splitting method while secretion of saliva was stimulated by dropping 2% citric acid solution on the tip of the tongue. Both unstimulated and stimulated saliva were obtained from all the subjects enrolled in the study.

Untargeted metabolomics analysis of saliva samples was performed by LC-ESI-QTOF-MS in positive and negative ion modes. Chemometric analysis of data generated in Profinder software was carried out separately for unstimulated and stimulated saliva samples. Unsupervised multivariate analysis (PCA) showed segregation between CKD patients and healthy controls. Supervised PLS-DA model (VIP>1.5) and Volcano plot (FC>2 or <0.5 and p value<0.05) found those metabolic features significantly up- or downregulated in CKD patients. The characteristic MS and MS/MS spectra of these features were compared with online databases. The results revealed different metabolomics profiles in unstimulated and stimulated saliva samples. This difference may result from the stimulating effect of citric acid on the parotid glands that increases saliva flow

and pH and changes its composition[3,4]. Hence, as long as the saliva collection method is considered, the salivary metabolome could be a potential tool of choice for pediatric CKD diagnosis.

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P32
METABOLOMIC AND TRANSCRIPTOMIC CHANGES OF IN VITRO AGED RETINAL PIGMENT EPITHELIAL CELLS

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Omics sciences with an integrative aim are a promising strategy to obtain a full picture of visual health and disease. Using simple models like cell cultures of the retinal pigment epithelium (RPE) serves as a first step to identify and understand the molecular processes, pathophysiological changes and potential biomarkers arising during ageing. In this work we established an in vitro model of human RPE cells to study their transcriptomic and metabolomic changes during a 6-month follow-up, at 4, 12, 17 and 25 weeks-old. Cell cultures were characterized by conventional immunocytochemistry and transepithelial electrical resistance measurement, observing progressive accumulation of extracellular material (apolipoprotein E and hydroxyapatite). Transcriptome was analysed by RNA sequencing (RNAseq) and differential expression analysis was conducted using the platform Dr. Tom. RNAseq showed alterations of cell migration, adhesion and differentiation, and oxidation-reduction, lipid metabolic and apoptotic processes during RPE ageing (4 vs 25 weeks, Q-value<0.001). Untargeted metabolomics was carried out by high resolution mass spectrometry (LC-IM-QTOF) and bioinformatic analysis performed using Mass Professional Profiler software (Agilent). Untargeted metabolomics provided 498 significantly different metabolites among time points (p-value < 0.05) in positive ionization mode, and 222 in negative ionization mode. An additional filter was used to select significant metabolites with a fold-change > 2, resulting in 283 and 100 features in both positive and negative ionization mode, respectively. Three clusters of compounds with different time-associated patterns were found, belonging to lipid metabolism and sphingolipids pathways. Joint pathway analysis of enriched metabolic routes was conducted with Metaboanalyst tool and confirmed the alteration of glycerolipid and glycerophopholipid metabolism at the end of the 25- week experiment in comparison with the beginning RPE cells, together with alteration of glutathione metabolism, lysine degradation and nitrogen metabolism.

P33
LONGITUDINAL METABOLOME CHANGES IN A DIET-INDUCED SUBCLINICAL NAFLD RAT MODEL ARE SEX-DIMORPHIC

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Non-alcoholic fatty liver disease (NAFLD) is a complex disease that comprehends a spectrum of metabolic liver imbalances. Nearly 30% of the world's population is affected by NAFLD. There is little information about the sequence of metabolic and microbiota changes that happen during the development of NAFLD. Metabolomics has become a powerful tool for studying the molecular signatures and metabolic dysregulations of diseases. We performed, for the first time to our knowledge, an extensive and intensive longitudinal characterization of blood, urine, and fecal metabolome in an HFD diet-induced subclinical early NAFLD Wistar rat model. Male and female Wistar rats 18 weeks old were fed a chow diet or a 45% high-fat diet for 21 weeks. Every 3 weeks, samples of serum, urine, and feces were collected. After 21 weeks, the animals were sacrificed. Serum, urine, feces, and liver tissue were studied by metabolomics using a nNMR spectrometer. Hemograms and liver biochemistry did not show statistically significant differences. However, the liver histopathological analysis revealed steatosis without the development of fibrosis. Metabolomic differences were found in urine and feces, which appeared as early as week 3. In the liver metabolome, the main differences were related to the lipid subtypes (PUFAs, SFAs, etc) suggesting that HFD impacts not only the amount but also the composition of hepatic lipids,

towards a detrimental profile. Non-lipidic liver metabolites were altered only in HFD males suggesting that the disease had a more severe pattern in males than in females. In serum, in addition to specific lipid subtypes, metabolomic patterns were not altered until week 9. Metabolites related to host-microbiota co-metabolism were altered in serum, urine, feces, and liver tissue. Our results may open new perspectives for identifying biomarkers for early detection and prediction of the disease and the development of new preventive strategies.

P34

MULTI-OMICS ANALYSIS TO ASSESS THE EFFECTS OF POLYPHENOL-RICH PLANT EXTRACTS ON OXIDATIVE AND INFLAMMATORY STRESS: STUDY IN CAENORHABDITIS ELEGANS MODEL

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Plants are source of numerous active ingredients, many of them unknown or difficult to produce at an industrial level. When these active ingredients originate from agricultural waste, they gain importance within a circular economy model. This is the case of red onion peels, of which every year large quantities are discarded as a residue. The evaluation of both therapeutic potential and safety of polyphenol-rich extracts obtained from red onion peels shows significant interest for their application in pharmaceuticals, cosmeceuticals, nutraceuticals and food products.

Caenorhabditis elegans (C. elegans) model is presented as an efficient model to evaluate the effects of plant extracts rich in polyphenols against both oxidative and inflammation stress. Specifically, the oxidation stress (OS) and inflammatory bowel disease (IBD) models were tested in C. elegans model to evaluate the potential beneficial effects of some extracts obtained from red onion peels. These extracts were produced by two eco-friendly techniques, namely Natural Deep Eutectic Solvents – NADES and Sub-critical Water Extraction – SWE. The use of multi-omics techniques (transcriptomics, metabolomics (NMR and LC-MS/MS) and lipidomics analysis (LC-MS/MS)) together with the assessment of the worm lifespan was applied to provide a global overview of the system and to evaluate the effects of the bioactive compounds present in these extracts.

The results obtained have demonstrated the usefulness of these models in elucidating the effectiveness of these extracts and the bioactive compounds thereof. The data obtained through multi-omics approaches have shown that these bioactive compounds could have promising use in different fields, as they exhibit potential beneficial effects in front of both oxidative and inflammatory stress.

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P35
COMPREHENSIVE EVALUATION OF RECOMBINANT HUMAN GROWTH HORMONE TREATMENT EFFECTS ON LIPID PROFILE, BROWN ADIPOSE TISSUE ACTIVITY, AND BATOKINES IN SMALL FOR GESTATIONAL AGE CHILDREN

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Introduction

Small for gestational age (SGA) children, constituting 3.1–5.5% of the population, often exhibit growth deficits despite early catch-up growth. Recombinant human growth hormone (rhGH) treatment has been approved for such cases, showing efficacy in promoting growth and normalizing body composition. However, the impact of rhGH treatment on lipid metabolism, brown adipose tissue (BAT) activity, and batokines remains poorly understood.

Objective

This study aims to comprehensively evaluate the effects of rhGH treatment on the lipid profile, BAT activity, and circulating batokines in prepubertal SGA children.

Methods

Serum samples were collected from average gestational age children (AGA), SGA children before rhGH treatment (SGA-GH 0m), and SGA children after 3 months of rhGH treatment (SGA-GH). An untargeted lipidomics-based approach using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6545 quadrupole time-of-flight (QTOF) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was employed to analyze lipid profiles. BAT thermogenic activation capacity was assessed using infrared thermography (IRT) with a cold stimulation protocol. Circulating levels of batokines were measured using ELISA assays and a soluble protein quantification system.

Results

rhGH treatment led to increased L-carnitine and decreased acylcarnitines serum levels initially, with acylcarnitines levels returning to baseline after 3 months. Elevated levels of adrenic acid and 1a,1b-dihomo-PGJ2/1a,1b-dihomo-15-deoxy- delta-12,14-PGD2 were observed post-treatment. No significant changes were observed in BAT thermogenesis measured by IRT, but a decrease in MPC1 and Meteorin-like levels was noted. Other batokines exhibited trends toward modulation without statistical significance.

Conclusion

This pilot study suggests that rhGH treatment influences the serum lipidome and may subtly affect molecules related to BAT activity and batokines in SGA children. Further research into the direct effects of rhGH on specific lipid species and batokines could offer new strategies for addressing metabolic disturbances associated with obesity and aging.

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P36

COMPARISON BETWEEN QUANTITATIVE SEMIAUTOMATIC PROFILING AND MULTIVARIATE ANALYSIS OF DECONVOLUTED SIGNALS WITH URINE AND PLASMA 1H-NMR SPECTRA

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Identification and quantification of metabolites in complex mixtures can be a tedious and time-consuming task, even with the help of specialized software. Here, we present a pipeline based on the deconvolution of 1D 1H-NMR spectra into individual Lorentz curves, calculation of their integrals, followed by the alignment of the data, which, together with the posterior multivariate analysis, provides a powerful fingerprinting tool for high-throughput metabolomic analysis of large datasets. Results were compared with quantitative semiautomatic profiling analysis using Chenomx software, where 22 metabolites were fitted and quantified in 200 spectra of human plasma. Additionally, the pipeline was tested with a set of 400 human urine spectra in order to check its performance in a more demanding scenario.

Bruker spectra from two sets of human samples were used as input, one consisting of plasma from 40 patients at 5 different times and another consisting of urine from two patient groups. The deconvolution, integral calculation, and alignment of the integrals were performed as indicated in the MetaboDecon[1] vignette. The optimal maxShift parameter for alignment[2] was determined using the CluPA algorithm, based on the highest median Pearson correlation coefficient. The pipeline's output is a matrix of samples and ppm values containing integrals of individual Lorentz curves, which are then subjected to multivariate analysis using PCA and PLS-DA[3].

Results from the multivariate analysis of aligned integrals are consistent with those based on Chenomx concentrations, showing similar group separation patterns. Furthermore, ppm that contribute more to the separation in the PLS-DA of the matrix of integrals mostly correspond to the metabolites with higher contribution to the loadings of the PCA of Chenomx concentrations.

As expected, the performance in urine spectra was inferior due to greater chemical shift variations, indicated by lower mean Pearson correlation in alignment and increased optimal maxShift values.

