24—28 February 2025

Lipidomic technologies and applications

Clinical applications
Data • Exposome
Technologies
Nutrition • Lipid Biology

iSLS13

The Ngee Ann Kongsi Auditorium
National University of Singapore

SLING
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Lipidomics
Incubator

MS ACL

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13th International Singapore Lipid Symposium 24th February – 28th February 2025

Organising Committee: Hyung Won CHOI, Amaury CAZENAVE GASSIOT, Anne BENDT, Bo BURLA, Federico TORTA, Huimin HONG.

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The Association for Mass Spectrometry & Advances in the Clinical Lab @ iSLS 13

Programme (click to download)

Time	MONDAY FEBRUARY 24		TUESDAY FEBRUARY 25	WEDNESDAY FEBRUARY 26	THURSDAY FEBRUARY 27	FRIDAY FEBRUARY 28
Topic	MSACL Asia Workshop			Exposome in health (chaired by Sartaj Mir)	Clinical Lipidomics/Metabolomics I (chaired by Xue Li Guan)	Metabolomics (chaired by Yi Yun Pang)
9:00 - 9:30	**Dramatic Change Offers Many Opportunities** **David Herold**		**Daryl Hee** Challenges & Opportunities in Clinical Mass Spec Labs	**Opening ceremony**	**Peter Meikle** - Defining the role of lipid metabolism in bridging diet and metabolic health	**Zheng-Jiang Zhu** - Mass spectrometry-based untargeted metabolomics and single-cell metabolomics
9:30-10:00				**Xu Guowang** - Study on the effect of exogenous chemical residues in serum on chronic diseases at the metabolic level	**Li Chen** - Longitudinal Landscape of Maternal Plasma Apolipoproteins from Preconception to Postpartum and Their Associations with Maternal Metabolic Risk Factors and Offspring Birth Outcomes	**Matej Oresic** - Chemical exposures and microbiome derived bile acids during early life: insights into the progression to islet autoimmunity
10:00-10:30			**Michael Vogeser** Basics of Quality in Routine Mass Spec Labs	**Brad Clarke** - PFAS Analytical Challenges: Navigating Complexities in Biological and Environmental Samples	**Nicola Gray** - Accelerating the Development of Targeted Metabolite Phenotyping Assays for Precision Population Health	**Sastia Prama Putri** - Metabolomics application for quality improvement of important agricultural and food products
Topic	Track 1 **LCMS 101** **Isabel Tsai and Chia-Ni Lin**	Track 2 **Metabolomics 101** **Timothy J. Garrett**		Exposome analysis (chaired by Brad Clarke)	Clinical Lipidomics/Metabolomics II (chaired by Jianhong Ching)	Standardization (chaired by Michaela Chocholouskova)
10:30-11:00				**Eric Chan** - Is it time to extend physiologically-based pharmacokinetic (PBPK) modeling and simulation to the investigation of drug–exposome interactions?	**Robert Gurke** - Clinical Lipidomics in Immune-Mediated Diseases: Challenges and Perspectives	**Elie Fux** - Bridging the Gap: Standardizing Metabolomics for Routine Clinical Use
11:00-11:30			**Katharina Habler** Practical example of validation and verification of LCMS methods: the TDM showcase	**Sartaj Mir** - Quantification of PFAS in human samples for population-scale studies	**Yu Guoqi** - Longitudinal changes of plasma lipidomics profile and pregnancy health: findings from a multiracial cohort	**Ondřej Peterka** - CLIG Interlaboratory Study on the Harmonization of Lipid Concentrations in Human Plasma
11:30-12:00				**Jeremy P. Koelmel** - FluoroMatch: A Comprehensive Solution for Non-Targeted PFAS	**Charna Dibner** - Longitudinal multi-omics in postpartum women identify a persistent signature of gestational diabetes	**Michael Vogeser** - Standardized description of LC-MS/MS measurement procedures
12:00-1:00	**Lunch**		**Lunch**	**Lunch** (12pm-1pm)	**Lunch** (12pm-1pm)	**Lunch** (12pm-1.30pm)
1:00-1:30			**Translational research & biomarker discovery using metabolomics: approaches & pitfalls** **Christiane Auray-Blais**	**Platinum Sponsor talk** **Agilent Technologies; David Beale** - Forever chemicals don't make hero mutant ninja turtles: Elevated PFAS levels linked to unusual scute development in newly emerged freshwater turtle hatchlings (Emydura macquarii macquarii) and a reduction in turtle populations	**Platinum Sponsor talk** **Waters; Lim Yanting** - Discovery lipidomics with the Xevo MRT and integrated informatics for confident lipid characterization and quantification	
1:30 - 1:45	Track 1 **LCMS 101** **Isabel Tsai and Chia-Ni Lin**	Track 2 **Metabolomics 101** **Timothy J. Garrett**		**Short talk award: Sarah Hancock** (Targeting desaturases to improve gemcitabine-based chemotherapy in pancreatic ductal adenocarcinoma)	**Travel award: Dana Hicks** (Organ-Specific Lipid and Lipoprotein Metabolism in Cardiovascular-Kidney-Metabolic Syndrome)	**Travel Award: Minhui Zhu** (Development of a Quantitative MRM assay from Skin Swabs Rich in Sebum to Diagnose Parkinson's Disease)
Topic				Nutrition science (chaired by Sarah Hancock)	Lipidomics and Biology II (chaired by Federico Torta)	MS technologies (chaired by Amaury Cazenave Gassiot)
1:45-2:15				**Lightning talk session for posters** **(selected presenters will be notified)**	**Ondřej Kuda** - Deciphering Diverse Obesogenic Strategies of TZDs through Complex Lipid Metabolic Flux Analysis	**Alaa Othman** - High throughput RPLC-MS/MS lipidomics, challenges and opportunities
2:15-2:45			**Katharina Habler** Keep it running! Lab organization, data quality for analytical series	**Mei Hui Liu** - Lifestyle factors and blood lipids: Results from dietary intervention studies on Singaporean men	**Christian Metallo** - Tracing and modeling compound lipid homeostasis to understand disease	**Takeshi Bamba** - Advancements in Lipidomics Platform Based on Supercritical Fluid Chromatography/Mass Spectrometry
2:45-3:15				**Jun Kunisawa** - Immunoregulatory Functions of Dietary and Microbial Lipid Metabolites and Their Application to Precision Nutrition	**Stephen Blanksby** - Isomeric discrimination by collision-/ozone-induced dissociation reveals the lipid complexity sn-onymous with triacylglycerols	**Sven Heiles** - Visualizing cellular lipid distributions by AP-MALDI mass spectrometry imaging
3:15 - 3:45	**Wei Guo** - The clinical use of LC-MS in China Moderated by Michael Chen		**Platinum Sponsor talk** **Roche; Elie Fux** Improving Accuracy in Clinical MS Laboratories: The Role of Standardization and Traceability in Ionify Assays for the Cobas Mass Spec solution	**Tea break (30min)**	**Tea break (30min)**	**Tea break (30min)**
Topic				Lipidomics and Biology I (chaired by Bo Burla)	Precision medicine / CVMD	Data processing (chaired by Hyung Won Choi)
3:45-4:15	**Huafen Liu** - The Challenges and Opportunities of Transitioning LC-MSMS from Research Scientific Application to In Vitro Diagnostics		**Michael Vogeser** - The Roche MS solution - key technological innovations and results of a pilot study	**Albina Nowak** - Fabry disease – the most frequent monogenetic sphyngolipidosis	**SysMeC launch & Cardiometabolic Research Landscape in Singapore** **Patrick Tan** (Senior Vice Dean of Research, Duke-NUS), **Roger Foo** (Vice Dean of Research, NUSmed), **Yibin Wang** (CVMD, Duke-NUS), **Derek Hausenloy & Mark Chan** (CADENCE)	**Laura Goracci** - Tracing lipid metabolism in Lipostar 2: from stable isotopes to ω-alkyne-containing species
4:15-4:35	**Zhouyang Kang** - CalibradX: The Evolution of IVD Kits for Mass Spec - from Development to Routine Use		**Meet the Experts:** 'Ask us Anything'	**Maria Fuller** - Complex glycosphingolipids in inherited neurodegenerative disease		**Nils Hoffmann** - Enabling FAIR Computational Comparative Lipidomics with LipidCompass
4:35-4:45						
4:45-4:55	**Ziqing Kong** - CalibradX: From Metabolomics to Clinical Applications - Insights and Practices from an Industry Perspective			**Christiane Auray-Blais** - Glycosphingolipids and rare diseases		**Dominik Kopczynski** - Status and Perspectives of the Lipidomics Reporting Checklist
4:55-5:15						
5:15-5:45	**Networking**	**Networking**	**Networking**	**Networking & Lab Tour of NUS-Agilent Hub at Cels** (5:15-6:30pm)	**Poster session & Networking** (4:45-6:00pm)	**Closing ceremony and awards** (Darlene Solomon FeMS and poster award)
5:45-6:00						
6:00-6:30						Note: This programme is susceptible to changed without prior notice
6:30-9:00			**Speakers Reception** **(by invitation only)**		**Conference Dinner (6:30 - 9:00pm)** **(by registration)**	

MSACL Asia Workshops

Workshop – **Dramatic Change Offers Many Opportunities**

Monday 24th February – 9h00-9h30

David HEROLD
Professor of Pathology, University of California, San Diego
Chair, Mass Spectrometry & Advances in the Clinical Lab

In the next few years, dramatic changes are expected to occur in the mass spectrometry clinical laboratory space. The most immediate change will likely be the introduction of the Roche i601 Cobas modular LC-MS/MS system. The release of this system has the potential to transform several important aspects of future clinical labs.

First, it will influence the selection of replacement automated lines in labs where competitors already have instruments installed. As the Roche system begins to dominate the market, it may prompt clinical laboratories to reassess their current automation and consider integrating Roche's technology to ensure they remain competitive.

Second, the workload and analyte composition necessary for labs with stand-alone mass spectrometry sections will need to be evaluated to maintain their operations. Labs may need to consider whether to replace their existing menu with the Roche instrument, especially since it will likely offer a more extensive testing menu, operate 24/7/365, and become linked to Roche's automation lines within 3 to 5 years.

Third, if labs decide to transition to the Roche system, this could free up existing instruments and staff, allowing them to focus on developing more advanced testing options. Specifically, while Roche markets, sells, installs, and validates their instrument, laboratories could pivot towards developing new clinical tests to ensure they continue to contribute to the quality of care provided to patients at their medical centers.

As you are attending the SLING conference, I believe you are consciously or subliminally anticipating this challenge. Therefore, we need to discuss the timeline for this transition, the development of a more complex and high-value menu, and the technologies that could facilitate this change.

Workshop – LCMS 101

Monday 24th February – 9h30-15h15

Isabel TSAI

Associate Professor, Department of Biochemistry and Molecular Cell Biology, School of Medicine, College of Medicine, Taipei Medical University

Chia-Ni LIN

Technical Director, Department of Laboratory Medicine, Chang Gung Memorial Hospital

Dive into the dynamic world of clinical mass spectrometry with a focus on liquid chromatography-mass spectrometry (LC-MS) for targeted analysis! This exciting course delves into four essential topics:

1) mass spectrometry principles
2) liquid chromatography techniques
3) ample preparation
4) data analysis with validation concepts.

Across 4 immersive sessions, you'll gain practical insights and expert knowledge to master complex analytical challenges in clinical and research applications. Elevate your skills and harness the power of cutting-edge LC-MS technology!
Each session will be 45-50 minutes talk, followed by 10-15 minutes for discussion.