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P37

ENHANCED METHODOLOGY FOR QUANTIFICATION OF SHORT CHAIN FATTY ACIDS IN MURINE LIVER TISSUE BY HS-SPME-GC/MS AND CHLOROFORMATE DERIVATIZATION

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The gut-liver axis involves a bidirectional interaction between the gut and the liver, primarily mediated by the portal vein. Some studies have evaluated the flux of short-chain fatty acids, SCFAs, across the liver by comparing their concentration in the portal and the hepatic vein, revealing that 40% of acetic acid and 80% of propionic acid are metabolized by the liver, while the non-metabolized SCFAs are released into the systemic circulation. Consequently, the liver serves as a major site for the metabolic processing of gut-derived SCFAs. However, the number of studies conducted on liver is limited due to the challenging determination of SCFAs coming from their low concentration along with their high polarity and volatility. Here, we propose a new method for the quantitation of SCFAs in liver by HS-SPME-GC/MS and chloroformate derivatization. For this, multivariate designs of experiments such as Plackett-Burman and Central Composite Design were employed to optimize conditions for derivatization with isobutyl chloroformate, as well as optimized the HS-SPME derivatization parameters to minimize both organic solvent waste and the displacement effects on the fiber coating. Matrix effects were assessed employing different types of liver samples (mouse, rat, chicken) to ensure method robustness. A comprehensive investigation of quantitation strategies for SCFAs was conducted and the pros and cons of each quantitation strategy were discussed. Method validation was carried out employing QC samples as surrogate matrix and isotopically-labeled SCFAs as surrogate analytes. LLOQ ranged from 0.006 to 0.019 ng/µL. Precision and accuracy were assessed in three concentration levels (low, mid, and high). Intraday and intermediate precision, expressed as %RSD, yielded results better than 5 and 13%, respectively. Recovery ranged from 72 to 124%. As a proof of concept, the method was applied to the analysis of mice liver, employing a study of control versus systemic lupus erythematosus animals.

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GC-MS-BASED MOLECULAR NETWORK AND PRINCIPAL COMPONENT ANALYSIS OF THE GREEN EXTRACTION OF ALKALOIDS WITH ANTICHOLINESTERASE POTENTIAL

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The study focuses on the Amaryllidaceae family, which is known for its variety of isoquinoline alkaloids with diverse biological properties[1]. The aim is to improve the extraction of these alkaloids from Worsleya procera leaves according to green chemistry principles2 and to analyze its fractions rich in candidate alkaloids for cholinesterase inhibitors. Three extraction methods were developed from a biphasic maceration. The traditional method used a mixture of hexane:methanol 80% (1:1v/v) for alkaloid extraction, followed by acidification (H2SO4 0.5M) and basification (NaOH 3M) of the polar extract (EMeOH). Alkaloids were recovered using hexane (FM_HEX) and AcOEt (FM_AcOEt). The alternative method used heptane:ethanol 70% (1:1v/v), citric acid 0.5M, and NH4OH 3M (EEtOH), resulting in FE_HEP and FE_AcOEt. An optimized method eliminated the use of acids in alkaloid concentration and performed partitioning with AcOEt only, resulting in EMAcOEt and EEAcOEt from EMeOH and EEtOH, respectively.

One-way ANOVA and Tukey post test ($p<0.05$) demonstrated that the yields obtained from the EMeOH and EEtOH extracts were equal. There was no difference in the total alkaloid content recovered using different partitions (FM_HEX+FMAcOEt=5.90±0.31^{a}; EMAcOEt= 5.84±0.14^{a}; FE_HEP+FE_AcOEt= 5.37±0.36^{b}; EEAcOEt= 5.16±0.32^{b}). PC1 (41%) formed clusters with all fractions except FE_HEP, which remained isolated, and another cluster with EMeOH and EEtOH. EEAcOEt and FE_AcOEt selectively inhibited BuChE in relation to AChE.

GC-MS-based molecular networking[3] resulted in 27 annotated alkaloids, predominantly belonging to the licorine, homolicorine, and tazetine subtypes[4]. FM_AcOEt, FE-AcOEt, and EEAcOEt detected the most alkaloids, while FE_HEP contained aliphatic compounds and fatty acids. The method consistent with green chemistry extracted and concentrated alkaloids similar to the other methods, reinforcing the importance of sustainable methods that are possible and emerging. Optimizated method obtained alkaloids similar to the others. Green methodologies are emerging and part of the sustainable development agenda for 2030[2].

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P39

TOWARDS THE IMPLEMENTATION OF MULTI-ANALYTICAL PLATFORMS IN METABOLOMICS: HILIC-HRMS AND CE-HRMS AS COMPLEMENTARY TECHNIQUES

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Cationic, anionic, zwitterionic and, partially polar metabolites are very important constituents of blood serum. Several of these metabolites underpin the core metabolism of cells (e.g., Krebs cycle, urea cycle, proteins synthesis, etc.), while others might be considered ancillary but still important to grasp the status of any organism through blood serum analysis. Due to its wide chemical diversity, modern metabolomics analysis of serum still struggles to provide a complete and comprehensive picture of the polar metabolome, due to the limitations of each specific analytical method[1]. In this study, two metabolomics- based analytical methods using the most successful techniques for polar compounds separation in human serum samples, namely hydrophilic interaction liquid chromatography (HILIC) and capillary electrophoresis (CE), are evaluated. Both are coupled to a high-resolution time-of-flight mass spectrometer via an electrospray ionization source (ESI-Q-TOF-MS). The performance of the two methods has been compared using five terms of comparison, three of which are specific to metabolomics, such as (1) compounds' detectability (2) Pezzatti score[2], (3) intra-day precision (repeatability), (4) ease of automatic data analysis (through a common deconvolution, alignment and extrapolation software, MS-DIAL, and (5) time and cost analysis. From this study, HILIC-MS proved to be a better tool for polar metabolome analysis, while CE-MS helped identify some interesting variables that contribute to completing metabolome coverage in metabolomics studies[3]. Finally, in this framework, MS-DIAL demonstrates for the first time its ability to process CE data for metabolomics, although it is not designed for this purpose.

Acknowledgments

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P40

HILIC LC-MS/MS METHODOLOGY FOR HIGH-CAPACITY METABOLOMICS ANALYSIS

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Mass spectrometry (MS) is routinely used as a highly sensitive analytical technique for metabolomics studies to target a wide variety of small molecules[1]. In order to study the metabolites, present in different biological matrices, targeted metabolomics are a promising tool. However, some analytical challenges persist, including retention of ionic metabolites, reproducibility in the presence of salts, broad coverage of metabolite classes, and the separation of relevant isomers[2-4]. To confront these issues,

we used a robust hydrophilic interaction chromatography (HILIC) LC/MS method using the InfinityLab deactivator additive as a mobile phase modifier to enhance the peak shape and detection signal of metal-chelating organic acids and phosphorylated analytes[4,5]. A set of metabolite standards, including physiological amino acids and organic acids of the TCA cycle, were prepared in purified water at 100 µM. The stock was then diluted to 0.5 µM in 80:20 acetonitrile/water and 50 µL was placed in an insert. Metabolites were separated using an InfinityLab Poroshell 120 HILIC-Z column on a LC system coupled with a 6495 QQQ (both form Agilent Technologies, Inc). 10X mobile phases buffered stock solutions (200 mM ammonium acetate, pH ranked from 9.1 to 9.5) in water were first prepared, and 1X solvents were made with water (containing 0.1% of deactivator reagent (p/n 5191-4506) (solvent A). The organic phase (solvent B) was pure acetonitrile[6]. In this study, a mobile phase at pH 9.3 provided the best overall results for chromatographic separation. Moreover, the addition of the deactivator additive improved the peak shape and intensity. As a conclusion, PEEK-lined HILIC-Z column coupled to the 6495 QQQ LC/MS provides excellent analytical performance for profiling across a broad range of metabolite classes in biological samples.

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P41
SHOTGUN LIPIDOMICS FOR HIGH THROUGHPUT ANALYSIS

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Shotgun lipidomics lean on direct infusion mass spectrometry and offers a rapid approach for assessing various lipid classes. This method merges swift analysis with minimal idle time by directly introducing lipids into the mass spectrometer[1]. The acquisition of data in both polarity modes[2] ensures ionization efficiency and detection specificity and improves the lipidome coverage because more lipid classes and more individual species within each class can be spotted. In this communication, we detail protocols for quantifying major lipid classes using nanospray direct infusion mass spectrometry, facilitating comprehensive and high throughput lipidomic analyses.

Sample preparation and analysis was performed as described in a previous method[3] for direct infusion (DI) lipidomics with some variations. A DI chip-based nano-electrospray device (TriVersa NanoMate; Advion Biosciences, Ithaca, NY), was interfaced to a Thermo QExploris 240 quadrupole-Orbitrap mass spectrometer, like that described previously[1]. Lipid extracts were polled and split for use as quality control (QC) and quality assurance (QA) following guidelines[4,5]. Samples were analyzed using lock masses of internal calibrator in full scan mode combining MS^1 and MS^2 data acquisition. DI data were processed by LipidXplorer 1.2.8.1[6] identifying lipids by their accurate mass and tandem mass spectra (tolerance 5 ppm for MS1 and 20 ppm for MS2) following guidelines for annotation[7] and reporting[8] lipidomic data. The output was manually filtered, and statistical analyses were performed online using MetaboAnalyst[9].

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P42
COMPARATIVE STUDY OF THREE BLOOD MICROSAMPLING DEVICES THROUGH UHPLC-MS LIPIDOMIC PROFILING

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To date, there is a need to make blood sample collection more accessible, to standardize, and harmonize sample handling/ treatment. Whit this aim, blood microsampling (BμS) — the process of collecting small volumes of blood (<100μL) — offers a smart alternative to conventional methods for profiling of human biofluids. This approach is minimally invasive, relatively pain-free, avoids displacements, improves analyte stability, and facilitates large-scale studies. However, qualitative and quantitative studies are necessary to acquire reliable knowledge of their capabilities for high metabolite coverage, as well as accuracy and precision in their determination.

Here, we present a lipidomics study of venous blood from 10 healthy individuals in three BμS devices: dry blood spot (DBS)-Whatman™, quantitative DBS (qDBS)-Capitainer® B and volumetric absortive microsampling (VAMS)-Neoteryx Mitra®. For sample preparation a single-phase lipid extraction protocol (MeOH/MTBE) was applied before the analysis by RP-LC-ESI(+/-)- QTOF-MS.

The lipid profiles were compared, and reliable identification of compounds was performed against our in-house human plasma MS database containing 592 molecular lipid species. For the targeted lipid extraction, an algorithm from Agilent MassHunter Profinder was used — based on the molecular formula, retention time and accurate mass — . In addition, evaluation of the lipid recovery for each BμS device was carried out with the pooled study samples spiked before and after the extraction with Avanti SPLASH LIPIDOMIX®. Finally, the precision of the determination with the three BμS devices was evaluated using three biological replicates from three individuals.

After data-preprocessing, we obtained recovery rates yielding 100-121% for Whatman, 99-113% for Capitainer, and 78-105% for Mitra, demonstrating good lipid coverage over the whole polarity range. Cholesterol esters and cholesterol display the highest standard deviation, due to its in-source fragmentation. Regarding the precision, Mitra and Capitainer were the best, with CV<25% for most of the identified lipids.

P43
COMPARATIVE ANALYSIS OF BLOOD MICROSAMPLING DEVICES FOR GLOBAL UNTARGETED METABOLIC PROFILING

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Microsampling technologies have emerged in bioanalysis and find applications in various fields of biomedical research including metabolomics studies. Although several devices share the same concept for sample collection, variations on their matrices might impact analytical outcomes from biological samples. The aim of the present work is to evaluate the information obtained by the different microsampling devices, namely Dried Blood Spots, Volumetric Absorptive Microsampling and Capitainer cards for the blood global metabolic profile.

To achieve this goal, a whole blood untargeted analysis method was developed, using an UHPLC with a Reverse Phase system, coupled to a TIMS-TOF working in DDA mode. MS conditions were optimized to increase sensitivity and reduce in- source fragmentation. Our aim was to obtain the most detailed chromatographic profile possible, while facilitating trustful annotations. Extraction protocols were optimized based on literature surveys [1][2]. Among the different solvents tested (acetonitrile, methanol and water, both individually and at different proportions), acetonitrile:methanol mixture in a ratio of 70:30 yielded the most favorable outcomes, considering number features, area of annotated peaks and reproducibility. Data processing and analysis was performed by different software (i.e. MSDial, XCMS, Metaboscape).