Workshop – **Metabolomics 101**

Monday 24th February – 9h30-17h15

Timothy J. GARRETT
University of Florida College

Metabolomics refers to the comprehensive measurement of small molecules in biofluids by either mass spectrometry (MS) or nuclear magnetic resonance (NMR) with the aim of covering multiple KEGG pathways, exposome products, and chemical reactions to provide new insights into disease etiologies. MS based metabolomics generally requires the use of liquid chromatography to separate metabolites based on polarity and high-resolution MS to accurately measure the mass-to-charge (m/z). The combination of retention time and m/z accuracy provides a reliable method to identify metabolites, which is critical for making disease marker discoveries. Understanding how data is generated is key to understand how to process data.

This short course will cover:

- Introduction to Metabolomics
- Instrument platforms
- Chromatographic separation
- Sample Extraction methods
- Metabolite Identification
- Experimental Design and Quality Control
- Data processing with hands on instruction using an open-source software package

Workshop – The clinical use of LC-MS in China moderated by Michael Chen

Monday 24th February – 15h15-15h45

Wei GUO

Director of Department of Laboratory Medicine, Zhongshan Hospital, Fudan University, Professor and doctoral advisor

Mass spectrometry (MS) has a history spanning over a century. However, it was not until the 21st century that liquid chromatography-tandem mass spectrometry (LC-MS) made its foray into clinical applications, thereby instigating a methodological revolution in the field of medical laboratory. Endowed with the merits of high sensitivity, specificity, and the ability to conduct multi-component analysis, LC-MS has rapidly emerged as the gold standard method for clinical quantification. It holds extensive and irreplaceable value in the realm of precise clinical diagnosis and treatment. Specifically, it plays a pivotal role in unearthing the etiology of patients, formulating therapeutic regimens, and proffering medication guidance. Although clinical MS in China is still in the developmental phase, more than 166 clinical testing projects predicated on LC-MS, encompassing hormones, therapeutic drug monitoring (TDM), neurotransmitters, vitamins, and other clinically essential tests, have been instituted to underpin clinical diagnosis and treatment in the medical laboratory department of Zhongshan Hospital in Shanghai, China. Nevertheless, the clinical application of MS technology proffers both opportunities and challenges and mandates standardized management under the aegis of guidelines to ultimately confer benefits upon a greater number of patients. In this presentation, practical cases from clinical practice will be amalgamated with the experience of LC-MS clinical application, with the aim of disseminating our insights and experiences in leveraging LC-MS technology to buttress precision medicine.

Workshop – The Challenges and Opportunities of Transitioning LC-MSMS from Research Scientific Application to In Vitro Diagnostics

Monday 24th February – 15h45-16h15

Huafen LIU
Calibra Diagnostics, Hangzhou, China

This presentation will outline the critical factors involved in adapting liquid chromatography-mass spectrometry (LC-MS) systems from research-grade scientific instruments to in vitro diagnostic (IVD) devices in clinical testing labs. This transition involves navigating complex differences across different regions, regulatory frameworks, IVD reagents, quality systems, standardization, medical reimbursement, physicians' medical interpretation and operator level technical needs.

Workshop – **Industry slot: CalibradX**

Monday 24th February – 16h15-17h15

The Evolution of IVD Kits for Mass Spec: from Development to Routine Use

Zhouyang KANG

From Metabolomics to Clinical Applications: Insights and Practices from an Industry Perspective

Ziqing KONG

Workshop – Challenges & Opportunities in Clinical Mass Spec Labs

Tuesday 25th February – 9h00-10h00

Daryl HEE
Changi General Hospital, Singapore

Round table discussion among experts in clinical mass spec. Moderated by Dr Daryl Hee, Changi General Hospital, Singapore).

This round table discussion will highlight the multiple challenges faced by scientists and lab operators when setting up a clinical laboratory leveraging on mass spec technology, including launching of new mass-spec routine clinical tests, in different continents. Following topics will be discussed during the round table:

1. Front load investment and cost recovery
2. Reagent rental
3. LDT and IVD test kits
4. MS and immuno-analyser
5. EQA and standardization
6. Regulation and accreditation
7. Automation and LIS
8. Technology acceptance, opportunities and advancement.

Workshop – **Basics of Quality in Routine Mass Spec Labs**

Tuesday 25th February – 10h00-11h00

Michael VOGESER
Institute of Laboratory Medicine, LMU University Hospital, LMU Munich, Germany

Consistently reliable results from LC-MS/MS analytical platforms are crucial for both patient care and clinical research.

This session will address the key pillars of high-quality testing in the MS laboratory:

1. method validation prior to implementation
2. specified processes for the release of analytical series and individual results
3. long-term internal and external analytical quality assurance
4. process-oriented general laboratory quality management (including e.g. training, authorization, self-inspection and CAPA processes).

Workshop – **Practical example of validation and verification of LC-MS/MS methods: the TDM showcase**

Tuesday 25th February – 11h00-12h00

Katharina HABLER
Institute of Laboratory Medicine, LMU University Hospital, LMU Munich, Germany

In this interactive session participants can gain practical insights into LC-MS/MS workflows, discuss typical issues encountered during method validation and verification.

1. Introduction to LC-MS/MS Method Validation and Verification:

Key principles of validation and verification of LC-MS/MS methods and LC-MS/MS systems, with a focus on therapeutic drug monitoring (TDM).

2. Step-by-Step Practical Application:

Detailed walkthrough of validation and verification processes using real-world TDM examples.

Workshop – Translational research and biomarker discovery using metabolomics: approaches & pitfalls

Tuesday 25th February – 13h00-14h15

Christiane AURAY-BLAIS

Full Professor, Researcher, Department of Pediatrics, Division of Medical Genetics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Clinical Research Centre-CHUS, Sherbrooke, Quebec, Canada

This workshop will focus on three aspects of translational research studies: 1) the discovery of biomarkers using semi- and untargeted metabolomic approaches; 2) the structural elucidation of targeted biomarkers; and 3) the quantitative analysis of biomarkers detected. Different steps involved in a metabolomic approach will be described. The focus on biomarker discovery will be on the choice, collection, and preparation of samples for optimal results. This should be done to prevent the risk of creating artificial biases between patient and control groups. Several aspects concerning the ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS/MS) analysis of samples will be addressed to increase the quality of the dataset and to obtain the necessary information to optimize the alignment (data mining) of the results. Other tools, such as various mathematical transformations applied to the marker peak areas to improve biomarker discovery based on their abundance will also be presented. Multivariate analyses, specifically the supervised orthogonal partial least-square discriminant analysis (OPLS-DA) statistical method, will demonstrate how to target biomarker candidates. Tips on the structural elucidation of biomarkers by tandem mass spectrometry will be revealed. Novel biomarkers are not often listed in tandem mass spectrometry data banks. Therefore, tools will be shown to perform structural elucidation of molecules. Means to resolve the presence of structural isomers interfering with the interpretation of a biomarker fragmentation spectrum will be shown. Examples of metabolomic studies performed in our laboratory where UPLC method optimization allowed the separation of a targeted biomarker from structural isomer interferences will be shown. The last part of the workshop will be dedicated to the development and validation of multiple reaction monitoring (MRM) methods for the quantitative analysis of biomarkers with a focus on the reduction of matrix effects. During this workshop, biomarker results for various genetic diseases will be presented aiming at a technological transfer to the clinical field for early detection, monitoring and follow up of patients.

Workshop – **Keep it running! Lab organization, data quality for analytical series**

Tuesday 25th February – 14h15-15h15

Katharina HABLER
Institute of Laboratory Medicine, LMU University Hospital, LMU Munich, Germany

This interactive session will explore practical advice for sustaining operational excellence in the lab.

1. Organizing Lab Workflow:

Training and competencies - strategies for efficient lab organization to maintain seamless operations.

2. Internal and External Quality Assurance:

Monitoring and maintaining a high quality of data results across analytical series.

3. Troubleshooting and Continuous Improvement:

How to use pipetting olympiads, system suitability tests and much more to keep my method running.

Workshop – Improving Accuracy in Clinical MS Laboratories: The Role of Standardization and Traceability in Ionify Assays for the Cobas Mass Spec Solution

Tuesday 25th February – 15h15-15h45

Elie FUX

Teamlead Quantitative Mass Spec, Roche Diagnostics GmbH, Penzberg, Germany

In recent years, clinical laboratory professionals have emphasized the need for the IVD industry to prioritize standardization to ensure consistent patient results across different times and locations. This standardization process involves assigning calibrator values that guarantee uniform and comparable test outcomes across various reagent-calibrator-analyzer combinations by aligning them with a specific reference system.

Mass spectrometry (MS) assays, including the Ionify assays for the Cobas® Mass Spec solution, stand out in this context, offering significant advantages. Beyond their high specificity, broad dynamic range, sensitivity, and multiplexing capabilities, these assays mitigate challenges related to batch-to-batch variations, which can affect the accuracy and consistency of results.

To support the standardization of Roche's automated Cobas Mass Spec solution and Ionify assays, we are developing, validating, and publishing LC-MS-based Reference Measurement Procedures (RMPs) for submission to the Joint Committee for Traceability in Laboratory Medicine (JCTLM).

An initiative to address the need for a greater number of RMPs for small molecules that meet ISO 15193 guidelines and are tailored to the manufacturer's specific needs was initiated. This included assigning SI-traceable values to calibrators through National Measurement Institute (NMI) certified reference materials or using qNMR-characterized materials, systematically assessing measurement uncertainty, and publishing the methods in peer-reviewed journals.

Several collaborations between industry, clinical laboratories, and hospitals have focused on developing and validating RMPs to standardize clinical IVD MS assays. This ongoing initiative has resulted in the publication of over a dozen methods listed with JCTLM.

This session will offer a comprehensive overview of our initiative's progress, detailing the methodology and validation processes that enhance standardization in laboratory medicine, ultimately helping to improve patient care.

Workshop – The Roche MS solution – key technological innovations and results of a pilot study

Tuesday 25th February – 15h45-16h15

Michael VOGESER
Institute of Laboratory Medicine, LMU University Hospital, LMU Munich, Germany

A first fully automated LC-MS/MS-system, which can be seamlessly integrated into a complete laboratory automation system, is being developed by Roche Diagnostics and is now close to being launched on the market (Cobas® Mass Spec solution, Roche Diagnostics International Ltd, Rotkreuz, Switzerland). An advanced prototype of this system was tested for the first time under intended-user conditions in our clinical laboratory. At the timepoint of study execution the reagents and the analyzer were still under development. The study was focused on the assessment of the system functionality and sample throughput in a stand-alone configuration. Assessment of analytical performance was n*ot* within the scope of this prototype study. Stable-isotope labeled internal standards were used for quantification. The instrument incorporates two distinct chromatographic configurations; one for conventional HPLC, and a second configuration based on Rapid LC, both with several HPLC streams configured in parallel. A triple-quadrupole mass spectrometer is used for analyte detection and quantification. For calibration of the assays, a pre-defined master calibration curve was adjusted with a two-point calibrator set. Evaluation of chromatograms and quantification were performed fully automated by the system, without peak-review or interpretation of meta-data by the operator. The following analytes were addressed in diverse experiments: Tacrolimus, Cyclosporine, Sirolimus, Everolimus, Mycophenolic Acid and its glucuronide, Voriconazole, Lamotrigine, Meropenem, Estradiol, Testosterone, and 25-OH- Vitamin D2/3. Routine simulations were performed in batch and random-access working mode. It was found that a 3-day training was sufficient to operate the system successfully, without the need for any previous skills in mass spectrometry or chromatography. Practicability on the level of state-of-the-art standard high-throughput analytical systems for clinical laboratories was observed, with automated, timed start-up procedures, automatic shutdown, and extended walk-away times. A high degree of calibration stability was observed. The time to the first result was ~37 minutes. A throughput of up to 100 injections per hour was observed in random access mode with 9 different analytes. An innovative key feature that enables high sample throughput in a random-access working mode implemented in the system is analyte extraction from diagnostic samples by use of functionalized paramagnetic particles. These particles can be immobilized by the application of a magnetic field, while they can be re-suspended and handled as a liquid by pipetting as long as no field is applied. The standard extraction steps can be applied to these particles, i.e. adsorption of the target analyte, removal of the depleted matrix, washing and elution of the target analytes from the MGPs. A variety of chromatographic surface modifications can be applied to MGPs, and sample preparation with MGPs can be parallelized. In turn, parallel sample extraction with MGPs can be coupled online with parallel chromatographic separation.

Abstracts of Oral Presentations

List of Speakers (Chronological)

List of Titles (Chronological by speaker)

Study on the effect of exogenous chemical residues in serum on chronic diseases at the metabolic level

Xu GUOWANG

Dalian Institute of Chemical Physics, Chinese Academy of Sciences

Human health or disease status is determined by the interaction of genetic and environmental factors. The exposure of toxic and harmful substances (exposomics) in the environment and food directly affects human health and quality of life. The relationship between these exogenous chemical substances and the occurrence and development of major chronic diseases can be revealed by analyzing the content of exposomics and the changes of metabolome in tissues or body fluids by mass spectrometry (MS).

This report will describe how we use MS to measure the metabolome and exposome and study the association between exogenous chemical residues including pesticides, veterinary drugs and organic chemical pollutants in serum from about 10,000 persons from 15 provinces in China and major chronic diseases including hypertension, diabetes, hyperlipidemia, hyperuricemia and obesity, find relevant risk factors and early warning markers, and clarify the molecular mechanism.

References

1. Lei You, et al. (2024) An exposome atlas of serum reveals the risk of chronic diseases in the Chinese population, Nat Commun, 15(1): 2268
2. Yuting Wang, et al. (2023) Screening strategy for 1210 exogenous chemicals in serum by two-dimensional liquid chromatography-mass spectrometry, Environmental Pollution, 331(Pt 2): 121914
3. Lei You, et al. (2022) Metabolome-wide association study of serum exogenous chemical residues in a cohort with 5 major chronic diseases, Environ. Int., 158: 106919
4. Yanfeng Wang, et al. (2022) Metabolomics insights into the prenatal exposure effects of polybrominated diphenyl ethers on neonatal birth outcomes, Sci Total Environ, 836: 155601.

PFAS Analytical Challenges: Navigating Complexities in Biological and Environmental Samples

Brad CLARKE

Associate Professor of Environmental Science and Analytical Chemistry, Australian Laboratory for Emerging Contaminants (ALEC), University of Melbourne, School of Chemistry

This presentation will discuss the pervasive use of chemicals in modern society and research efforts to combat the urgent health threat posed by 21st-century synthetic chemicals. Alarmingly, children across the planet (including in Australia) are born with hazardous substances in their bodies, impacting development and long-term health. Per- and polyfluoroalkyl substances (PFAS) serve as a case study for understanding the broader issue of chemical production and its hidden environmental costs. PFAS, introduced in the 1940s, remained in use for decades before their adverse health effects and persistence in the environment were realized. Current Australian and international regulations focus on a small subset of PFAS, but thousands of variants exist with limited data on their production or health impacts. This talk will examine the presence of PFAS in the Australian environment, including analysis methods such as targeted LC-QqQ-MS and non-targeted analysis (NTA) using high-resolution mass spectrometry (LC-QTOF). A particular focus will be placed on the challenges of detecting and analyzing PFAS in biological tissues, which is crucial for understanding human exposure and health risks. The discussion will also cover the environmental sources of PFAS in Australia, including wastewater treatment plants (WWTPs) and novel sources like warehouse fires and high-performance motor vehicles.

Wednesday 26th February – 10h00-10

Is it time to extend physiologically-based pharmacokinetic (PBPK) modeling and simulation to the investigation of drug–exposome interactions?

Eric CHAN

Deputy Head (Research), Department of Pharmacy and Pharmaceutical Sciences, National University of Singapore

Drug-exposome interactions are an elusive and neglected area of pharmacology. Notwithstanding that the constantly evolving field of untargeted metabolomics/exposomics has been applied to decipher the impact of the exposome on biological systems and human health, the mechanistic and quantitative outcomes of drug-exposome interactions have not been systematically investigated. In this talk, Professor Eric Chan will present the application of physiologically-based pharmacokinetic (PBPK) modeling and simulation of complex disease-drug-drug interactions in pharmaceutical drug development. He will be using potential examples to advocate the application of PBPK modeling and simulation for the investigation of drug-exposome interactions.

Wednesday 26th February – 10h30-11h00

Quantification of PFAS in human samples for population-scale studies

Sartaj MIR

Singapore Lipidomics Incubator, National University of Singapore, Singapore

Environmental chemical exposures are intricately linked to human health with lifelong consequences. Perfluoroalkyl and polyfluoroalkyl substances (PFAS) exposure represents one of the critical components of the chemical exposome. PFAS have been linked to several adverse health effects, including metabolic diseases. However, the molecular basis of PFAS exposure on human health outcomes is not completely understood. Integrative omics analysis of environmental chemicals including PFAS with metabolomics and lipidomics offers a suitable avenue for population-scale exposome studies. For this, workflows have to be established for PFAS analysis in human samples for large-scale studies to match that of other omics approaches. We have developed and validated a simple and robust quantitative method for PFAS analysis from human plasma/serum. We conducted an integrated analysis of plasma PFAS and lipidomics profiles in multi-ethnic Asian subjects. From this study, we observed that women had lower levels of PFAS compared to men and Asian-Indians had lower levels of PFAS compared to both Chinese and Malay subjects. Association studies revealed both shared and distinct relationship of PFAS with plasma lipidomic profiles. We are conducting similar large-scale multi-omics studies that will help in better understanding of the molecular basis of PFAS exposure on human health outcomes.

Wednesday 26th February – 11h00-11h30

FluoroMatch: A Comprehensive Solution for Non-Targeted PFAS

Jeremy J. KOELMEL

Environmental Health Sciences, Yale School of Public Health, USA

Per- and polyfluoroalkyl substances (PFAS) are a pervasive and persistent class of anthropogenic chemicals which are used in a plethora of industrial and consumer applications. With over 15,000 PFAS contained in the EPA CompTox Database, the diversity of PFAS synthesized and their transformation products is astounding. Furthermore, toxicological profiles and fate and transport properties are highly dependent on structure, necessitating the need for full structural characterization of complex mixtures. With targeted mass spectrometric methods covering less than 10% of the total PFAS burden in various matrices including sewage, soil, and industrial effluent, there is an essential need for non-targeted analysis. Therefore, we will discuss FluoroMatch Suite, an open-source, free, and comprehensive software for annotating PFAS from complex non-targeted mass spectrometry datasets. First introduced in 2020, over the past 5 years FluoroMatch has been downloaded by over 500 users, and has grown to cover the entire PFAS data-processing workflow from vendor file conversion to visualization and validation of annotations. FluoroMatch includes homologous series detection, retention time trends analysis, Kauffman analysis, isotope filtering, formula prediction, rule-based MS/MS annotation use a database of over 13,000 PFAS, fragment screening, biotransformation product prediction, CCS matching, and more. All evidence is displayed in an interactive visualization platform which can be distributed online. Similar frameworks exist for lipidomics and polymers, and the software platforms support LC-HRMS/MS, LC-IM-HRMS/MS, and progress in GC-HRMS is also being made. This talk will discuss various use cases and features of FluoroMatch.

Wednesday 26th February – 11h30-12h00

PLATINUM SPONSOR TALK – AGILENT TECHNOLOGIES

Forever chemicals don't make hero mutant ninja turtles: Elevated PFAS levels linked to unusual scute development in newly emerged freshwater turtle hatchlings (Emydura macquarii macquarii) and a reduction in turtle populations

David J. BEALE

CSIRO, Australia

Per- and Polyfluoroalkyl substances (PFAS) are pervasive, persistent synthetic contaminants. While PFAS exposure can cause toxic effects in wildlife, little is known about their accumulation, developmental and maternal effects on egg-laying species. We can address this gap and provide ecosystem health metrics using omics-based ecosurveillance, which assesses chemical contamination using proteomics, lipidomics, and metabolomics. Herein, we collected 26 freshwater turtles (*Emydura macquarii macquarii*; 50% male and 50% female) from three different waterways; a PFAS-impacted, a PFAS-control, and a PFAS-free site (reference site). PFAS were measured in serum, tissues, and eggs using a validated LC-TQ-MS method. A streamlined and automated one-pot extraction workflow was used to obtain multi-omics data via LC-TQ-MS and LC-QToF-MS. Metabolites and lipids were identified following the Metabolomics Standards Initiative. Protein identification was performed using draft turtle genome-translated sequences.

Serum PFAS concentrations were ten-fold greater at the impacted site relative to the control samples. The reference site turtles had no measurable PFAS. The distribution of PFAS within tissues demonstrated a high bioaccumulative effect of perfluorooctanesulfonic acid (PFOS) in the liver (3.3-fold) and ovary tissues (8.0-fold) compared to serum values, while perfluorobutane sulfonamide (FBSA) and perfluorohexanesulfonic acid (PFHxS) were more abundant in the serum compared to tissues. Specific biochemical profiles (proteins, lipids, and metabolites) of serum, tissues, eggs, and hatchlings demonstrated a positive correlation in the impacted turtles exposed to elevated PFAS with enhanced purine metabolism, glycerophosphocholines, and an innate immune response, suggesting inflammation, metabolic preservation, and re-routing of central carbon metabolites. Lipid transport and binding activity were negatively correlated. Purine metabolism metabolites were significantly elevated in the PFAS-impacted eggs, while yolks were depleted in lipids tied to growth and development. This study reveals the impacts of PFAS exposure risks in wildlife, opening the scope for data-driven ecosystem management and enabling informed decision making for regulators.

SHORT TALK AWARD

Targeting desaturases to improve gemcitabine-based chemotherapy in pancreatic ductal adenocarcinoma

Sarah HANCOCK

Victor Chang Cardiac Research Institute, Australia

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with a survival rate of less than 10% at 5 years, a figure that has not improved in more than 25 years. This poor prognosis is due to late diagnosis, its asymptomatic and heterogeneous nature, and resistance to common chemotherapies like gemcitabine and newer combination therapies like gemcitabine/nab-paclitaxel. New treatments are desperately needed to improve PDAC patient survival time. To discover new metabolic targets that can improve chemotherapy efficacy in PDAC we generated gemcitabine-resistant (GEMR) Panc1 and MiaPaCa2 pancreatic cancer cells and discovered upregulation of de novo lipogenesis in GEMR cells through untargeted lipidomics, immunoblotting for total and activated lipid synthesis enzymes and by tracing radiolabelled glucose incorporation into lipids. Knocking down key lipogenic enzymes sensitised PDAC cells to gemcitabine treatment, with fatty acid synthase (FAS) knockdown producing the largest decrease in cell growth in both cell lines and stearoyl-CoA desaturase 1 (SCD1) knockdown significantly impacting cell growth in MiaPaCa2 cells only. Using ozone-enabled fatty acid discovery we found that Panc1 cells have significant apocryphal fatty acid desaturase 2 (FADS2) activity, producing 16:1 and 18:1 n-10 monounsaturated fatty acids (MUFA). FADS2-derived MUFA were also apparent in patient-derived organoid models. Knockdown of either SCD1 or FADS2 resulted in a concomitant increase in the alternate desaturase activity in Panc1 cells, which explained why Panc1 cells were relatively insensitive to SCD1 knockdown. Dual desaturase knockdown successfully sensitised cells to gemcitabine treatment. Furthermore, chronic treatment with combination gemcitabine/paclitaxel chemotherapy resulted in selective downregulation of FADS2 expression and activity which sensitised Panc1 to SCD1 knockdown. Therefore we propose that SCD1 inhibitors could be explored in combination with gemcitabine/nab-paclitaxel to improve chemotherapy efficacy in PDAC.