Each individual device underwent a similar optimization process. Methanol: H2O demonstrated to be the most efficient solvent, when allied to vortex agitation and sonication with beads. To increase sensitivity and improve chromatographic profile, samples underwent evaporation and reconstitution in a reduced volume of water-methanol mixture at a ratio of 95:5.

A similar procedure was done using a GC/MS method. Samples underwent an analogous process for solvent optimization, and derivatization was performed after sample evaporation using Methoxyamine and Trimethylsilane [3]. Finally, we compared the untargeted profiles obtained from ten individuals using the three different microsampling devices and plasma samples, under the optimized analysis conditions at both chromatographic systems.

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P44
DEVELOPMENT AND APPLICATION OF A WORKFLOW FOR CELL METABOLOMICS ANALYSES

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Cell metabolomics comprises several challenges, particularly for scarce populations, such as obtaining similar numbers of cells or establishing the optimal and minimal number of cells necessary for analysis. Moreover, there is no established methodology to differentiate metabolites derived from cells (intra- or extra-cellular) from those of culture media. Here, we propose a novel workflow for cell metabolomics analyses.

CD3+ cells were isolated from peripheral blood of a healthy donor, resuspended in methanol and distributed in 30 vials, each containing 5 replicates of 6 different, increasing numbers of cells: 25.000, 50.000, 100.000, 250.000, 500.000 and Metabolite extraction was performed using a 4:1 mixture of methanol:methyl-tert-butyl ether, followed by untargeted lipidomics using liquid chromatography coupled to mass spectrometry (LC-MS QToF 6550, Agilent) in positive and negative electrospray ionization modes (ESI+/ESI-).

Raw data was deconvoluted using MassHunter Profinder B.10. and subsequently filtered according to blank subtraction, presence/absence in quality controls (QCs) and samples and coefficient of variation in QCs, obtaining 1191 and 251 features for ESI+ and ESI-, respectively. Then, correlation analyses between cell numbers and metabolite abundances within specified ranges (e.g., for all samples between 50.000 and 750.000 cells) were conducted. Principal Component Analysis (PCA) and clustering analysis were also conducted. The highest number of features that significantly correlated ($p<0.05$, $\rho>0.7$) was obtained for the 250.000-750.000 range (n=237) for ESI+ and for the 50.000-250.000 range (n=99) for ESI-. Furthermore, PCA models using only correlated metabolites showed a clear clustering for all replicates.

This strategy was applied to analyse differences between memory effector (Teff) and regulatory (Treg) T cells from healthy subjects (n=6), successfully discriminating intracellular metabolites from those of the cell media. We obtained 195 metabolites for Teff and 233 for Treg, with 133 shared between both cell types.

P45

QCOMICS: RECOMMENDATIONS AND GUIDELINES FOR ROBUST, EASILY IMPLEMENTABLE AND REPORTABLE QUALITY CONTROL OF METABOLOMICS DATA

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The implementation of quality control strategies is crucial to ensure the reproducibility, accuracy, and meaningfulness of metabolomics data. However, this pivotal step is often overlooked within the metabolomics workflow and frequently relies on the use of non-standardized and poorly reported protocols. To address current limitations in this respect, we have developed QComics, a robust, easily implementable and reportable method for monitoring and controlling data quality. The protocol operates in various sequential steps aimed to (i) correct for background noise and carryover, (ii) detect signal drifts and "out-of-control" observations, (iii) deal with missing data, (iv) remove outliers, (v) monitor quality markers to identify samples affected by improper collection, preprocessing, or storage, and (vi) assess overall data quality in terms of precision and accuracy. To simplify its implementation, this protocol can easily be performed in software that is available to most researchers, without the need of advanced statistical and programming skills. Notably, this tool considers important issues often neglected along quality control, such as the need of separately handling missing values and truly absent data to avoid losing relevant biological information, as well as the large impact that preanalytical factors may elicit on metabolomics results. Altogether, the guidelines compiled in QComics might contribute to establishing gold standard recommendations and best practices for quality control within the metabolomics community.

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P46

DECODING THE HUMAN SEMINAL PLASMA METABOLOME: ASSESSMENT OF THE PERFORMANCE OF DIFFERENT SAMPLE PREPARATION STRATEGIES

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The global decline in birth rates observed over the past five decades persisted in 2023, driven by both sociocultural changes and rising fertility challenges [1]. Indeed, more than half of the fertility problems in couples arise from the male partner. Traditional semen analysis fails to diagnose the etiology of male fertility conditions [2]. Therefore, new omics approaches are emerging as an alternative toolbox to retrieve more information from seminal liquid and, thus, to better understand and diagnose the phenomena behind male sub- and infertility [3]. From an analytical point of view, seminal fluid represents a rich and easily accessible source of metabolites [4]

In the present work, we first compared different sample preparation techniques enabling the analysis of oxylipins in human seminal plasma. Samples were analyzed using a targeted metabolomics strategy for 79 molecules. Different protein precipitation treatments, optionally followed by solid phase extraction (SPE), yielded similar but distinctive profiles. Each protocol was characterized on the base of their alpha diversity, which measures the richness of metabolites within each sample. Our results confirmed the presence of a panel of other oxylipins in addition to the expected prostaglandins. Finally, SPE and solid phase microextraction (SPME) using HLB sorbent were compared aiming at a broader metabolomic seminal plasma analysis. SPE yielded a slightly more diverse profile of compounds for untargeted analyses, but at the expense of a complex and time-consuming protocol.

In all, our results show that the oxylipin profile obtained from seminal liquid remains quite unaffected by variations to the sample preparation protocol such as deproteinization solvent, with SPE providing a characteristic coverage when untargeted analyses are performed. In this direction, comparison of SPE versus SPME revealed a better removal of interferents with SPE providing a better coverage over the more polar region.

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P47

QUANTITATIVE ANALYSIS OF POLAR METABOLITES WITH COMPREHENSIVE PLASMA AND CELL TARGETED METABOLOMICS WORKFLOW

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Incorporating Quantitative Analysis in a HILIC Polar Metabolomics Workflow with the New 4th Generation 6495 LC/Triple Quadrupole.

Targeted metabolomics methods provide a sensitive and precise measurement of metabolites with a large dynamic range. Previously described is a HILIC polar metabolite workflow using Bravo Sample Prep Platform with cells or plasma, a 1290 Infinity II Bio LC for improved performance of metal sensitive analytes, and a 6495 LC/TQ mass spectrometer with a database of ~500 polar metabolites and retention times1. The speed of the 6495 LC/TQ allows for hundreds of analytes in both positive and negative ion mode to be analyzed with precision in the same injection. This workflow and database can be deployed in several ways, from metabolite pathway discovery (profiling) to semi quantitative analysis of hundreds of analytes in a sample, or for absolute quantitation using isotopically labeled internal standards.

Described here is an update to the HILIC polar metabolite workflow showcasing the new 4th generation 6495 LC/TQ which has improved ion optics for increased sensitivity. The workflow update also incorporates the use of commercially available isotopically labeled internal standards for absolute quantitation of certain analytes. New 13C transitions were added to the database and quantitative performance was evaluated and described here. These transitions provide easy adoption of semi or absolute quantitative studies for researchers wanting even more precision and accuracy in their studies.

Furthermore, analyte quantitation was completed on cell and plasma sample extracts processed using the Bravo Sample Prep Platform, showcasing a comprehensive quantitative workflow of complex samples.

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P48

DISTRIBUTION OF METALS IN SPOONBILLS FROM NATURAL AREAS OF THE SOUTHWEST SPAIN. THE IMPACT ON THE MICROBIAL-PRODUCED METABOLITES AND GUT MICROBIOTA

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The presence of industrial and urban centers in Andalusia, which are significant sources of pollution with environmental effects, justifies the need to investigate and evaluate the presence, mobilization and accumulation of different xenobiotics in the tissues of living organisms in particularly vulnerable ecosystems. The pollution caused by heavy metals are widely recognized at global, regional and local levels [1] and their influence on ecosystems [2], especially in areas with abundant water, which acts as a carrier of these pollutants. The wading bird known as the common spoonbill (Platalea leucordia) resides in wetlands and shows a high sensitivity to pollution, which positions it as a valuable bio-indicator for assessing the ecological vitality of ecosystems. It also acts as a living indicator of the impact of human activities on the natural environment [3]. To this end, 143 fecal samples were analyzed from four different birds: spoonbill, stork, yellow-legged gull and black-headed gull, in three selected areas: The Marismas del Odiel Biosphere Reserve (Huelva), Salina the Tapa (Puerto de Santa María, Cádiz) and Salina the Cetina (Puerto Real, Cádiz). An untargeted metabolomic study was performed on faeces to determine microbially produced metabolites that could potentially be related to the presence of environmental contaminants. The metabolomic analysis was carried out by combining gas chromatography with mass spectrometry and ultra-high performance liquid chromatography with quadrupole time-of-flight coupled (UHPLC-QTOF). Possible metabolic alterations caused by environmental pollution at the different sampling points, as well as possible associations with pollutants, are being investigated.

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IMPACT OF ARSENIC EXPOSURE AND SELENIUM SUPPLEMENTATION ON MICE GUT METABOLOME, PLASMA SELENOPROTEOME AND ARSENIC METABOLISM

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Every year, arsenic (As) contaminates water and food, leading to poisoning in numerous individuals. Controlled experiments exposing As combined with metabolomics enable the assessment of pollutants' mode of action and effects in mammals, often interacting antagonistically or synergistically. Selenium (Se) acts as a well-known antagonist against various pollutants, crucial for health and nutrition due to the antioxidant properties of selenoproteins. This study aimed to assess As impact on mice gut microbiota and the potential role of Se supplementation as an antagonist. Sixty BALB/c Mus musculus mice were divided into six groups: (i) Control (C), (ii) mice fed a regular diet with microbiota depleted by antibiotics (Abx), (iii) mice fed a regular diet and exposed to As (As), (iv) mice fed a regular diet exposed to As after microbiota depletion with antibiotics (As-Abx), (v) mice fed a Se-supplemented diet exposed to As (As-Se), and (vi) mice fed a Se-supplemented diet and exposed to As after microbiota depletion with antibiotics.

Gut contents were analyzed by ultra high-pressure liquid chromatography coupled with quadrupole time-of-flight (UHPLC-QTOF) after metabolite extraction from lyophilized samples with 250 µl of methanol, followed by vortexing and centrifugation. Mice plasma selenoproteome was assessed by direct injection of samples into HPLC coupled with inductively coupled plasma mass spectrometry with triple quadrupole (ICP-MS). As speciation in plasma was conducted via HPLC-ICP-MS after extraction

with 125 µl trichloroacetic acid, 20 µl acetonitrile, and 20 µl deionized water. Our findings demonstrated a significant impact of As on microbial-produced metabolites (gut metabolome) and mouse plasma selenoproteome, along with As speciation profiles. Se supplementation mitigated several impairments caused by As exposure, and notably, we observed a competition for Se between the host and gut microbiota, reflected in the plasma selenoproteome.

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ASSOCIATION BETWEEN HUMAN MILK MICROBIOTA AND THYROID HORMONES BY COMBINING HETEROATOM-TARGETED METABOLOMICS, UNTARGETED METABOLOMICS AND METATAXONOMICS

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Breastfeeding is considered the gold standard of infant nutrition, exerting a profound influence on both infant growth and development. Iodine, an essential nutrient solely obtained through the diet, assumes heightened importance during pregnancy to accommodate altered maternal thyroid physiology and fetal development. Previous investigations have delved into the repercussions of maternal iodine deficiency on the metabolome of human milk (HM) [1]. Thyroid hormones (TH) represent the sole iodine-containing compounds with biological activity, pivotal for infant health. Given the chiral nature of TH, their enantiomers exhibit different biological and metabolic activities. Moreover, HM microbiota significantly contributes to gastrointestinal colonization in infants, particularly within the initial 100 days of life [2]. Herein, we analyzed TH and microbiota in 250 samples of HM of iodine deficient women and controls. TH were extracted by Hollow Fiber-Liquid Phase Microextraction (HF-LPME), separated by a column switching including both, chiral and reversed stationary phases, and then detected by quadrupole time-of-flight (QTOF) tandem mass spectrometry, inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ-MS) and ion mobility mass spectrometry (IMMS). Iodine was used as a "tag" into the ICP-MS to determine TH. TH concentrations (ng/g) ranked as follows: D-T4 (96.78 ± 164.00) > L-T4 (71.23 ± 112.65) > 3,3',5'- triiodothyronine (65.22 ± 112.54) > 3,3',5-triiodothyronine (43.85 ± 68.43) > 3,5-diiodothyronine (43.42 ± 56.35) > thyronine (19.94 ± 34.41) > 3,5-diiodotyrosine (9.86 ± 21.86) > 3-iodotyrosine (9.53 ± 26.27). Furthermore, microbiota profiling analyzed by 16S amplicon sequencing on the Illumina platform, revealing numerous associations that depends on maternal iodine levels.

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P51

EFFECT MANGANESE EXPOSURE IN THE METABOLISM OF SELENIUM-SUPPLEMENTED MICE MODEL COMBINING SERUM HETEROATOM-TAGGED PROTEOMICS AND METATAXONOMICS IN THE GUT CONTENT

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Manganese (Mn) is an environmentally abundant essential metal required for numerous biochemical processes throughout the human body and an important cofactor of several proteins [1]. However, exposure to Mn can induce neurotoxicity in humans that progresses from early psychiatric abnormalities to very severe symptoms including Parkinson's disease [2]. On the other hand, it is well known that selenium (Se) is an antagonist against several pollutants such as mercury (Hg), arsenic (As), and Cd [3]. However, there is little evidence about the protective role of Se against Mn. For this reason, 270 serum samples were divided into 9 groups: a control group with mice fed a rodent diet, a group of mice fed a rodent diet and antibiotics for the depletion of microbiota, a group of mice fed a Se-normal diet, a group of mice fed a high Mn-diet a group of mice fed a high Mn-diet and antibiotics, a group of mice fed a normal Se and high Mn-diet, a group of mice fed a Se- supplemented and high Mn-diet, and a group of mice fed a Se-supplemented and high Mn-diet and antibiotics.

In this study, we have optimized a heteroatom-tagged proteomics method based on size exclusion chromatography (SEC) coupled to inductively coupled plasma mass spectrometry with triple quadrupole (ICP-QQQ-MS) to determine the Mn-species (Mn-α-2-macroglobuline, Mn-β-lactoglobulin, Mn-transferrin, Mn-albumin and Mn-citrate) in the serum from microbiota depleted mice fed Se-supplemented diet and exposed to Mn, to evaluate the protective effect of Se against Mn, the depletion of microbiota and the alteration of Mn species. Moreover, the gut microbiota profile obtained by 16S rRNA amplicon sequencing was analyzed to identify specific associations between Mn species and gut microbes shaped by the animal supplementation groups.

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P52
FECAL BIOMAKERS AS A TOOL TO DESCRIBE THE TRANSFORMATION FROM WILD-PIG TO DOMESTIC PIG

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Along the development of the domestication, wild species suffer profound transformations, adapting to human environments and purposes. The deliberate taming and breeding of animals for human use purposes led to a fundamental change in human-animal relationships during the Neolithic age. Through domestication, humans gained control over selected species, shaping their biology and behavior to suit the livestock necessities. This age witnessed the emergence of numerous domestic species, including cattle, sheep, goats, and pigs, which played pivotal roles in shaping human economies, social structures, and ecological landscapes.

Among these domesticated species, the pig stands as an interesting case of study, showcasing the complex interaction between humans and animals during the Neolithic period. From its wild ancestor, the wild boar, to the domesticated pig, this species underwent remarkable evolutionary changes shaped by human intervention. Recent advances in lipid analysis have enhanced our understanding of pig domestication by allowing us to identify pig feces in archaeological sites using bile acids or sterol. However, there remains a notable gap in research regarding the differentiation between pig and wild-pig feces

In this research work, after a lipidomic study of domestic pig and wild-pig feces through a liquid chromatography quadrupole Time of Flight method, a Partial least squares-discriminant analysis model (PLS-DA) model has been created by Matlab capable of distinguishing between wild and domesticated specie. Afterwards, a feature selection of biomarkers presents in just one of the animals has been carried out and the MS/MS spectra of the selected biomarkers were obtained. Finally, the identification of selected biomarkers was carried out in LipidsMaps, Metfrag and MassBank database using their exact mass, formula, and MS/MS spectra.

The analysis of these biomarkers was done in archaeological samples from El Mirador cave in Sierra de Atapuerca (Burgos, Spain).

P53

EXPLORING THE METABOLOMIC RESPONSE OF AZELAIC ACID TREATMENT IN ARABIDOPSIS THALIANA SEEDLINGS

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In this study, we investigated the impact of low concentrations of the dicarboxylic acid azelaic acid (AZA) on the metabolism of the model species Arabidopsis thaliana. Previous studies already demonstrated the phytotoxic potential of this molecule, suggesting auxin imbalance on its mode of action. The untargeted metabolomic analyses were conducted through GC/MS on both untreated and AZA-treated (44 µM) A. thaliana seedlings at two time points: 7 and 14 days post-treatment initiation. Our results revealed a gradual accumulation of starch content (glucose, mannose, xylose, etc.), amino acids (lysine, GABA, threonine, glutamine, etc.), and organic acids (glutaric acid, shikimic acid, succinic acid, etc.) in AZA-treated seedlings over time. This accumulation of osmoprotectants suggests a potential induction of stress in Arabidopsis seedlings by AZA. Additionally, ionomic analysis demonstrated that AZA treatment induced phosphorus deficiency, which plants compensated for by increasing malate content within the roots. Furthermore, AZA treatment resulted in a gradual accumulation of putrescine over time, serving as a metabolic biomarker indicative of potassium deficiency and plant stress. Metabolomic profiling further revealed an elevation in the biosynthesis of specialized metabolites, such as nitrogen-containing and sulfur- containing compounds, and alterations in the levels of diverse phytohormones, including jasmonate and brassinosteroids, implicated in plant protection under biotic and/or abiotic stresses. These findings support the hypothesis that the mode of action of AZA involves an auxin imbalance, indicating its potential as an auxinic herbicide. The observed changes in starch and jasmonate levels, coupled with alterations in potassium homeostasis, directly correlate with previously reported disruptions in the auxin transport system, root architecture, and gravitropic root response.

P54

PLANT CELLS AS BIOFACTORIES FOR THE PRODUCTION OF SPECIALIZED METABOLITES AND THEIR USE IN DERMOCOSMETIC AND AGRI-FOOD PRODUCTS

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Plants synthesize an enormous variety of compounds which have beneficial effect on human health, and therefore, they are considered as bioactive compounds, and constitute the most important ingredients for pharmaceutical, agri-food and cosmetic industries. We have developed different strategies to increase the production of these specialized metabolites. One of these strategies consists of the use of plant cell cultures under elicitation. In fact, modified β-cyclodextrins (mβ-CD) are used as elicitors since they increase bioactive compound production in a wide variety of plant cell cultures. This process is due to mβ-CD properties, specially, their hydrophobic central cavity which trap nonpolar compounds, making them highly water-soluble compounds.

This process is independent of cell biomass generation since as bioactive compounds are produced by cells, they are secreted and easily recovered from the medium without biomass destruction. Also, the production of these metabolites is further increased when mβ-CD are combined with methyljasmonate.

Using these elicitors, the biosynthesis pathways of different specialized metabolites are induced. Thus, using cell cultures, the production of stilbenes in Vitis vinifera, indole alkaloids in Catharanthus roseus, and carotenoids, phytosterols, tocopherols, phenolic compounds and glucosinolates in other plant cell cultures are increased.

Currently, we are generating these bioactive metabolites for their use in dermo-cosmetic and food companies, generating new bioactive ingredients for functional foods. Also, agricultural companies are looking for solutions to reduce the use of chemical and achieve more resistant plants with a greater capacity to respond to biotic and abiotic conditions. These companies are testing our extracts derived from elicited plant cell cultures as biostimulants to strengthen seeds and seedlings against unfavourable edaphoclimatic conditions.

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P55
EXPLORING CITRUS POSTHARVEST DECAY: METABOLOMICS ANALYSES OF BLUE MOLD DISEASE

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Penicillium italicum is the causal agent of the blue mold disease, which causes citrus postharvest decay, reducing fruit quality and productivity[1]. In this study, the pathosystem *Citrus sinensis-P. italicum* was investigated in different times (6, 9 and 13 days of infection) using a mass spectrometry-based untargeted metabolomics workflow. The oranges used in the study were successfully infected with the pathogen *P. italicum*. After 6-dpi, characteristic symptoms of blue mold disease were observed. No symptoms of the disease were observed in the control oranges. To evaluate intergroup metabolic differences associated with *P. italicum* infection in oranges, we employed PCA analyses. The PCA analysis revealed a significant separation between the control and infected groups based on their metabolic characteristics, thus reflecting metabolic differences between these two groups. The metabolites annotations were performed using Global Natural Product Social Molecular Networking (GNPS) platform, enabled the identification of thirty metabolites in the UPLC-MSMS analyses conducted on the groups of oranges control and infected by *P. italicum*. Citrus defense metabolites such as nobiletin ([M+H]+ m/z 403.1388), a polymethoxyflavone, were annotated. Although nobiletin's role in fruit defense against pathogens like P. digitatum is known, its association with defense against P. italicum is novel. Other polymethoxyflavones like tangeretin ([M+H]+ m/z 373.1289) and tetramethyl-O-scutellarein ([M+H]+ m/z 343.1175) were also identified, with their intensities increasing at 6 dpi according to chemical imaging data obtained by desorption electrospray ionization mass spectrometry imaging analyses (DESI-MSI). These findings underscore the active contribution of citrus secondary metabolites to host defense mechanisms. Importantly, previous studies on the metabolic interaction between *P. italicum* and citrus have not been reported, highlighting this research a fundamental basis for future investigations in the development of control strategies.