We also describe some of the newer work our lab has done to better understand the mechanism of selective downregulation of FADS2 by paclitaxel treatment, including timing and proposed mechanisms. Furthermore, we are also exploring the use of online RNAseq databases to determine specific cancers that might have high apocryphal FADS2 activity and therefore might benefit from a dual desaturase targeting approach.

Wednesday 26th February – 13h30-13h45

Lifestyle factors and blood lipids: Results from dietary intervention studies on Singaporean men

Mei Hui LIU

Department of Food Science & Technology, National University of Singapore, Singapore

Diets, their compositions and exercise interventions can impact obesity and blood glucose control. We will first report the results of a 16-week diet and exercise intervention on Singaporean Chinese with obesity. Significant changes were observed in nearly half of the measured clinical traits and several blood lipids. Observed alterations in fatty acid oxidation and lipid levels suggest potential improvements in metabolic health and reduced inflammation due to lifestyle modifications. We will also examine the influence of diet compositions on postprandial glucose control from a separate study and discuss the implications of lifestyle factors and food choices on blood glucose management.

Wednesday 26th February – 14h15-14h45

Immunoregulatory Functions of Dietary and Microbial Lipid Metabolites and Their Application to Precision Nutrition

Jun KUNISAWA

Microbial Research Center for Health and Medicine, National Institutes of Biomedical Innovation, Health and Nutrition

The gut environment, shaped by dietary components and gut microbiota, plays a crucial role in the control of host health. Among dietary components, oils are often discussed in terms of quantity, but the quality determined by the fatty acid composition is equally important. We have focused on the quality of dietary oils, particularly linseed oil rich in omega-3 fatty acids, and demonstrated that metabolites derived from dietary lipids function as suppressive factors for allergies and inflammatory diseases. Furthermore, it has been revealed that not only host enzymes but also microorganisms such as gut bacteria are involved in lipid metabolism, contributing to the production of unique lipid metabolites that regulate host immune responses and influence disease development. In addition to basic research, analysis of human samples has also shown individual differences in the patterns and quantities of metabolites derived from dietary oils, suggesting these differences may account for variations in the effects of dietary oils. We further developed alternative methods utilizing fermented foods for individuals who may not benefit from dietary oils. This presentation will introduce the immunoregulatory mechanisms of dietary oils and explore the potential of personalized nutrition based on precision nutrition research.

Wednesday 26th February – 14h45-15h15

Fabry disease – the most frequent monogenetic sphingolipidosis

Albina NOWAK

Department of Endocrinology, Diabetology and Clinical Nutrition, University of Zurich, Switzerland

Fabry disease (FD) is a rare X-linked glycosphingolipid storage disease and a result of mutations in the α-galactosidase A gene (*GLA*), which leads to reduced activity of the encoded lysosomal exoglycohydrolase, α-galactosidase A (α-Gal A). The α-Gal A defect causes the progressive accumulation of globotriaosylceramide (Gb3) and other related glycosphingolipids in lysosomes of patients affected with FD, leading to systemic manifestations such as chronic kidney disease, cardiomyopathy, and stroke.

The deacylated derivative of Gb3, globotriaosylsphingosine (Lyso-Gb3), has been identified as a hallmark of FD. Further analogues and isoforms of Gb3 and Lyso-Gb3 were recently detected in sera and urine of FD patients. Discovery of new biomarkers is needed to improve newborn and high-risk group screening, to facilitate the diagnosis and therapeutic monitoring, to predict the risk of severe clinical events.

Wednesday 26th February – 15h45-16h15

Complex glycosphingolipids in inherited neurodegenerative disease

Maria FULLER

National Referral Laboratory, Genetics and Molecular Pathology, SA Pathology; Adelaide Medical School and School of Biological Sciences, University of Adelaide, Australia

Gangliosides are a subset of complex sphingolipids defined by the presence of sialic acid residues. Present in all cells they predominate in the brain, 10 times more abundant in neuronal than non-neuronal cells. During neuronal development, the pattern of ganglioside expression changes from simple gangliosides predominating in the embryonic brain to more complex structures at maturation. Testament to their role in proper neurological development and function, pronounced neurodegeneration is a phenotypic hallmark of inborn errors of ganglioside metabolism, a group of disorders known as the gangliosidoses. Measuring the numerous different species of gangliosides in biological samples is not easy. Although liquid chromatography mass spectrometry methods have largely replaced traditional thin layer chromatography, and improved the quantitative nature of ganglioside assessments, significant challenges remain due to their complexity, size, and negative charge. Our laboratory developed a method to measure 30 species of ganglioside by mass spectrometry for the biochemical diagnosis of the gangliosidoses. Additionally, we showed that gangliosides are also altered in other neurodegenerative disorders and have clinical utility as surrogate biomarkers of disease activity in cerebrospinal fluid and plasma. Using a mouse model of a neurodegenerative disorder, we found that gangliosides are altered in the brain of the diseased model, and elevation of the simple gangliosides suggests impaired neurodevelopment. Given the brain is a mixed cell population, primary corticoid neurons are being isolated to determine what cell types harbour these ganglioside alterations.

Wednesday 8th February – 16h15-16h45

Glycosphingolipids and rare diseases

Christiane AURAY-BLAIS

Full Professor, Researcher, Department of Pediatrics, Division of Medical Genetics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Clinical Research Centre-CHUS, Sherbrooke, Quebec, Canada

Clinical applications of different mass spectrometry methodologies will be presented for the evaluation of glycosphingolipids in rare diseases. Novel biomarkers for Fabry disease and Gaucher disease detected by metabolomic studies using time-of-flight mass spectrometer in urine and plasma will be reported as well as tandem mass spectrometry methods developed and validated for targeted glycosphingolipids. The effect of various treatments on glycosphingolipids such as enzyme replacement therapy, chaperone therapy, and gene therapy for Fabry disease patients will be presented. The results of a study on the chromatographic separation of glucosylceramide and galactosylceramide for a Gaucher disease patient type 3 having a vitrectomy will be shown.

Wednesday 26th February – 16h45-17h15

Defining the role of lipid metabolism in bridging diet and metabolic health

Peter J. MEIKLE

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Baker Department of Cardiovascular Research Translation and Implementation, La Trobe University, Bundoora, Victoria, Australia

Dysregulation of lipid metabolism underpins multiple diseases, including type 2 diabetes and cardiovascular disease. While the metabolic pathways of lipids are well characterised, dysregulation of the same pathways due to diet, lifestyle and genetic influences is not well understood. We have applied our high throughput lipidomics platform (> 750 lipid species, 10 µL plasma, 15 minutes) to large clinical and population studies (>75,000 individuals). These data have enabled us to develop lipidomic scores for metabolic BMI[1], metabolic age[2], a plasmalogen score[3] and most recently a lipidomic risk score for cardiovascular disease[4].

Diet is a key factor influencing the risk and development of multiple diseases, yet the underlying metabolic pathways involved remain poorly understood. We analysed data from 13,335 participants across two large Australian cohorts examining comprehensive lipidomic profiles in relation to diet. In both cohorts we observed characteristic lipidomic profiles linked to dairy (sphingomyelins and lipids esterified with 14:0, 15:0, 17:0 or 17:1 fatty acid), red meat and poultry (alkyl- and alkenyl-phosphatidylcholine and phosphatidylethanolamine), and fish (higher 22:6, and lower 22:4 fatty acids) as well as many other foods.

We then assessed the link between metabolic signatures of diet quality with cardiometabolic health and all-cause mortality. The metabolic signatures of diet quality showed improved performance compared to the food frequency questionnaire (FFQ) derived diet quality scores in the prediction of metabolic and cardiovascular risk. Similarly, they showed improved prediction of all–cause mortality with hazard ratios (95% confidence interval) of 0.89 (0.84–0.93), 0.87 (0.83–0.92), and 0.88 (0.84–0.93) for the Australian Dietary Guideline Index (DGI), the Global Diet Quality Score (DGQS), and the MIND score respectively.

These studies provide valuable insights into the impact of diet on metabolic health and disease risk. A web-based resource tool, developed with these data now offers a comprehensive platform for guiding research aimed at developing preventive and therapeutic strategies to improve human metabolic health.

[1] Beyene HB *et al.* Metabolic phenotyping of BMI to characterize cardiometabolic risk: evidence from large population-based cohorts. *Nature Communications* **14**, 6280 (2023).
[2] Wang T et al. A lipidomic based metabolic age score captures cardiometabolic risk independent of chronological age. *EBioMedicine* **105**, 105199 (2024).
[3] Beyene HB. *et al.* Development and validation of a plasmalogen score as an independent modifiable marker of metabolic health: population based observational studies and a placebo-controlled cross-over study. *EBioMedicine* **105**, 105187 (2024).
[4] Wu, J.*, et al.* Lipidomic Risk Score to Enhance Cardiovascular Risk Stratification for Primary Prevention. *Journal of the American College of Cardiology* **84**, 434-446 (2024).

Thursday 27th February – 9h00-9h30

Longitudinal Landscape of Maternal Plasma Apolipoproteins from Preconception to Postpartum and Their Associations with Maternal Metabolic Risk Factors and Offspring Birth Outcomes

Li CHEN

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Apolipoproteins, essential components of lipoproteins, play a critical role in lipid transport and metabolism. However, there is limited research on how maternal apolipoproteins change from preconception to postpartum and their links to maternal metabolic health and offspring birth outcomes. In this study, we quantified apolipoproteins in maternal plasma from 243 trios at three time points: preconception, 26–28 weeks of pregnancy, and three months postpartum, as part of the Singapore PREconception Study of long-Term maternal and child Outcomes (S-PRESTO). Linear regression models and network analysis were used to explore associations between maternal apolipoproteins, genetic variants, biochemical measures, metabolic risk factors, and offspring birth outcomes. ApoC-III, ApoB, and ApoL1 concentrations increased significantly during pregnancy compared to preconception and postpartum. Genome-wide association studies identified multiple genetic variants linked to apolipoproteins. Plasma apolipoproteins were strongly associated with biochemical measures including lipidomic profiles, lipoprotein features and fat-soluble vitamins, as well as metabolic risk factors including glycaemic traits, liver enzymes, inflammatory markers, albumin, and blood pressure. Integrative network analysis revealed pronounced interrelations within the apolipoproteins and with cholesterols, triglycerides, alpha tocopherol and GlycA. Notably, higher preconception concentrations of maternal ApoC-I and ApoC-III were linked to shorter gestational age in offspring. This study provides a comprehensive view of maternal circulating apolipoproteins from preconception to postpartum and their associations with metabolic health and pregnancy outcomes. The findings offer insights into the molecular mechanisms underlying maternal metabolic flexibility and pregnancy-related outcomes.

Thursday 27th February – 9h30-10h00

Accelerating the Development of Targeted Metabolite Phenotyping Assays for Precision Population Health

Nicola GRAY

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Computational and Systems Medicine, Health Futures Institute, Murdoch University Perth WA Australia

Introduction

Metabolite phenotyping is an important tool in translational biomedical research. Targeted approaches, typically relying on liquid chromatography-tandem mass spectrometry (LC-MS/MS), focus on the quantification of predefined metabolites which are often chosen for their biological relevance, association with specific diseases, or pathways of significance. Sample preparation is a crucial step in LC-MS to remove matrix effects and ensure accurate, precise and reliable analyses with enhanced sensitivity, reproducibility and instrument protection. Solid phase extraction (SPE) is a common technique that permits rapid, selective sample preparation prior to analysis, yet requires significant optimisation to maximise analyte recovery and minimise matrix interference. In metabolic phenotyping workflows, where sample matrices are often complex, traditional SPE methods do not efficiently capture the entire metabolome, resulting in bespoke assays for a limited range of analytes or compromised performance to capture a broad spectrum of analytes under sub-optimal conditions. Hence, generally multiple targeted methods are employed to analyse focused compound panels owing to distinct chemical properties and polarity differences. To address this, we propose a comprehensive screening approach delivering optimal SPE and LC-MS conditions for target metabolites across a diverse range of physicochemical properties, enabling automated method selection for large-scale analysis.