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P56
EXPLORING METABOLIC ALTERATIONS IN RESPONSE TO SALINITY, DROUGHT, AND COMBINED STRESS ACROSS DIFFERENT CARPOBROTUS SP.PL. POPULATIONS: AN UNTARGETED METABOLOMICS APPROACH

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Abiotic stresses such as salinity and drought pose significant challenges to plant growth and survival in the context of climate change, affecting metabolic processes essential for adaptation and resilience. *Carpobrotus* sp.pl., an invasive genus of succulent plants know for this ability to tolerate adverse environmental conditions, serves as an excellent model for studying stress responses. This study employs an untargeted metabolomics approach, employing Gas Chromatography-Mass Spectrometry (GC-MS), to investigate metabolic alterations in response to salinity, drought, and combined stress across four populations of *Carpobrotus* from two different genetic clusters (Cluster A and B) and from two bioclimatic regions (Atlantic and Mediterranean).

A total of 158 metabolites were detected and identified in *Carpobrotus* shoots. Principal Component Analysis (PCA) revealed clear differences between populations regardless of treatment, with treatments distinctly separated within each population. Nicotinic acid, hexadecane and leucine the primary metabolites contributing to population differences, while sorbitol and L- proline were notable under stress conditions. Analysis of metabolite changes between treatments highlighted significant differences, with

146 metabolites identified as significantly altered. Notably, L-proline emerged as a principal metabolite associated with abiotic stress treatments. Further investigation revealed distinct metabolic responses to individual stressors across populations, with notable variations in metabolite accumulation. Under salinity stress, significant increases in L- proline and uric acid were observed across all populations, indicating common adaptive responses. Conversely, drought stress elicited population-specific responses, with Samil and Cádiz populations exhibiting similar metabolic patterns. Combined stress conditions resulted in differential metabolite accumulation across populations, with L-proline, 1,6- anhydroglucose, and uridine significantly altered.

These results elucidate the complex metabolic mechanisms involved in stress responses in *Carpobrotus*, highlighting both shared adaptive strategies and unique responses specific to different populations. Our study provides valuable insights into the resilience of *Carpobrotus* populations in the face of environmental stressors and challenges.

P57
IN-VITRO FERMENTATIONS TO STUDY THE INHIBITORY EFFECT OF A THYME EXTRACT ON MICROBIAL TRIMETHYLAMINE PRODUCTION FROM L-CARNITINE

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Epidemiological studies have revealed that high concentrations of circulating trimethylamine-N-oxide (TMAO) are strongly associated with the development of major adverse cardiovascular events and all-cause mortality. Several dietary precursors can be metabolized by specific gut bacteria to produce trimethylamine (TMA), which is subsequently oxidized in the liver to TMAO by flavin-containing monooxygenase 3. Among dietary precursors, L-carnitine, which is generally found at high concentrations in foods of animal origin, is an important dietary precursor of TMA. A multistep microbial pathway has been recently proposed as the principal route for TMA production through L-carnitine metabolism by the gut microbiota. L- carnitine is first converted into γ-butyrobetaine (γ-BB) by multiple human commensals that possess the cai gene operon, and γ-BB is further metabolized into TMA by the enzymes encoded by the *gbu* gene cluster.

Nowadays, there is great interest in the discovery of food compounds that might inhibit gut microbial TMA production from its trimethylamine precursors. In this work, we have tested the inhibitory effect of a thyme extract in a complex microbiome community. For that, we collected the feces from five TMA-producing participants and cultured them in nutritional broth supplemented with L-carnitine. Modulation of the microbial community was analyzed by short-read amplicon sequencing of 16S rRNA gene for taxonomic profiling of microbial communities. Fecal microbiota functional activity based on targeted metabolomics analysis of microbiota-derived metabolites was carried out by LC-QqQ-MS/MS for highly selective and sensitive quantification of microbial metabolite analysis. Despite the interpersonal differences, our study showed that thyme extract reduced the TMA-producing capacity in vitro through L-carnitine metabolism in TMA-producing participants. This study showed an increase in γ-BB in the presence of thyme extract or Meldonium (control) in the growing medium, suggesting that both treatments might inhibit the conversion of γ-BB to TMA, resulting in the accumulation of γ-BB.

P58
METABOLOMIC INVESTIGATION OF THE INTERACTION BETWEEN A NOVEL PHYTOPATHOGENIC FUNGUS AND ITS HOST CITRUS SINENSIS

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Fungal phytopathogens cause various diseases in global citrus cultivation, resulting in low productivity and significant economic losses[1]. Studies usually focuses on treating the disease symptoms, however the metabolites involved in the pathogen-host interaction are underexplored[2]. In this study, we applied an untargeted metabolomics workflow based on mass spectrometry to investigate the metabolites involved in the interaction between the phytopathogen (LaBioQuiMi01) and its citrus host. Two groups were evaluated: infected leaves and healthy leaves at different time points after inoculation (5, 10, and 15 dpi). Through multivariate

analyses PCA and PLS-DA discrimination between control and infected plants was observed, indicating metabolic differences between the groups. In the infected group, alterations in the biological system were observed, with elevated levels of the amino acid phenylalanine (*m/z* 166.0865) and isocoumarin (*m/z* 207.0652), as well as the flavonoids Nobiletin (*m/z* 403.1388), Hesperidin (*m/z* 611.1968), Tangeretin (*m/z* 373.1287), Gardenin B (*m/z* 359.1130), and Hesperetin (*m/z* 303.0860). These compounds may be involved as plant defense or resistance mechanisms. Other compounds were also observed only in the infected group, such as indole acid (*m/z* 203.1179) and the mycotoxin (*m/z* 374.2331). This latter metabolite was also detected in the fungus in vitro culture. To evaluate the influence of this mycotoxin (obtained through in vitro extract isolation) on the pathogen-host interaction, we conducted a biological assay to test its phytotoxicity on citrus seed germination. The mycotoxin exhibited moderate phytotoxic effects at a concentration of 500 ppm and high phytotoxicity at a concentration of 1000 ppm on citrus seed germination. Our biological assays suggest that the mycotoxin may play a significant role in the virulence of the new phytopathogen. Therefore, this research serves as a foundation for further investigations into the interaction of the fungus LaBioQuiMi01 with its citrus host.

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P59
METABOLOMIC RESPONSE OF TWO VICIA FABA GENOTYPES TO CLIMATE-INDUCED STRESS: DROUGHT AND WATERLOGGING

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Understanding how plants respond to climate change is necessary for developing resilient crops, and metabolomics can offer valuable insights into this task. In this study we investigated the metabolomic responses of two faba bean (*Vicia faba*) genotypes, Lynx and Zoran, to drought and waterlogging situations.

Plants were grown under controlled conditions with three irrigation regimes: optimum, drought, and waterlogging. Root samples were taken for untargeted metabolomic GC/MS analysis according to Lisec (2006)[1]. Compounds were identified using the software MS-DIAL based on EI-MS and Retention Index public libraries. Annotated metabolites data were analysed using Metaboanalyst 5.0, assessing both multifactorial (genotype and irrigation effects) and one-factor (irrigation effects on each genotype) approaches.

All the 77 annotated metabolites were found significant after two-way ANOVA, and 50 of them were simultaneously affected by the two factors and its interaction, indicating that irrigation treatments clearly affected the metabolites accumulation, and that each genotype responded differently to the treatments. While drought treatment induced up-accumulation of many metabolites in the root of both genotypes, including many well-known osmoprotectants, waterlogging induced a general down-accumulation of most metabolites in Zoran and varied accumulation of those metabolites in Lynx.

Correlation analysis indicated that Zoran roots could inherently accumulate more phenolic compounds than Lynx, mainly under drought stress. One-factor analyses revealed that ornithine-urea cycle metabolites up-accumulated under drought conditions in both genotypes and Lynx-waterlogging, but down-accumulated in Zoran-waterlogging. Similarly, ascorbate cycle metabolites up-accumulated in Lynx-drought and Lynx-waterlogging but down-accumulated in Zoran-waterlogging.

This study demonstrated that drought stress strongly activates the metabolism of both faba bean genotypes, while Lynx is potentially activating a more intense response to waterlogging than Zoran. Phenolic compounds, nitrogen mobilization, and ascorbate oxidative processes could be behind the different responses of Lynx and Zoran to waterlogging conditions.

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P60

UNTARGETED METABOLOMIC APPROACHES FOR THE DETECTION OF COMMON CIRCULATING METABOLITES DERIVED FROM VARIOUS BIOACTIVE PLANT EXTRACTS

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Clinical evidence suggests similar benefits of bioactive-rich extracts in chronic diseases. Although they share chemical families such as phenolic compounds, the specific compounds vary widely between species. In fact, more than 8000 phenolic structures have been reported in literature. In addition, these compounds can also be metabolized in the body through different pathways. For that, our hypothesis relies on that common bioavailable metabolites may contribute to reported bioactive effects from diverse plant compounds. Therefore, the aim of this study was to detect bioavailable common metabolites from 5 bioactive extracts (*Hibiscus sabdariffa, Lippia citriodora, Olea europaea, Silybum marianum* and *Theobroma cacao*) rich in phenolic compounds but with different chemical compositions. To achieve this goal, we proposed an acute double-blind intervention study in humans in which blood samples were collected at different times. These samples were analysed using an untargeted metabolomics workflow based on HPLC-ESI-QTOF-MS. Data processing was performed using mzMine software and notame R package. The most relevant result was a total of 9 common bioavailable Phase-II metabolites found for the *L. citriodora* and *O. europaea* matrices. Despite being the same metabolites, these presented statistically different Tmax depending on the source of origin, revealing different metabolization mechanisms depending on the ingested extract. This highlights the potential of combining both extracts in the development of nutraceuticals to allow circulating metabolites to reach the bloodstream for a longer period, and therefore increase the chances of reaching target tissues. We are currently exploring the bioactive anticancer properties of the common bioavailable metabolites to better understand the mechanisms driving their bioactivity. Some of the common metabolites that have been shown to possess anti-cancer effects in pancreatic cancer cells are vanillic acid sulphate and homovanillic acid sulphate. This research holds promise for future applications, potentially facilitating the use of these compounds in functional foods or pharmaceuticals.

P61

AN INTEGRATED PIPELINE TO UNRAVEL PLANT EXTRACTS METABOLOME

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The enormous richness of plant secondary metabolism is a unique source of metabolites with a wide range of biological activities. Besides the scientific interest of metabolomics characterisation of extracts derived from different plant species, the knowledge extracted often results in applications in the pharmaceutical, cosmetic and agronomical fields. Determining the metabolomics composition of extracts is also one of the major bottlenecks for product standardization and quality improvement.

Due to the chemical diversity of the different families of metabolites, the plant metabolome cannot be addressed by a single technique, being essential a combination of liquid and gas chromatography techniques coupled to mass spectroscopy dedicated methods. This is why the characterization of plant-derived extracts is a major challenge for metabolomics.

In particular, plant hormones play a pivotal role in several physiological processes and their quantification is crucial to understand the biological activity of plant extracts. Since plant hormones are present at very low concentrations in plant tissues (10-9 M to 10-6 M), the development of a high-throughput and comprehensive method for the determination of hormones is challenging.

The HORMONE ANALYSIS and METABOLOMICS FACILITIES installed at the IBMCP (UPV-CSIC) have developed a combined pipeline for complete metabolomics characterisation of plant extracts including:

- Analysis of more than 24 plant hormones, Auxins, Cytokinins, Gibberellins, Abscisic Acid, Jasmonic Acid, ethylene and Salicylic Acid in small volumes samples
- Primary metabolism targeted analysis
- Analysis of chlorophylls, carotenoids and xanthophylls by
- Analysis of volatile compounds
- Targeted and untargeted analysis of semipolar secondary metabolites (phenolics, diterpenoids and alkaloids)

We combine broad experience in extraction techniques with our knowledge in both LC and GC methods coupled to mass spectrometry. Our equipment includes two UPLC-MS/MS Thermo Scientific™ Q Exactive™ Mass Spectrometers, a LECO Pegasus GC-MS, a Waters2965 LC-PDA-Fluorescence and an Agilent Q5975B inert XL M GC-MS.