Methods

Data was generated from screening 700 reference standards spanning a range of metabolite classes (eicosanoids, bile acids, microbial metabolites, acylcarnitines, amino acids and steroids) across a variety of conditions; four SPE phases (strong anion, strong cation, weak anion, weak cation) each with two eluents and four LC conditions (reversed-phase and hydrophilic interaction LC each at pH 4 and pH 9). A Sciex Exion UHPLC with 6 column switching capability coupled to a 6500+ QTRAP MS was used for analysis. Analytes were spiked into plasma at three concentrations to assess recovery, linearity, matrix effects, and metabolite performance under each condition was ranked by these criteria.

Results

Our results provide a deep understanding of analyte behaviour in diverse extraction and chromatographic conditions to enable more efficient assays for comprehensive targeted metabolic phenotyping. We generated a database of optimal conditions across a broad spectrum of analytes to facilitate method development for accurate and precise metabolite phenotyping, leading to new tools for discovery and translation. The set of optimal conditions yielding the greatest number of metabolites achieving the assessment criteria were selected for assay translation targeting 196 analytes and internal standards. To demonstrate application to large cohorts, we evaluated 780 human plasma samples with our optimised workflow, highlighting the precision and reproducibility of metabolite measures based on physiochemical properties as an alternative to biological pathways.

Conclusions

The presented strategy improves the targeted method development workflow, predominantly improving analyte coverage and throughput whilst maintaining the accuracy and rigor of bespoke assays in discovering reliable potential biomarkers in various applications.

Thursday 27th February – 10h00-10h30

Clinical Lipidomics in Immune-Mediated Diseases: Challenges and Perspectives

Robert GURKE

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Fraunhofer Cluster of Excellence for Immune Mediated Diseases (CIMD), Germany
Goethe University Frankfurt, Institute of Clinical Pharmacology, Faculty of Medicine, Germany

Lipids are essential components of cell membranes and play a crucial role in energy metabolism. Involvement in various signaling pathways makes them key players in the immune system, closely implicated in immune-mediated diseases (IMDs) such as rheumatoid arthritis, inflammatory bowel diseases, plaque psoriasis, and psoriatic arthritis. Analyzing lipid profiles offers promising prospects as biomarkers for early diagnosis, monitoring disease progression, and guiding therapeutic decisions in IMDs. However, significant challenges must be addressed to achieve accurate analysis and interpretation of lipid data. One primary challenge is the need for specific procedures for sampling, sample preparation, and analysis to ensure reliable determination of lipid molecules. Lipids exhibit highly variable concentrations in different biological matrices, necessitating robust methodologies to account for this variability. Additionally, ensuring compound stability throughout the analytical workflow is critical, as lipids are susceptible to factors such as oxidation and hydrolysis. Therefore, thorough attention must be paid to pre-analytical sample handling, requiring close collaboration between researchers and clinicians to establish standardized protocols. If these necessities are considered, lipidomics can provide immense value in the search for biomarkers in IMDs. However, current methods primarily focus on systemic changes in plasma, serum, or urine, rather than local changes in tissue. To complement these classical approaches, a novel method in clinical lipidomics is the application of imaging mass spectrometry to analyze tissue biopsies from patients. This technique enables spatial mapping of lipid distributions and provides valuable insights into the pathophysiological mechanisms underlying IMDs. While this innovative method offers new opportunities for understanding disease processes, it also introduces additional challenges in sample preparation and data interpretation. In conclusion, while lipidomics in the context of IMDs offers exciting possibilities, it is accompanied by substantial challenges. Overcoming these obstacles is essential to fully harness lipidomic analyses as diagnostic and prognostic tools.

Thursday 27th February – 10h30-11h00

Longitudinal changes of plasma lipidomics profile and pregnancy health: findings from a multiracial cohort

Guoqi YU

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Background: Maternal lipidomic profiling presents a valuable approach for characterizing lipid metabolite dynamics during pregnancy, yet longitudinal data remain scarce. This study investigates the associations between lipidomic profiles across gestation and pregnancy outcomes in a diverse cohort.

Methods: We analyzed untargeted plasma lipidome profiles from 321 pregnant women enrolled in the NICHD Fetal Growth Study-Singletons. Plasma samples were collected at four gestational windows: 10–14, 15–26, 23–31, and 33–39 weeks. Individual lipid metabolites at each visit were assessed for associations with gestational diabetes (GDM) and neonatal anthropometry, while longitudinal analyses employed weighted correlation network analysis and consensus network analysis. Linear mixed-effects models were applied with false discovery rate adjustments to evaluate lipid network associations across gestation.

Findings: Lipid networks primarily characterized by elevated plasma diglycerides and short, saturated/low unsaturated triglycerides and lower plasma cholesteryl esters, sphingomyelins and phosphatidylcholines were associated with higher risk of developing GDM (false discovery rate (FDR) <0.05). Among individual lipids, 58 metabolites at visit 0 and 96 metabolites at visit 1 (40 metabolites at both time points) significantly differed between women who developed GDM and who did not (FDR <0.05). Several triglycerides (TG) exhibited positive associations with birth weight (BW), BW Z-score, length, and head circumference, whereas certain cholesteryl esters (CE), phosphatidylcholines (PC), sphingomyelins (SM), phosphatidylethanolamines (PE), and lysophosphatidylcholines (LPC 20:3) demonstrated inverse associations with BW, length, and abdominal and head circumference at various gestational periods. Longitudinal lipid trajectories revealed associations between TG, PC, and glucosylceramides (GlcCer) with BW, CE (18:2) with BW Z-score, length, and skinfold sum (SS), and specific PC and PE species with abdominal and head circumference. Notably, TG modules at gestational weeks 10–14 and 15–26 were primarily linked to BW, while at 33–39 weeks, networks of LPC (20:3), PC, TG, and CE displayed inverse associations with abdominal circumference. Distinct lipid trajectory patterns within consensus modules highlighted significant variations in BW and length.

Conclusion: This study provides novel insights into the dynamic lipidomic changes throughout pregnancy and their associations with GDM and neonatal anthropometry. The findings suggest that specific lipid subclasses, particularly TG, PC, CE, and LPC, may serve as potential biomarkers for fetal growth trajectories. Further investigations are warranted to elucidate the underlying mechanisms linking maternal lipid metabolism to neonatal outcomes.

Thursday 27th February – 11h00-11h30

Longitudinal multi-omics in postpartum women identify a persistent signature of gestational diabetes

Charna DIBNER

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Aims/Hypothesis: Gestational diabetes mellitus (GDM) poses long-term health risks as evidenced by an increased incidence of cardiometabolic diseases, yet the lasting metabolic imprint from GDM remains to be defined. We aimed to establish a detailed multi-omics signature in a longitudinal cohort of postpartum women after GDM (GDM group) compared to women after normoglycemic pregnancies (non-GDM group).

Methods: In this prospective observational study, we collected blood samples 1-2 months after delivery (baseline) and 6 months later (follow-up) from the GDM and non-GDM groups (n = 15 and 14, respectively). Samples were processed for serum metabolomics, lipidomics, and gene expression analyses of isolated monocytes by NanoString. Univariate, multivariate and machine learning analyses were used to identify the factors involved in the classification of GDM status, based on the combined multi-omics data.

Results: Serum metabolomics analysis revealed decreased bile acids (taurocholic acid, cholic acid, and deoxycholic acid, FDR-adjusted $p<0.05$) in the GDM group. Pathway analysis of the metabolome uncovered impaired glucose homeostasis and bile acid synthesis. Lipidomics analysis identified 302 lipid species across 19 classes, with an increase in the lysophosphatidylinositol (LPI) family in the GDM group at baseline ($p<0.05$). Lactosylceramides were the most decreased lipid family in the GDM condition (FDR-adjusted $p<0.05$). NanoString analysis of monocyte RNA showed altered core-clock and cytokine genes such as *NR1D1*, *PER1*, *PER2*, *PER3, IL8 and TNFa* between GDM and non-GDM groups (FDR-adjusted $p<0.05$), with *PER2* correlating to fasting glucose. Machine learning analyses combining the three data sets into a single model revealed a stronger classification accuracy of GDM than HbA_{1c} or fasting glucose at both baseline (82%) and follow-up (72%). This signal included altered core-clock and cytokine transcripts at baseline, and differences in metabolites and lipid species at follow-up. To validate our findings, we compared our mass spectrometry metabolomics data to NMR metabolomics of 6-month postpartum samples from women with and without GDM in the UPBEAT cohort (n = 332), revealing consistent alterations across the two cohorts in hexose/glucose, isoleucine, leucine and valine.

Conclusions/interpretation: We identify a novel GDM signature based on multi-omics analyses of serum metabolites, lipids, and monocyte transcripts in a prospective longitudinal cohort of postpartum women with and without GDM. This post-GDM imprint includes decreased bile acids and lactosylceramides, and increased lysophosphatidylinositols and core-clock transcripts. Together, these findings reveal a lasting imprint from GDM, warranting further validation in larger GDM cohorts.

Thursday 27th February – 11h30-12h00

PLATINUM SPONSOR TALK – WATERS CORPORATION

Discovery lipidomics with the Xevo MRT and integrated informatics for confident lipid characterization and quantification

Yan Ting LIM

Waters Corporation, Singapore

Lipids play a crucial role in various cellular functions, such as survival, proliferation and death. These processes are closely linked to the development of cancer, including transformation, progression and metastasis. Despite developments in analytical technology, the detection and identification of lipids remains a significant challenge.

We developed a lipidomics workflow that takes advantage of the speed, sensitivity and resolution of the novel Xevo™ MRT (multi reflecting time-of-flight) mass spectrometer to increase lipidome coverage and identification confidence. This, in combination with data analysis powered by the waters_connect™ Software Platform and the Mass Analytica™ Lipostar2 software (Mass Analytica, Barcelona, Spain), provides streamlined identification review and integrated analytics . The benefits of this lipidomic workflow are demonstrated using spiked lipid extracts to demonstrate the high throughput afforded by the faster gradient profiles, and with colorectal cancer-based study samples to demonstrate its relevance for biomarker discovery.

Thursday 27th February – 13h00-13h30

TRAVEL AWARD

Organ-Specific Lipid and Lipoprotein Metabolism in Cardiovascular-Kidney-Metabolic Syndrome

Dana HICKS

Australian National Phenome Centre, Murdoch University

Introduction

Cardiovascular-kidney-metabolic (CKM) syndrome, as defined by the American Heart Association, encompasses interrelated health conditions like obesity, diabetes, chronic kidney disease, and cardiovascular disease. The cardiovascular and renal systems are closely linked through metabolic processes, with diseases often arising from shared pathophysiological mechanisms and risk factors such as dysglycemia, dyslipidemia, hypertension, and obesity. To better understand these biological processes, especially the roles of lipids and lipoproteins, examining organ-specific arteriovenous gradients and assessing the differences in lipid composition between venous blood representing the heart and kidney is crucial for developing targeted therapies and improving patient outcomes.

Methods

Samples from a double-blind crossover trial were analysed to assess lipid metabolism disparities between the heart and kidneys. Participants (n=18) with Stage 2 CKM received either Empagliflozin or placebo daily for 4 weeks, then swapped treatments for another 4 weeks, separated by a 4-week washout. At the end of each phase, coronary sinus (CS), renal vein, and matching radial artery samples were obtained via catheterisation. Lipidomic analysis was performed using liquid chromatography-mass spectrometry (LC-MS), and lipoprotein analysis using nuclear magnetic resonance (NMR) spectroscopy. Arteriovenous gradients from paired CS, renal, and arterial samples were determined to assess organ-specific differences between the heart and kidney. The lipids and lipoproteins identified as significant by the Friedman test, paired Wilcoxon signed-rank test, and variable importance in projection (VIP) scores >1 from partial least squares discriminant analysis (PLS-DA) were further investigated to provide insights into the differences in lipid composition between the heart and kidney.

Results

Data analysis revealed significant differences in lipoprotein subfractions between CS venous and matched radial artery samples. Specifically, the subfractions of very-low-density lipoproteins (VLDL) free cholesterol (VLFC), apo-B100 (VLAB), and VLDL1 triglycerides (V1TG), cholesterol (V1CH), and phospholipids (V1PL) showed a net uptake by the heart, as indicated by arteriovenous gradient analysis. This finding aligns with previous research showing that the heart utilises VLDL particles as an energy source through the net uptake of triglycerides and other components. The heart also demonstrated significant uptake of long-chain fatty acyls (FA), including FA(18:0), FA(18:1), FA(20:0), FA(20:1), FA(20:2), FA(20:3), FA(22:4), and FA(22:5). In contrast, a net organ release of lysophosphatidylethanolamine (LPE) 18:2 was observed.

Analysis of the renal vein and matched radial artery samples revealed significant differences for several lipid species, including diacylglycerol (DG) (14:0_14:0), DG(16:0_20:4), FA(14:0), FA(14:1), FA(16:1), FA(16:2), FA(18:3), LPE(20:4), LPE(22:6), and lysophosphatidylinositol (LPI) 16:1, all reflecting a net uptake by the kidney. In contrast, the kidney observed a net organ release of FA(18:0).

Conclusion

This sample collection is the first to use arteriovenous sampling for lipidomic analysis across the heart and kidney, revealing organ-specific differences in lipid and lipoprotein metabolism associated with Stage 2 CKM.

Thursday 27th February – 13h30-13h45

Deciphering Diverse Obesogenic Strategies of TZDs through Complex Lipid Metabolic Flux Analysis

Ondřej KUDA

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Complex lipid metabolic flux analysis (MFA) has emerged as a powerful tool for elucidating the intricate dynamics of lipid metabolism in biological systems. We present a novel approach to unravel the differential lipogenic strategies triggered by various thiazolidinediones (TZDs) in preadipocytes and bone marrow stromal cells.

Liquid chromatography-mass spectrometry (LC-MS) serves as the cornerstone of our lipid flux analysis, enabling precise quantification and identification of lipid species at specific hierarchical levels, including detailed structural information. The integration of stable isotope labeling, particularly 13C and 2H, with LC-MS has enhanced our ability to track lipid synthesis, degradation, and interconversion rates with unprecedented accuracy. We developed a comprehensive pipeline for complex lipid MFA by leveraging the standardized Grammar of Succinct Lipid Nomenclature (Goslin) and the Elementary Metabolite Unit (EMU) framework. This approach allows for lipidome-wide flux analysis, providing a holistic view of lipid metabolism dynamics.

Applying this methodology, we investigated the metabolic effects of various TZDs, known for their insulin-sensitizing properties but associated with increased risk of bone fractures. While TZDs generally promote adipogenesis, our analysis revealed distinct metabolic pathways leading to this outcome for different TZDs in preadipocytes and bone marrow stromal cells.

Our findings suggest that targeted therapeutic intervention with specific TZDs could potentially improve peripheral insulin sensitivity while preserving bone structure. This nuanced understanding of TZD-induced metabolic changes opens new avenues for personalized treatment strategies in metabolic and bone diseases, potentially mitigating the adverse effects on bone density and quality associated with current TZD therapies. This study demonstrates the power of advanced lipid MFA in deciphering complex metabolic processes and highlights its potential in developing more targeted and effective therapeutic interventions for metabolic disorders.

Thursday 27th February – 13h45-14h15

Tracing and modeling compound lipid homeostasis to understand disease

Christian METALLO

Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies

Metabolism is central to virtually all cellular functions and contributes to diseases like cancer, metabolic syndrome, and neuropathy. My laboratory applies stable isotope tracers, mass spectrometry, and metabolic flux analysis (MFA) to quantify metabolism in cells, animal models, and human patients. We are particularly interested in understanding how amino acid and lipid metabolism are coordinated in the context of cancer, diabetes, and neuropathy. A central focus of these studies is sphingolipid biosynthesis via serine palmitoyltransferase (SPT), which integrates amino acid and fatty acid signals into the membrane lipidome through its promiscuity and responsiveness to substrate availability. Here I will detail how we apply MFA and related methods to decipher why serine and glycine are reduced in diabetes and contribute to co-morbidities of diabetes through lipid metabolism. I will also describe efforts in tracing the fates of monounsaturated fatty acids in the context of high-fat diets. We demonstrate that saturated and trans-mono-unsaturated fatty acids are distinctly metabolized by SPT to promote hepatic steatosis and atherosclerosis in low-density lipoprotein receptor (Ldlr)-deficient mice. Finally, I will introduce a framework for applying 13C MFA to elucidate lipid synthesis and recycling pathways in cultured cells and precision-cut tumor slice cultures. We validate this method through characterization of lipid biosynthesis and recycling pathways within lung and pancreatic adenocarcinoma tumors. Collectively, these data provide mechanistic insights into the roles of SPT flux and lipid diversity in driving disease states.

Thursday 27th February– 14h15-14h45

Isomeric discrimination by collision-/ozone-induced dissociation reveals the lipid complexity *sn*-onymous with triacylglycerols

Stephen BLANKSBY

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Triacylglycerols (TGs) serve critical cellular functions as both energy reserves and reservoirs of fatty acid (FA) building blocks for membrane phospholipids. Both the identity of the fatty acid and their stereo-specific numbering (*sn*-) position on the glycerol backbone of TGs are (i) critical in understanding their biological functions and (ii) structural hallmarks of their biosynthesis. Advancement of this understanding has been impeded by the complex array of lipids present in nature and the limitations of contemporary lipidomic technologies to resolve and structurally discriminate between TG regioisomers. Here, we introduce ion mobility-aligned collision-(CID)/ozone- (OzID) induced dissociation for detailed TG structural analysis on liquid chromatography timescales.

While MS^n approaches based on varying combinations of collision- (CID), ozone- (OzID) and photo- (PD) dissociation have been demonstrated to achieve isomeric-discrimination of TGs, these are most-often characterised by low duty cycle and thus an incompatibility with high-throughput analysis of complex lipidomes. To overcome these impediments, we enabled CID/OzID as a *pseudo*-MS^3 sequence on a traveling-wave ion-mobility mass spectrometer. In this approach, mass-selected $[TG+Na]^+$ precursor ions are subjected to CID in the pre-mobility trap, with the resulting product ions separated in the mobility region prior to OzID in the post-mobility transfer array. Extraction of the resulting data in the mobility-time domain enables assembly of OzID mass spectra of individual CID product ions and consequent assignment of both fatty acyl-chain identity and *sn*-position in a single composite acquisition achieved on the native duty cycle of the instrument. The acceleration of the CID/OzID acquisition in this manner allows for hyphenation with the reversed-phase chromatographic workflows widely deployed in lipid analysis. In application of this sequence to isomeric reference standards of both AAB- and ABC-type TGs (*e.g.*, TG 16:0_16:0_18:1 and TG 16:0_18:0_18:1, respectively) diagnostic CID/OzID product ions were observed that uniquely identify the position of each acyl chain on the glycerol backbone and thus enable discrimination between isomers even where chromatographic resolution is incomplete. Application of this workflow to lipid extracts, including those from edible nuts and milk fat, will be presented. Preliminary results using this novel approach have confirmed the enrichment of short-chain fatty acids at the *sn*-1/3 positions of milk-derived TGs (*e.g.*, TG 4:0/18:0/18:1), while pinolenic acid –a characteristic polymethylene interrupted polyunsaturated FA– is localised this to the *sn*-1/3 positions of TGs extracted from pine nuts (*e.g.*, TG 16:0/18:2/18:3*n*-6,9,13).

Thursday 27th February – 14h45-15h15

Mass spectrometry-based untargeted metabolomics and single-cell metabolomics

Zheng-Jiang ZHU

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Mass spectrometry-based untargeted metabolomics is constantly challenged by large-scale and unambiguous metabolite annotation in complex biological matrices, due to the enormous chemical and compositional diversity of metabolome. In my presentation, I will introduce the metabolic reaction network-based strategy, namely MetDNA, developed in my group for accurate and large-scale metabolite annotation. The recent development of MetDNA3 employed a new metabolic networking strategy, to enable global metabolite annotation from knowns to unknowns in untargeted metabolomics. Further, I will introduce an ion mobility-resolved mass cytometry technology that integrates flow cytometry for high-throughput single-cell injections and ion mobility–mass spectrometry for multidimensional metabolomic profiling. Our technology achieves an unprecedented depth of coverage, detecting over 5,000 metabolic peaks and annotating more than 800 metabolites at the single-cell level. We demonstrated accurate cell type and subtype annotation using single-cell metabolomics and unveiled distinct metabolic states and heterogeneity of hepatocytes during aging.

Friday 28th February – 9h00-9h30

Chemical exposures and microbiome derived bile acids during early life: insights into the progression to islet autoimmunity

Matej ORESIC

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Professor of Biochemistry (Metabolomics). Turku Bioscience Centre and Department of Life Technologies, University of Turku, Turku, Finland

Recent studies suggest that gut microorganisms produce a wide range of bile acid conjugates, commonly referred to as microbially conjugated bile acids (MCBAs). Regulation and health impacts of MCBAs are poorly understood, although chemical exposures to per- and polyfluoroalkyl substances (PFAS) are known to interfere with microbial bile acid metabolism. In a longitudinal birth cohort setting, we studied MCBA trajectories in early childhood as well as investigated their link to islet autoimmunity, an early sign of progression to type 1 diabetes (T1D). We developed a targeted metabolomics method to profile 110 MCBAs in 303 stool samples from 74 children, collected longitudinally (3-36 months of age). Participants included those developing single (P1Ab) or multiple (P2Ab) islet autoantibodies in the follow-up, and controls (CTR) who remained islet autoantibody-negative. We found that MCBAs have distinct age-dependent trajectories which are dysregulated preceding islet autoimmunity. Stool MCBA levels associated with gut microbiome composition and impacted the innate (LPS-induced signaling) and adaptive immune responses including Th17 and Treg cell differentiation, suggesting MCBAs are potential modulators of immune system development and T1D risk.

Friday 28th February – 9h30-10h00

Metabolomics application for quality improvement of important agricultural and food products.

Sastia Prama PUTRI

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Metabolomics is the study of global quantitative assessment of metabolites in a biological system. Metabolites are the result of the interaction of the system's genome with its environment and are not merely the end product of gene expression but also form part of the regulatory system in an integrated manner. The exhaustive profiling of metabolites is an advantageous feature for analysis of various kinds of organisms including microbes and plants compared to the conventional analysis that only targets specific compounds. The capacity of metabolomics to measure metabolites as well as to identify food components favor its usefulness for detection of adulterated crops or food products as well as for food quality improvement. Our group has pioneered the application of metabolomics for quality assessment of various important tropical food and crops. Here, a novel approach to identify underexplored compounds having beneficial effects to human health through an integration of nutritional epidemiology and food metabolomics will be discussed.

Friday 28th February – 10h00-10h30

Bridging the Gap: Standardizing Metabolomics for Routine Clinical Use

Elie FUX

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Effective translation of lipidomics and metabolomics into routine clinical laboratories requires a robust standardization approach to ensure harmonization and reliability of data. Clinical laboratory professionals and the IVD industry have long emphasized the importance of standardization to ensure consistent patient results across different times and locations. This involves assigning calibrator values that guarantee uniform test outcomes across various reagent-calibrator-analyzer combinations by aligning them with a specific reference system.