P62
METABOLOMIC APPROACH FOR HOP'S PHYTOCHEMICAL CHARACTERIZATION RELATIVE TO ITS PHENOLOGICAL STAGE

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Humus lupulus L., the common hop, is grown mainly for beer production. Female inflorescences, commonly referred as hop cones, are added to the wort for beer bitterness and their aromatic and antiseptic properties, linked to their original composition. The growing popularity of craft beer and the increasing interest of natural medicine is widening appealing scenarios for aromatic hop varieties. Phenolic compounds, including prenylated chalcones as xanthohumol, α-acids (humulones) and β-acids (lupulones) are produced at lupulin glands at the base of the bracts. Hop essential oil is also rich in monoterpenes and sesquiterpenes, which are responsible of the "hoppy" aroma of beer. The brewing value of hops is determined by the α-acid content and the composition of essential oil. Even metabolite's profiles have been exhaustively characterized in different hop varieties [1,2] and their contribution to the hop quality is also well established [3]. However, when it comes to metabolites involved in hop development, literature is scarce. In this sense, evaluating the metabolic differences between phenotypic stages of growth becomes of upmost importance to control and monitoring the optimal hop development.

Our study aims at deciphering the chemical characterization of hop's cones from the Sladek variety at three different phenological states. An untargeted analysis based on an hydroalcoholic extraction followed by ultra-high performance liquid chromatography-high resolution mass spectrometry (UPLC-HRMS) acquisition was performed in the negative and positive ion modes to obtain an exhaustive characterization of non-volatile profile for comparative purposes. The annotated metabolome dataset was generated by Compound Discoverer 3.3 (Thermo Fi. Sci.). The datasets were compared by different multivariate statistics approaches to identify chemical markers with may influence the phenological stage development.

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P63

1H NMR-BASED FINGERPRINTING AND CHEMOMETRICS AS A PROMISING NON-DESTRUCTIVE METABOLOMICS APPROACH FOR THYME AUTHENTICATION

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Aromatic herbs and spices have recently received more attention because of the increasing adulteration, deliberate origin mislabeling, and other fraud activities detected in the quite long and complex supply chains of the condiment industry. In this context, proton nuclear magnetic resonance spectroscopy (^{1}H NMR) is currently receiving more attention in food authenticity applications due to its advantages, including its non-destructiveness, simple sample preparation, less-solvent consumption, rapid analysis, high reproducibility and robustness [1]. This study [2] highlights the potential of ^{1}H NMR-based fingerprinting analysis to ensure the authenticity and traceability of thyme based on the geographical region of production (cultivation areas located in Morocco, Spain, and Poland), as well as the processing practices (comparison between sterilized vs. non-sterilized herb), providing statistical models through orthogonal partial least squares discriminant analysis (OPLS-DA) with high-quality parameters (R^2Y = 0.920-0.990 and Q^2 = 0.875-0.978) and high predictability (full correct classification rate of 100%). Moreover, almost 30 predominant ^{1}H NMR signals were associated with 18 thyme metabolites, including amino acids, organic acids, and carbohydrates, among others. As a further step, this approach allowed the putative identification of most discriminant metabolites (markers), noticing 8 key compounds such as α-glucose, acetic acid, chlorogenic acid, thymol, and choline, among other markers. The findings highlighted the effect of the region of cultivation and post-harvest practices of the major composition of thyme, pointing out the possibility of exploiting this data for product traceability. This study provides a metabolomics insight into the origin and processing effects on thyme fingerprinting, opening the path to new metabolomics approaches for quality control in the spice industry. This research was funded by "MCIN/AEI/ 10.13039/501100011033 (Grant FPU18/05133)".

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P64

VOLATILOME OF SHADE-TOLERANT AND SHADE-AVOIDING PLANTS UPON PROXIMITY SHADE

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Light is essential for photosynthesis and the integration of environmental information. In their natural habitats, plants grow in close proximity to each other. The presence of nearby plants can impact both light quantity and quality, causing a response in shaded plants known as Shade Avoidance Syndrome (SAS). Plant species show different responses to proximity shade: either avoidance or tolerance. Responses in shade-avoiding species include hypocotyl elongation, while shade- tolerant species do not show any elongation response. It is also known that plants under different abiotic stresses emit Volatile Organic Compounds (VOCs) as signals for plant-to-plant communication.

To study the role of plant-to-plant communication in response to shade, a GC-MS analysis was performed to identify the VOCs produced by one shade-avoiding species (*Arabidopsis thaliana*) and one shade-tolerant species (*Cardamine hirsuta*) exposed to 24 hours of low ratio of Red to Far Red light (R:FR) to simulate proximity shade. Both species show a change in VOCs produced under shade, with greater changes in A. thaliana. Among the VOCs differentially emitted by A. thaliana under low R:FR conditions,

nonanal, decanal and phenylacetaldehyde were detected. Moreover, levels of apocarotenoids such as β- ionone were higher in *C. hirsuta* in white light when compared to proximity shade, suggesting a role for these compounds in shade adaptation. An RNAseq analysis was performed to understand the relationship between mRNA abundance and VOCs released. Interestingly, an increase in the expression of pipecolic acid biosynthesis was observed. Treatments with this compound induce the emission of nonanal, observed in *A. thaliana* under W+FR.

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P65
ENGINEERING TOMATO FRUIT METABOLISM

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The tomato fruit is an example of how plants adapt their developmental program to specific purposes. The fruit is an organ that has evolved to protect and disperse the seeds and for this the fruit has to be attractive and to provide a reward to dispersers. Small molecules confer the tomato with bright colors (mainly carotenoids) and with a characteristic and appealing flavor (sugars, acids and volatiles) and as additional reward with healthy compounds. For this once the fruit has reached its final size it enters the ripening process where basically nothing else but a dramatic reorganization of metabolism occurs.

In addition to the use of natural diversity and molecular breeding, metabolic engineering allow us not only to expand the range of variation of compounds normally present but to a lesser extent but to enlarge the metabolomics profile t to include new exotic compounds not found normally in the tomato clade. These engineering also reveal novel / unexpected interactions between the metabolic pathways and new opportunities for biotech and breeding.

We will present examples on how specific branches of the endogenous metabolite network of tomato fruit can be activated by targeted intragenic approaches to reach levels of specific metabolites never reached by natural variations and how this also results in unexpected effects in other branches. We have also used transgenic approaches to engineer the tomato fruit metabolisms to produce high levels of exotic apocarotenoids of interest for the industry with also unexpected results in the endogenous carotenoid pathway.

P66
METABOLIC DIVERSITY IN TOMATO FRUITS ACCUMULATING SAFFRON APOCAROTENOIDS

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Tomato ripe fruit is rich in liposoluble antioxidants, mainly lycopene, and low levels of β-carotene. Both carotenoids and vitamin C contribute to tomato antioxidant activity and benefit human health. Nevertheless, the water-soluble fraction of tomato is poor in antioxidant compounds. In our laboratory, we produced Tomaffron (TF), a transgenic tomato line expressing Crocus sativus CCD2 and accumulating high levels of saffron apocarotenoids, including crocins and picrocrocin, which are water-soluble apocarotenoids with high antioxidant activity. TF showed a higher overall antioxidant activity than its wild-type, MoneyMaker (MM). The precursors of CsCCD2 are lutein and zeaxanthin; the second is not detected in MM ripe fruit, and the first is detected at low levels and disappears by the expression of CsCCD2. Saffron apocarotenoids are also produced in Gardenia jasminoides. In addition to zeaxanthin and lutein, GjCCD4a uses β-carotene and lycopene as substrates to produce saffron apocarotenoids.

We aimed to increase saffron apocarotenoid content in TF by crossing it with Xantomato, a quadruple mutant that accumulates high levels of zeaxanthin and β-carotene in the fruit. In addition, we expressed GjCCD4a in MM and hp3/BSh, an intermediate mutant of Xantomato that accumulates high levels of β-carotene, to obtain saffron apocarotenoids from different carotenoid substrates.

From the cross of TF and Xantomato, we obtained different tomato plants accumulating higher levels of saffron apocarotenoids than TF and showing differences in carotenoid accumulation. In the T0 generation of the GjCCD4a transformants, the accumulation of saffron apocarotenoids was 15 times higher in the hp3/BSh than in MM transgenic plants.

This work resulted in a wide diversity of tomato plants accumulating high levels of saffron apocarotenoids with different carotenoid profiles in ripe fruit, in addition to an accumulation of safranal, which is not present in wild-type fruit, and α and β-cyclocitral.

P67
QUALITATIVE AND QUANTITATIVE CHANGES IN THE PHENOLIC COMPOSITION OF VIRGIN OLIVE OIL DURING FRYING

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Extra Virgin Olive Oil (EVOO) is considered an excellent product due to its healthy and organoleptic properties, which are related to its chemical composition, with particular emphasis on phenols. These healthy properties have been amply demonstrated. The European Food Safety Authority highlights that phenols are responsible for the health claim included in the European Regulation EU 432/2012, being exclusive of VOO. This claim associates a specific phenolic content with the protection against lipid oxidation in blood. Furthermore, previous metabolomics research has studied the phenolic behavior when the EVOOs are subjected to high temperature, such as that reached when frying.

In this research, metabolomics was used to monitor qualitative and quantitatively phenolic compounds in three oils with different phenolic profiles subjected to 10 frying cycles. Complementary, we quantified the phenolic transfer from EVOOs to six foods. The oils selected were Picual EVOO, Arbequina EVOO and ordinary olive oil. Moreover, two types of vegetables (potato and eggplant), two fishes (anchovy and fish finger) and two meats (chicken wings and chicken nuggets) were used in this frying study.

After the frying process, phenols were extracted from oils and foods and, the resulting extracts were analyzed by LC-MS/MS using a targeted approach. Phenols enriched in foods were hydroxytyrosol, tyrosol, oleuropein and ligstroside aglycone. In addition to the above list, we quantified oleocanthal and oleacein in EVOOs. We observed that the phenols associated with the phenolic degradation (hydroxytyrosol, tyrosol and oleocanthalic acid) were increased during frying. By contrast, the aglycones, oleocanthal and oleacein were decreased during frying. This study reveals that the phenolic enrichment in each food depended on the type of oil and the food matrix. In the same way, the degradation of phenols in oils was conditioned by the type of food.