For lipidomics and metabolomics, adopting a similar standardization approach will offer significant benefits. These technologies provide invaluable insights into a patient's metabolic state, essential for accurate medical interventions and prognosis. However, without standardization, data generated may be inconsistent and incomparable.

In IVD applications, standardization includes ensuring traceability of results to higher-order references, agreeing on measurement units, and providing reliable reference materials. Mass spectrometry (MS) is the workhorse of many lipidomics and metabolomics assays and needs to be carefully calibrated and standardized to ensure consistent and reliable data across laboratories and over time.

This presentation will cover fundamental standardization principles as applied to IVD assays, illustrating how these processes can benefit metabolomics. Key aspects include developing and validating reference measurement procedures (RMPs), assigning SI-traceable values to calibrators through certified reference materials, and systematically assessing measurement uncertainty. Such standardization will enhance the comparability and reliability of metabolomics data, supporting personalized and precision medicine with more accurate diagnoses, therapeutic interventions, and improved patient management.

By highlighting the importance and methodology of standardization used in the IVD industry, this presentation aims to inspire the metabolomics community to adopt similar approaches, ensuring consistent and reliable data for clinical applications.

Friday 28th February – 10h30-11h00

CLIG Interlaboratory Study on the Harmonization of Lipid Concentrations in Human Plasma

Ondřej PETERKA

University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Pardubice, Czech Republic

Lipidomics is one of the most rapidly growing omics field today because many researchers have recognized the importance of lipidomic data in their research. However, there are still some limitations complicating interlaboratory studies, such as the lack of standardized protocols for preanalytical part, sample preparation, selection of internal standards (IS), and data processing, which leads to slightly different values of molar concentrations for identical samples. The new interlaboratory study on the harmonization of lipid concentrations in human plasma was opened for all members of the Clinical Lipidomics Interest Group (CLIG) in the International Lipidomic Society (ILS). Phase I is based on the publicly available data on lipid concentration in human blood samples using various IS mixtures, which allowed to select the overlapping lipid species for the next phases. In Phase II, instructions for measurements of molar concentrations of 157 lipid species from 11 lipid subclasses were prepared for individual workflows based on mass spectrometry (MS), liquid chromatography/MS (LC/MS), or supercritical fluid chromatography/MS (SFC/MS). All groups obtained 4 human plasma samples (NIST SRM 1950, Diabetic, High triacylglycerol, and Young African American) and centrally prepared extract of NIST SRM 1950 provided by NIST and the unique IS mixture that included 37 IS from 11 lipid subclasses prepared by Avanti Polar Lipids. The goal of this interlaboratory ring trial is to harmonize molar concentrations reported by individual laboratories using different workflows. 24 participated groups from four continents reported 31 data sets for four basic lipidomic workflows: a/ lipid species separation using reversed-phase (RP)-LC/MS, b/ direct infusion MS, c) lipid class separation using hydrophilic interaction liquid chromatography (HILIC), and d) lipid class separation using SFC. The effects of various parameters on the determined concentrations are studied. The centrally prepared extract (unified double Folch protocol for all laboratories) was used for the comparison of individual workflows and IS to eliminate potential errors in the sample preparation by individual laboratories. The lipid extraction was performed using the double Folch extraction protocol by all participating laboratories and was compared with the centrally prepared extract and optionally with their own chloroform-based protocols, MTBE-based protocols, or protein precipitation. The results for some sphingolipid and phospholipid classes are highly consistent among individual laboratories, but there are also lipid classes with significant differences, especially for cholesteryl esters, where the in-source fragmentation and the selection of inappropriate IS could seriously complicate the quantitation of individual lipid species. The results were compared with the lipid concentrations in human plasma reported in the previous ring trials. In phase III, the participants can revise their issues in the methodology to determine the consensus values for 4 human plasma samples. This interlaboratory study allows the visualization of various effects, which can be generalized and used for future improvements in the experimental design of large-scale, multi-laboratory studies.

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Friday 28th February – 11h00-11h30

Standardized description of LC-MS/MS measurement procedures

Michael VOGESER

Institute of Laboratory Medicine, LMU University Hospital, LMU Munich, Germany

Quantitative biomedical LC-MS/MS measurement procedure are highly complex and involve a large number of different variables. The description of the respective procedures in scientific publications or in the manufacturers' technical notes is usually insufficient when it comes to implementation in different environments. Therefore, we propose a highly standardized description of LC-MS/MS methods. In doing so, we distinguish between truly fundamental features of a procedure (e.g., ionization mode and mass transitions) and contrast these with features that can be described in an individual implementation but can hardly be standardized in the longer term (e.g. the brand of an instrument, column or solvent). Such a largely abstracting approach to the description of measurement procedures seems necessary to us in order to standardize procedures over time and space in the long term, especially for epidemiological research (see Clin Mass Spectrom. 2019 Apr 25;13:36-38. doi: 10.1016/j.clinms.2019.04.003.).

Friday 28th February – 11h30-12h00

TRAVEL AWARD

Development of a Quantitative MRM assay from Skin Swabs Rich in Sebum to Diagnose Parkinson's Disease

Minhui ZHU

University of Manchester, UK

Parkinson's Disease (PD) is the second most common neurodegenerative disorder worldwide, for which there is no diagnostic test so far but relying on the manifestation of motor symptoms. Skin swab sampling offers a non-invasive and accessible approach for biomarker discovery and diagnostics, enabling at-home collection, ambient storage, and analysis via mass spectrometry. We have recently shown that swabbing skin areas rich in sebum with both gauze and Q-tips can provide sufficient material to allow classification of PD and controls 1,2. This study first evaluates the robustness and quantifiability of sebum components from skin swabs using mass spectrometry, establishes a reliable normalization framework, and explores lipid profiles for biomarker discovery. We have utilised QToF instruments for discovery (Synapt G2-S and Select Series Cyclic, Waters Corp.) and triple quadrupoles (Xevo TQ-S and TQ-XS, Waters Corp.) for targeted work. In all cases Acquity UPLC I-class (Waters Corp.) was employed for chromatographic separation.Robust linearity was demonstrated for both spiked deuterated lipid standards and endogenous lipids with the square of correlation coefficient > 0.99 across most of them in different ranges. Matrix effects, including both signal suppression and enhancement, were mitigated using targeted MRM analysis, which improved precision and reduced interference in lipid-rich matrix. Endogenous triglyceride (TG) pairs, such as TG 46:1 and TG 48:1, were quantified with MRM assay and identified as robust normalization references (CV < 15%) with stable ratio between subjects, enabling reliable quantification across samples.Based on these findings, we proceed to explore disease-specific biomarkers, lipid profiles from quality control (QC) samples for PD and healthy controls, separately, were compared. A total of 300 samples (200 PD and 100 controls) were assessed, revealing significant differences in lipid profiles. From these, the top 50 features were identified for further characterization and development of MRM methods. These features hold promise for distinguishing between disease and health states, supporting clinical diagnostics for PD.This work demonstrates the feasibility of skin swab sebum sampling for PD diagnostic applications, combining robust quantification with the identification of disease-specific signatures, proving its potential for advancing accessible and reliable diagnostics.

References: (1) Sinclair, E.; Trivedi, D. K.; Sarkar, D.; Walton-Doyle, C.; Milne, J.; Kunath, T.; Rijs, A. M.; De Bie, R. M. A.; Goodacre, R.; Silverdale, M.; Barran, P. Metabolomics of Sebum Reveals Lipid Dysregulation in Parkinson's Disease. Nat. Commun. 2021, 12 (1), 1592. https://doi.org/10.1038/s41467-021-21669-4.(2) Trivedi, D. K.; Sinclair, E.; Xu, Y.; Sarkar, D.; Walton-Doyle, C.; Liscio, C.; Banks, P.; Milne, J.; Silverdale, M.; Kunath, T.; Goodacre, R.; Barran, P. Discovery of Volatile Biomarkers of Parkinson's Disease from Sebum. ACS Cent. Sci. 2019, 5 (4), 599–606. https://doi.org/10.1021/acscentsci.8b00879.

Friday 28th February – 13h30-13h45

High throughput RPLC-MS/MS lipidomics, challenges and opportunities

Alaa OTHMAN

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High-throughput molecular profiling is critical for studying biological heterogeneity, particularly in single-cell omics and personalized medicine. Mass spectrometry-based lipidomics is the gold standard for lipid profiling, with reverse-phase liquid chromatography coupled to tandem mass spectrometry (RPLC-MS^2) offering key advantages such as isomer separation and reduced ion suppression. However, conventional methods (20–30 min per run) limit large-scale studies. Achieving high-throughput RPLC-MS2 lipidomics requires addressing three challenges: (i) developing robust, fast LC-MS methods that retain chromatographic advantages, (ii) optimizing electrospray ionization (ESI) to match the fast LC methods for high ionization efficiency with minimal in-source fragmentation, and (iii) accelerating MS2 acquisition (speed and efficiency) for reliable lipid identification via diagnostic fragment rule-based matching.

To address this, we first optimized gradient lengths from 30 to 1.5 minutes using the Vanquish Binary Horizon LC system with ACQUITY Premier C_{18} BEH columns (1.7 µm, 2.1 × 30–150 mm) at 0.3–1.5 mL/min flow rates. Mobile phases included IPA: ACN (9:1) and ACN: H_2O (6:4) with various modifiers using isotopically labeled standards and different lipid extracts (NIST SRM 1950 and tissue extracts). Remarkably, all gradients demonstrated excellent reproducibility retention time reproducibility (CV = 1–2%, 0.1–0.8 s) and reproducible backpressure (CV = 0.1–0.5%). Carryover was below 1% even after loading 10 pmol of class-specific standards, with peak widths of 1.5–2 seconds at baseline at the shortest gradients, reaching a peak capacity of 300-500 in less than 2 minutes.

Secondly, to optimize electrospray ionization (ESI) for high sensitivity and minimal in-source fragmentation, we tested the S-Lens-based Orbitrap Exploris 240, ion funnel-based Orbitrap Exploris 480, and Orbitrap ASTRAL, all equipped with the NGS HESI probe. Optimized tuning parameters achieved a linear dynamic range from 1 fmol to 10 pmol on-column with minimal to acceptable in-source fragmentation across multiple lipid classes.

Based on the LC and ionization optimal methods, we finally evaluated different MS2 acquisition modes and parameters on the three mass spectrometers first in DDA(TopN or fixed cycle time) to fit the cycle time constraints (200-250 msec). In the shortest gradients, 200-500 unique lipids could be identified reüroidcilibty on the sum composition level, with Obrpitrap ASTRAL having the highest number of annotated lipids on the shortest gradients. We then explored whether the MS2 scan time could be more efficiently used on the Orbitrap ASTRAL by combining DDA and PRM methods, significantly improving the annotation rates.

In summary, we systematically addressed several challenges to achieving an ultrafast RPLC-MS2 lipidomics method, which presents a unique opportunity for large-scale lipidomics studies.