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P68
SEARCH OF COMMON METABOLITES FROM OLEA EUROPAEA AND LIPPIA CITRIODORA LEAF EXTRACTS IN HUMAN URINE THROUGH UNTARGETED METABOLOMIC APPROACHES

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Olea europaea and *Lippia citriodora* are two plant sources that share several bioactive properties. Despite sharing some positive health effects and similar families of phenolic compounds, their chemical compositions exhibit differences. Since compounds

in these plants undergo metabolism in the organism, especially through phase I and phase II metabolic processes, we hypothesize that they may produce common derivatives contributing to their shared bioactivities. Therefore, our study aims to identify common metabolites from *O. europaea* and *L. citriodora* leaf extracts utilizing human urine samples. We conducted an acute double-blind dietary intervention involving twenty-five volunteers divided into three groups: 8 received an encapsulated O. europaea leaf extract, 8 received an encapsulated L. citriodora leaf extract, and 9 consumed a placebo capsule. Urine samples were collected pre- and post-supplement intake at various intervals and analyzed using an untargeted metabolomic methodology based on HPLC-ESI-QTOF-MS. Data processing involved MZmine 3.4.27. and R packages, primarily *notame*. Initial analysis revealed 18,823 molecular features in urine samples, after which signals were filtered to identify exogenous metabolites originating from the extracts. Preliminary findings showed 280 and 147 significant signals related to exogenous compounds from *O. europaea* and *L. citriodora* extracts. Significant molecular features identification was conducted by comparing MS and MS/MS data with information available in open-access metabolomics-related databases and previous literature. Successful annotation yielded 138 compounds for the O. europaea extract (e.g., elenolic acid, oleuropein aglycone, 10-hydroxyoleuropein) and 65 for the *L. citriodora* extract (e.g., ethyl vanillate, aromadendrin, hydroxypinoresinol glucoside). Interestingly, 37 signals were common between both extracts, including diferulic acid, gardoside, or homovanillic alcohol glucuronide, among others. Our ongoing work focuses on identifying additional common and unique metabolites derived from the extracts in urine samples, enhancing the understanding of their bioactive mechanisms for future applications in functional foods or pharmaceuticals.

P69

EXPLORING THE IMPACT OF FERMENTATION TIME AND AGROCLIMATIC VARIABILITY ON THE METABOLOMIC FINGERPRINT OF COCOA BEANS

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The market for fine flavor cocoa provides significant economic and non-economic benefits to farmers. However, identifying flavor precursor metabolites and measuring how its biochemical compounds may be affected by climate differences and post-harvesting practices remains a challenge. This study investigates how fermentation duration and agroclimatic conditions in Colombia´s fine cocoa producing region of Arauca, influence the untargeted metabolomic profile of cocoa beans.

Untargeted metabolomic analysis was performed by UHPLC-ESI-Orbitrap-MS to obtain the metabolomic fingerprint of cocoa beans fermented for 0, 24, 48, 72, 96, and 120 hours in 9 cocoa production zones. Analysis of data, applying a PLS-DA model, highlighted separation by fermentation time and we observed that metabolomics fingerprint change through fermentation time. Among the most discriminant metabolites, 18 oligopeptides, sucrose, glucose, fructose, flavanols, and acids were tentatively identified. The identification and analysis of these 18 oligopeptides during the fermentation process is highly interesting because they are the most important precursors for the development of cocoa aroma. The results showed that fermented cocoas can be differentiated from unfermented cocoas due to differences in peptide concentration. These results provide new insights into cocoa fermentation and may also contribute to the development of new alternatives for cocoa processing based on the tracking of biochemical and functional compounds (quality biomarkers).

P70

ASSESSING COCOA METABOLOMIC FINGERPRINT UNDER CONTROLLED PROCESSING CONDITIONS THROUGH INNOVATIVE STAINLESS-STEEL TANK TECHNOLOGY

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The cocoa flavor quality depends on the precursor's metabolites, which are conditioned by the processing variables that cannot be easily controlled during fermentation. This issue has inspired research aimed at influencing the cocoa biochemistry by transforming it under controlled conditions. The objective was to evaluate the effects of cocoa processing under controlled temperature, mechanical mixing and pH on flavor precursors metabolites linked to flavor formation in stirred tank bioreactors.

Untargeted metabolomic analysis through UHPLC-ESI-Orbitrap-MS was performed to obtain the metabolomic fingerprint of cocoa during the transformation time. Eight treatments in bioreactors and one control treatment in a wooden fermenter box were carried out. The process variables evaluated were the temperature using constant temperature at 45°C throughout the process and a temperature gradient like observed in spontaneous fermentation. The frequency of mechanical agitation was carried out applying 2 and 24 cycles/day. Finally, to modulate the initial pH of the medium, an acidic solution at 9 g/l was used. When we assess the effect of the process variables using multivariate statistics, through non supervised and supervised analysis, PCA and PLS-DA, respectively, temperature and initial pH control at the beginning of cocoa transformation are the variables that have the highest influence on the formation of the cacao metabolome clusters. Metabolomes were clearly grouped in function of initial pH control and temperature treatments, but process time also has a strong influence. In general, there is a higher dispersion of metabolomes between 24 and 72 h of processing in treatments with gradient temperature and no pH control. In contrast, the mechanical mixing frequency did not have a strong influence on cocoa metabolome formation. Consequently, optimal temperature (45°C) and acidic conditions at the beginning of the process, are required for the enzymatic biochemical transformation inside seed develops quickly.

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P71
EXPLORING THE IMPACT OF POSTHARVEST TREATMENTS ON BLUEBERRIES THROUGH UNTARGETED METABOLOMIC ANALYSIS

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Berry fruits, such as blueberries, are rich sources of bioactive compounds, mainly phenolic acids, flavonoids, and anthocyanins, among others, that have been demonstrated to have beneficial effects on human health. Blueberry anthocyanins have been used as a nutritional supplemental product for improving visual acuity and treating circulatory disorders. However, the short shelf life of fresh blueberries limits their availability and consumption for all seasons [1]. Post- harvest studies of fruits are of great importance to fully understand the changes in their biochemical components during fruit maturation in the plant, using physical, chemical, and physiological analysis.

A metabolomic platform combining GC-MS and UHPLC-QTOF was used to delve into the impact of four postharvest treatments on the metabolic profile of blueberries. Herein, we compared the results obtained between liquid-liquid extraction with a new analytical extraction method for untargeted metabolomics using a non-selective procedure based on three-phase hollow fiber liquid phase microextraction aided by ultrasound (three-phase-UA-HF-LPME), followed by gas chromatography-mass spectrometry [3]. The study led to the annotation of 116 metabolites that belong to aminoacyl-tRNA biosynthesis, especially in the ethylene absorption sheet (EAS), and alanine, aspartate, and glutamate metabolism in all the treatments, except for the modified atmosphere packaging (MAP) that mainly alters galactose metabolism. Pulsed electric field (PEF) significantly increased a wide number of bioactive compounds (e.g., terpene glycosides, isoflavonoids, phenols) compared to the other treatments. The metabolomic profiles showed a differential impact of the assayed postharvest treatments related to bioactive compounds with antioxidant activity or influencing fruit metabolism, flavor, timing, and the process of fruit maturation, among others. The study reveals novel bioactive compounds of blueberries after postharvest treatments that should be further investigated due to their potential influence on fruit quality and health-promoting effects.

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P73
EVALUATION OF THE EFFECT OF FUNGICIDES ON THE VOLATILOME OF SACCHAROMYCES CEREVISIAE EC1118™ DURING THE WINEMAKING OF AIREN GRAPES

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Evidence suggests that fungicides may influence the secondary metabolism of yeast, which may result in changes in the final quality of wine [1, 2]. In this study, the effect of fungicide residues on the production of aromatic secondary metabolites by S. cerevisiae EC1118™ was investigated using gas chromatography coupled to flame ionisation detector (GC-FID) and GC coupled to mass spectrometry (MS). The volatile profile of those wines obtained in the presence of fungicides was compared with that obtained for the control wine produced in the absence of fungicides.

For this, different vinification assays (n=3) were performed in the experimental cellar using Airen grape must inoculated with the selected yeast. The commercial formulations Frupica® (mepanipyrim 50 % w/w) and Domark® EVO (tetraconazole 12.5% w/v) were separately added to the grape must at concentrations corresponding to 2 mg/kg of mepanipyrim and 0.5 mg/kg of tetraconazole.

In the presence of the fungicide Frupica®, the content of acetate esters increased by 20%. This increase was mainly due to the higher content of isoamyl acetate and 2-phenylethyl acetate. However, this increase did not affect the odour intensity of the final wines, which were dominated by notes of fresh and ripe fruit. However, when Domark® EVO was added to the grape must, the content of higher alcohols decreased by 22 %.

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MODULATION OF THE YEAST EXO-METABOLOME BY FUNGICIDES

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Residues of phytosanitary products in grape must, even at trace levels, may interfere with yeast fermentative activity and alter the fermentation process [1]. Additionally, the biosynthetic pathways of aromatic compounds may also be affected [2]. These changes may be attributed to the antifungal substances and adjuvants present in commercial formulations.

Synthetic grape must was spiked with the target fungicides (tetraconazole and mepanipyrim) and their adjuvants (sodium docusate and sodium dodecyl sulphate-SDS) separately and jointly. Microfermentations were carried out in triplicate after inoculation with the yeast S. cerevisiae EC1118™. Control microfermentations (without fungicides) were also conducted for comparative purposes. Liquid samples were taken throughout the fermentation process to monitor its progress and the biosynthesis of aromatic compounds.

The rate of sugar consumption increased when sodium docusate was present, as well as when a mixture of tetraconazole and sodium docusate was used. However, this rate decreased when must was spiked separately with mepanipyrim or SDS. SDS increased

the rate of ethanol production, while tetraconazole increased the biomass to ethanol yield. As for the aromatic profile, tetraconazole caused significant changes, increasing the floral and fruit nuances of the fermented must.

The effects observed were dependent on the fungicide used. Generally, the addition of sodium docusate has an antagonistic effect on tetraconazole, while SDS has a synergistic effect with mepanipyrim.

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P75

MODULATION OF THE METABOLOME OF SACCHAROMYCES CEREVISIAE EC1118 IN RESPONSE TO THE PRESENCE OF TETRACONAZOLE AND DOCUSATE SODIUM

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Yeasts can modulate their genome by overexpressing or repressing several genes as an adaptive response to the presence of fungicides in the fermentation medium [1]. It is very important to study the effect that these substances have on the secondary metabolism of yeasts, as they have an impact on the final wine aroma profile. Due to the lack of studies on this subject, it is necessary to pay attention to the processes of extraction of the yeast endometabolome to elucidate the metabolic pathways affected by the presence of fungicides. The adaptability of many micro-organisms and the complexity of their metabolic pathways provide many opportunities for interaction with fungicides.

The main objectives of this study were twofold. First, to optimise an extraction protocol of intracellular metabolites for untargeted metabolomic analysis of the yeast Saccharomyces cerevisiae EC1118TM. Several parameters such as disruption conditions and extraction solvents were tested. Secondly, the effect of tetraconazole (0.5 mg/L) and its adjuvant sodium docusate (0.4 mg/L) on the metabolome of S. cerevisiae Lalvin EC1118™ was evaluated. The individual and combined effect of both substances was evaluated at laboratory scale by microvinification tests on synthetic must (n=3) [2]. Control fermentations (without fungicides) were also carried out for comparative purposes. Prior to yeast inoculation (106 cells/ml), the ethanol concentration was standardised in all fermentations. Yeast cells were harvested at different fermentation times, centrifuged and snap frozen in liquid nitrogen to avoid degradation of labile metabolites.

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P76

CHARACTERIZATION OF GENOME REGIONS INVOLVED IN THE PRODUCTION OF VOCS IN INTROGRESSION LINES OF SOLANUM PIMPINELLIFOLIUM

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Tomato is a popular vegetable consumed worldwide, and the improvement of its organoleptic quality is an important objective in biotechnological research. The production of volatile organic compounds (VOCs) in tomato (Solanum lycopersicum) is a complex process that significantly influences its flavor. Specific genome regions involved in VOC production are investigated in introgression lines of Solanum pimpinellifolium, a wild species related to cultivated tomato. In a previous study, introgression lines (IL) were generated through interspecific crosses between Solanum lycopersicum cv. Moneymaker as the recurrent parent and Solanum pimpinellifolium accession TO-937 as the donor parent of wild genes. In this study, introgression lines carrying a single TO-937 introgression for chromosome 9 (IL SP9-2) were characterized. The characterization of these ILs was performed using high-resolution genotyping techniques, and their metabolomic profiles were evaluated using refractometry, solid-phase microextraction (HS-SPME), and gas chromatography/mass spectrometry (GC/MS). Using metabolomic analysis, genetic mapping methods and whole-genome association analysis two Quantitative trait locus (QTL) was reduced, the M18-QTL1 was reduced by 2.4 Mb, and Brix-QTL was reduced by 430 Kb. The metabolic fingerprints demonstrate a clear distinction between tomatoes carrying the P allele and those carrying the L allele. Principal component analysis (PCA) of metabolic characteristics showed clear discrimination between samples. A heatmap was generated displaying the effects of the L and P alleles for each volatile compound in the IL population. Our results revealed several genome regions of Solanum pimpinellifolium that are positively implicated in VOC production in tomato. These findings provide valuable information for identifying candidate genes within these regions that may be directly involved in VOC production. Additionally, they enhance our understanding of the genetic mechanisms underlying variability in VOC profiles in tomato and have significant implications for improving its sensory qualities.

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P77
METABOLIC PROFILING OF MEDITERRANEAN FISH SPECIES: A 1H NMR-BASED STUDY

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Fish constitutes a significant component of the Mediterranean diet, renowned for its high nutritional value. Greece stands as a prominent contributor to Mediterranean aquaculture production, accounting for approximately 20% of the total production. The present study highlights the potential of NMR-based metabolomics in enhancing our understanding on the nutritional characteristics and differences among Mediterranean fish species. Metabolic profiling using ^{1}H NMR of a total of 118 fish samples comprising eleven Mediterranean fish species was performed. Multivariate statistical analysis was employed to explore the information acquired by ^{1}H NMR spectral data on metabolite content with biological relevance to the species variations. The results revealed significant differentiation of metabolic profiles in relation to taxonomic classifications, including family and genus, as well as geographical origin and sampling date. The major groups of metabolites responsible for differentiation in PLS-DA score plots were organic acids, free amino acids, and amines These results suggest that metabolic content analysis can provide significant information on the fishes characterisation which could have implications for areas such as aquaculture management, food quality control, and dietary recommendations.

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P78
METABOTYPING CHILDREN WITH OBESITY: FACTOR ANALYSIS- BASED MULTIPLATFORM DATA FUSION AND GENOME-SCALE METABOLIC NETWORKS

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The increase in childhood obesity prevalence has resulted in specific needs in defining obesity-associated entities. Comprehensive metabolic phenotyping (“metabotyping”) of children with obesity will also increase the understanding of the development of the disease. Integrating data from different analytical platforms enhances the coverage of complex sample metabolomes, allowing better understanding of the underlying biological mechanisms associated with a given phenotypic pattern. Factor Analysis makes it possible to integrate data, reduce dimensionality, and identify underlying factors that explain variance in metabolomic data.

The combination of Factor Analysis and hierarchical clustering algorithms allows metabotypying of the individuals according to the information from a multi-platform analysis.

Children with obesity (110) were analyzed by multiplatform untargeted metabolomics and 5 data sets (matrices) were obtained: RP-U(H)PLC-QTOF-MS/MS (positive and negative modes), GC-q-MS, and CE-TOF-MS (positive and negative modes). Data were integrated based on dimensionality reduction to combine all metabolomic information. Six independent factors were identified, permitting the classification (hierarchical clustering) of patients into three distinct metabotypes, which could not have been previously unveiled on the basis of clinical parameters, or genetic variants in the leptin-melanocortin pathway.

The use of genome-scale metabolic networks built with MetExplore and Met4J Galaxy workflow revealed significant alterations in lipid and BCAA metabolism in one specific metabotype. These alterations could lead to an increase in oxidative stress, insulin resistance, and proinflammatory markers, indicating a more disrupted metabolic phenotype in these patients. This information is highly relevant in the clinical management of patients, allowing establishment of individualized treatment choices. The integration of data from different analytical platforms provides the opportunity to obtain a more complete understanding of biological systems and to identify different metabolic phenotypes for personalized therapy.

P79
DATOMA: A CLOUD COMPUTING PLATFORM FOR HIGH- PERFORMANCE METABOLOMICS DATA ANALYSIS

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Cloud computing responds to the ever-expanding need for fast, scalable and reproducible data analysis workflows. Using cloud computing, research groups can process large data throughputs without maintaining their own in-house clusters. In the field of metabolomics, several web-based and cloud-based data analysis platforms have arisen in the past years: Worfkflow4Metabolomics (Giacomoni et al., 2014), Metaboanalyst (Pang et al., 2022), Phenomenal (Peters et al., 2018), XCMSonline (Tautenhahn et al., 2012), METLIN and GNPS (Aron et al., 2020). However, to this date, all such platforms have been designed with a rather narrow use scope, offering a limited set of data analysis tools.

We present Datoma, a cloud-native computing platform that allows users to execute >20 curated metabolomics bioinformatics tools using an intuitive web-based interface or programmatically via a dedicated Python package and API. Datoma has been designed with modularity and interoperability in mind: any bioinformatics tools (R-based or Python-based) can be easily migrated to Datoma by creating a reproducible Docker image and a parametric task script. Complex workflows can then be easily defined by mapping tool output files to the inputs of other tools using regular expressions. Cost-efficiency has also been considered in Datoma: tasks dynamically scale the required compute resources needed based on the inputs.

We processed a large-scale (>100GB) imaging-MS dataset with the tool rMSI (Ràfols et al., 2017) and achieved a >10x speed-up compared to a regular workstation (from 8-10 hours, to <30 minutes). Datoma thus allows reproducible, scalable and faster data analysis while avoiding dependency management and using tool-specific APIs.

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P80
IMPROVING INSIGHTS FROM METABOLOMIC FUNCTIONAL ANALYSIS COMBINING UNIVARIATE AND MULTIVARIATE ANALYSIS

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Metabolomic data provides valuable insights into the functional processes of biological systems, often analyzed through univariate and multivariate approaches, and well as with functional or pathway analysis. Yet, the integration of results from these sources to aid the interpretation of their biological significance remains challenging. Pathway and functional analysis typically involve the use of univariate statistical tests, such as t-tests or ANOVA, to identify significant features or metabolites using differences in the distribution of concentrations or intensities of features between different experimental groups. However, although univariate tests are useful for identifying features with significantly different concentration distributions between groups, they do not describe the associations that may exist between multiple variables. This represents a significant bottleneck limiting the applicability and interpretability of multivariate models of metabolomic data, despite its potential for providing deep biological insights.

Here we describe two straightforward methods to facilitate integrating results from univariate and multivariate analysis with functional metabolic analysis: i) using combined p-values from univariate and multivariate tests as input in functional analysis to help in generalizing conclusions beyond the scope of a single test, and ii) using cluster-CV to assess the impact on the predictive performance of a multivariate model at the pathway level.

Through simulated data, we show how these approaches, alone or in combination, enhance the interpretation of biological effects driving multivariate models and support the identification of altered pathways not detected by univariate analysis. By providing a deeper understanding of metabolic phenotypes, these methods might improve the biological insights derived from statistical and functional analysis from future or previous studies.

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TRIALS AND TRIBULATIONS OF STATISTICAL SIGNIFICANCE IN OMICS

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Introduction and Aim

Statisticians and researchers have recently raised concerns about the misuse of hypothesis testing, p-values, and statistical significance in biochemical sciences. Misapplication of these statistical tools can lead to irreproducible conclusions and impede scientific progress. This document presents three key recommendations to enhance statistical methods in these fields, improving research reproducibility.v

Methodology

We conducted a comprehensive review of the literature and current practices in biochemical and omic studies. This involved:

1. Analyzing common statistical practices and their shortcomings.
2. Reviewing guidelines from statistical and scientific organizations.
3. Synthesizing insights from recent research articles and expert opinions.
4. Formulating practical recommendations based on this analysis.

Findings and Significance

Our review identified three critical areas for improvement in statistical practices:

1. **Avoiding thresholding with p-values:** Relying on p-values as thresholded values for selecting biomolecules can lead to biased and irreproducible biological interpreations of data. We recommend avoiding this practice to enhance biological interpretation.
2. **Complete data reporting**: Incomplete data reporting hinders study comparisons and robust knowledge building. Full disclosure of data is essential for accurate comparisons and the comparison of studies.
3. **Detailed statistical reporting**: Reporting exact numbers instead of asterisks or inequalities provides clearer and more precise results.

These findings can significantly improve the reproducibility of research in biochemical sciences and omics.

Contribution to Discipline

Implementing these recommendations will enhance the reproducibility of future research findings. This contributes to the discipline by:

1. Promoting reproducible biological interpretations.
2. Facilitating better comparisons across studies.
3. Ensuring clearer communication of statistical results, aiding in replication and validation.

These improvements will lead to more robust biochemical sciences. Because of the high number of variables, these practices are of special importance in omics studies.

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P82

METABOLOMICS AS A HYPOTHESIS-GENERATING TOOL TO UNDERSTAND THE MECHANISM OF ACTION OF PROBIOTIC LACTOBACILLUS PLANTARUM 299V IN IMPROVING COGNITIVE FUNCTIONS IN PATIENTS WITH MAJOR DEPRESSION

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Major Depressive Disorder (MDD) presents a significant global health challenge due to its widespread prevalence, substantial disability impact, and link to suicide. Despite current first-line treatments such as antidepressants and psychotherapy, the heterogeneity of MDD symptoms complicates accurate diagnosis and treatment efficacy. Understanding the multifactorial nature of MDD is crucial in tailoring treatments; however, a complex interplay of various factors underlying the development and progression of MDD makes their exploration challenging. Among available approaches, metabolomics offers a promising avenue for understanding MDD's metabolic alterations and improving diagnostic precision. Our prior research indicated that patients with MDD showed cognitive benefits from treatments that combine SSRIs with Lactobacillus Plantarum 299v (LP299v). This study delved into the metabolic alterations associated with these cognitive improvements to uncover the biochemical processes at play. To achieve this, we employed multi-platform metabolomics, including plasma LC-QTOF and CE-TOF profiling, alongside chiral LC-QqQ analysis for amino acids. This approach reveals subtle but significant metabolic differences associated with MDD and LP299v supplementation. LP299v

supplementation increased the reduction of long-chain acylcarnitines, potentially demonstrating improved mitochondrial function with SSRI treatment. LP299v supplementation reduced N-acyl taurines more than four times compared to the placebo, suggesting a substantial impact on restoring biochemical balance. The supplemented group showed increased oxidized glycerophosphocholine (oxPC) levels. Furthermore, LP299v elevated sphingomyelins, L-histidine, D-valine, and p-cresol levels. Although the changes observed were subtle, our study effectively linked these metabolic variations to the disruptions in MDD, underlining the complex interaction between metabolism, gut microbiota, and mental health. These insights advocate further research into how probiotic treatments could influence MDD management. Our findings contribute to understanding the microbiome-gut-brain axis, suggesting new avenues and offering potential implications for complementary depressive disorder therapeutics.

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