Friday 28th February – 13h45-14h15

Advancements in Lipidomics Platform Based on Supercritical Fluid Chromatography/Mass Spectrometry

Takeshi BAMBA

Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

With the advancement of chromatographic separation techniques utilizing HPLC and mass spectrometry, the comprehensive measurement of lipid molecular species has become feasible. Among these techniques, reversed-phase liquid chromatography coupled with mass spectrometry (RP-LC/MS) is widely employed due to the hydrophobic interactions between octadecylsilyl (ODS) supports and lipid molecules, enabling the effective separation of individual lipids, including isomers. However, this approach poses challenges for quantitative analysis due to matrix effects in mass spectrometry, which are difficult to correct accurately. In contrast, normal-phase liquid chromatography (NPLC) and hydrophilic interaction liquid chromatography (HILIC) facilitate the separation of lipid classes by targeting the headgroup structures of lipids, such as glycerophospholipids and glycolipids. By introducing internal standards for each lipid class into the sample extract, accurate quantification becomes feasible. Nevertheless, both NPLC and HILIC encounter limitations in achieving comprehensive separation and retention time reproducibility. Additionally, the highly hydrophobic mobile phase used in NPLC makes it less suitable for mass spectrometry applications. Supercritical fluid chromatography (SFC) has emerged as a promising method for lipidome analysis, offering high-resolution separation of lipid classes with a normal-phase column and a mobile phase compatible with sensitive mass spectrometry. Compared to conventional NPLC and HILIC, SFC provides the capability to separate a broader range of lipid classes with higher speed and efficiency. Our research group has been developing a novel lipidome analysis method leveraging the unique separation characteristics of SFC. Specially, we have established a quantitative lipidome analysis system that combines an SFC platform utilizing a diethylamine (DEA) column with a triple quadrupole mass spectrometer (QqQ-MS). This system achieves high-resolution separation of 22 lipid classes in less than 20 minutes. To further expand lipid class coverage, we are currently screening new columns for SFC separation. Additionally, we are developing an automated preprocessing system and software for generating MRM transitions and quantitative data analysis based on in silico databases. These efforts aim to establish a comprehensive lipid analysis platform. In the presentation, I will share the latest outcomes of our research and development.

Visualizing cellular lipid distributions by AP-MALDI mass spectrometry imaging

Sven HEILES

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This contribution will outline emerging perspectives of atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry imaging (AP-MALDI MSI) platforms to study lipid alterations between individual cells. In this talk, I will detail the methodological improvements of AP-MALDI MSI that enable single cell investigations and I will showcase our most recent single cell studies. In a first proof-of-concept study human microglia cell lines, cells that are part of the nervous system with multiple functions such as synapse maintenance and defending against bacterial infections, will be investigated. By employing high lateral resolution, MSI is capable to statistically distinguish four naïve and four lipopolysaccharide (LPS)-stimulated cell lines based on lipid profiles in untargeted investigations. The single-cell resolution of the methodology additionally enables to visualize the molecular response of individual cells upon LPS stimulation. We were able to identify individual cells with elevated triacylglycerol (TG) levels and show that the number of these TG-enriched cells increased with LPS stimulation as a hallmark for a proinflammatory phenotype. Additionally, local abundance alterations of phosphatidylinositols (PIs) are observed that indicate a cell-specific regulation of the PI metabolism motivating detailed future investigations. In a second study, the possibility to correlate cellular lipid compositions to individual cell states will be investigated. First results for cancer cell lines indicate that cellular lipid content can not only change based on cell culture confluence but can also depend on the cell cycle state and differences between cell cytoplasm and nuclei are observed.

Friday 28th February – 14h45-15h15

Tracing lipid metabolism in Lipostar 2: from stable isotopes to w-alkyne-containing species

Laura GORACCI

Organic Chemsistry, DCBB, University of Perugia, Italy

Lipids play a crucial role in maintaining cellular homeostasis, serving as structural elements of membranes, energy stores, and regulators of signaling pathways. To fully understand the role of lipid metabolism in health and disease, it is vital to explore the dynamic nature of the lipidome. Although progress in lipidomics has yielded detailed profiles of lipid species, these analyses often fall short in providing temporal insights and pathway-level resolution.

Typically, analysis of metabolic flux requires the use of stable isotope tracers.[1] More recently, recent progress in click chemistry has enabled novel approaches for tracing lipid metabolism and detection of low abundant metabolites.[2,3] Thus, computational tools to decipher data from stable isotope labelling studies as well as from click-chemistry based derivatization are needed. Here we will describe the in-silico strategies we developed in Lipostar 2 to automatically detect modified lipids. Firstly, the workflows designed to monitor stable-isotope labelled species will be described. Secondly, we will present the new tool for the automatic detection and identification of "clicked" lipids generated by derivatization of w-alkyne-containing lipids with an azido-quaternary ammonium reporter, C171 (previously proposed by Thiele and colleagues for direct injection mass spectrometry),[3] when reverse phase liquid chromatography is coupled with mass spectrometry. This approach could also be extended to other azido-based reporters and derivatization agents.

[1] X. Han, R. W. Gross. J. Lipid Res., 63, 2, 100164
[2] C. Thiele, C. Papan, D. Hoelper, K. Kusserow, A. Gaebler, M. Schoene, K. Piotrowitz, D. Lohmann, J. Spandl, A. Stevanovic, A. Shevchenko, L. Kuerschner, ACS Chem. Biol. 2012, 7, 2004–2011.
[3] C. Thiele, K. Wunderling, P. Leyendecker, Nat. Methods 2019, 16, 1123–1130.

Friday 28th February – 15h45-16h15

Enabling FAIR Computational Comparative Lipidomics with LipidCompass

Nils HOFFMANN

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Lipidomics research has surged over the past decade, driven by advancements in high-throughput metabolomics technologies. This growth necessitates a FAIR, accessible resource for comparative lipidomics, embodied by LipidCompass. Our platform simplifies the analysis of lipidomics data from diverse perspectives, organized by the lipid shorthand nomenclature and cross linking data to LIPID MAPS, SwissLipids, ChEBI and other resources. LipidCompass accepts data from tools supporting mzTab-M, standardizing terminology with controlled vocabularies like NCIT and PSI-MS. Goslin integration ensures usage of the latest lipidomics shorthand nomenclature, thereby simplifying accurate mapping and integration with external databases. The platform's comprehensive exploration and visualization tools empower researchers to detect sample differences and perform large-scale comparative analyses across studies. As a central component of the Lipidomics Informatics for Life-Science (LIFS) project, LipidCompass will connect with services like LipidXplorer and LipidSpace. Collaborations with the International Lipidomics Society will enhance standardization and support, incorporating the lipidomics checklist. Results from public clinical lipidomics trials are available for interactive comparative analysis and new data can be submitted for review and curation, solidifying LipidCompass as a pivotal resource in computational comparative lipidomics.

Friday 28th February – 16h15-16h35

Status and Perspectives of the Lipidomics Reporting Checklist

Dominik KOPCZYNSKI

University of Vienna, Austria

The number of lipidomics experiments was rapidly growing within the recent 10 years and so did the frequency of published articles in scientific journals describing these experiments. However, many publications do not provide complete or transparent set of information to either comprehend, reproduce, or even reuse the data or results from these experiments. These reports may lack a controlled vocabulary, a clear structure, or even no access to the raw data. Another issue emerges, when non-lipidomics specialized journals send articles for peer review to non-domain experts for a sophisticated assessment of the described methods. In a community-wide effort, we implemented an interactive lipidomics reporting checklist [1, 2] to overcome these limitations. The Lipidomics Standards Initiative (LSI) compiled guidelines for minimum reporting standards with the assistance of the lipidomics community of the International Lipidomics Society (ILS). The interactive checklist is a web-based questionnaire. Users can select several lipidomics methods such as *direct infusion* or *separation* to customize their report. The checklist guides the user through eight sections, i.e., *Overall study design*, *Preanalytics*, *Lipid extraction, Analytical platform, Lipid Identification & Quantification, Quality control, Method qualification and validation, and Reporting.* Each section demands a minimum set of information in order to continue. The resulting pdf report can further be, e.g., attached as supplementary to an article, and/or published separately via the Zenodo platform. This system intends to support analytical chemists to ensure that their provided data are of highest level as well as educate young scientists in this field to learn the crucial requirements within this discipline. The questions in the checklist provide profound explanations and link to literature for further details. The checklist is free of charge and can be used without any limitations [3].

[1] MacDonald, J.G., Ejsing, C.S., Kopczynski, D. et al. (2022). *Nature Metabolism*. **4**, 1086-1088.
[2] Kopczynski, D. et al. (2024). *Journal of Lipid Research*. **65**, 100621.
[3] https://lipidomicstandards.org/reporting_checklist/

Abstracts of Poster Presentations

List of Poster Presentations (Alphabetical by presenting author's surname)

Lightning talks: Wednesday 26th February – 13h45-14h15

Metabolomics Profiles in EBV-Positive B-Cell Lymphomas

Abdullah ALFAIFI[1,2], Timothy J. Garrett[3], Ishtiaq Qadri[1]

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2 Fayfa General Hospital, Jazan Health Cluster, Jazan, Saudi Arabia
3 Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA

B-cell lymphoma accounts for 7.5% of Saudi cancers and ranks third. The prevalence of lymphoma in Saudi Arabia is significant, which may support the presence of certain etiological factors such as Epstein-Barr virus (EBV). EBV was the first oncovirus associated with human cancer. Along with its other well-known effects, recent studies have shown that EBV causes major changes in cell metabolism, which may cause/contribute to the proliferation of cancerous cells in humans. This study aimed to identify and characterize the metabolomics profile of EBV-positive B-lymphomas.

Materials and Methods

We conducted metabolomics analyses on plasma samples from 18 patients recently diagnosed with B-cell lymphoma, including 12 EBV-positive patients. The cross-sectional study was conducted from September 2022 and September 2023, at Prince Mohamed Bin Nasser Hospital (PMBNH) in Saudi Arabia. We employed ultra-high-performance liquid chromatography and a Thermo Q-Exactive Orbitrap mass spectrometer to investigate the plasma samples at the Southeast Center for Integrated Metabolomics at the University of Florida. MetaboAnalyst 6.0 was used to carry out the exhaustive quality of metabolomics assessment. After the successful quality parameters, 309 unique known metabolites were identified out of a total 7,676.

Results

Several critical important metabolites were identified including adipic acid, glycoursodeoxycholic acid, 2-hydroxyglutarate, monobutylphthalate, hippuric acid, hydroxyhippuric acid, and 2-hydroxyglutarate, These were distinct in EBV-positive BCL. Furthermore, EBV elevates LDH-A and lactate levels, resulting in the acidification of the extracellular microenvironment. This may provide new insights into the pathophysiology and therapy of EBV-induced lymphoma.

Conclusion

These metabolic profile findings aid in the clinical field by enabling earlier prediction or detection of B-cell lymphoma. EBV infection also induces changes in the gut microbiota's metabolic profiles. Therefore, further research is required to comprehend the gut microbiota and metabolic alterations induced by Epstein-Barr virus infection. Additionally, innovations in metabolism will lead to novel remedies like targeted therapy in precision medicine, prompting further studies.

Key words: Epstein-Barr virus; B-cell lymphomas; Metabolomics, Metabolic Biomarkers; Diagnosis; and Prognosis

Integrative plasma and fecal metabolomics identify functional metabolites in adenoma-colorectal cancer progression and as early diagnostic biomarkers

Amy CHENG[1]

1 Biotree Metabolomics Technology Research Center

Microbial metabolites are closely associated with colorectal cancer (CRC), one of the most common cancer types and the leading cause of cancer deaths worldwide. Previous studies have reported the enrichment of pro-tumorigenic gut metabolites including trimethylamine N-oxide (TMAO) and deoxycholic acid in patients with CRC. 1–3 In contrast, short-chain fatty acids and indole derivatives are depleted in CRC patients, which are known to exhibit antitumor functions and capable of improving cancer treatment efficacy. 4,5 Meanwhile, gut metabolites can be absorbed into the circulation to modulate host immunity. Indeed, metabolites in the bloodstream have received increasing attention. These blood microbial metabolites are significantly altered in both CRC and colorectal adenoma (CRA) patients, 6,7 suggesting their potential contribution to colorectal tumorigenesis. However, it is unclear how fecal and blood metabolomes are altered in the adenoma-carcinoma sequence, and further works are needed to depict whether metabolites in circulation could impact CRC progression. Colonoscopy remains the standard procedure for CRC diagnosis, yet its invasiveness and high operating cost have restricted its application for large-scale screening. 8 Given the advance in metabolomic profiling technology, multiple studies have shown the feasibility of detecting fecal metabolites as noninvasive diagnosis in CRC. For example, we previously reported that a panel of combined fecal metabolites and bacteria biomarkers could discriminate patients with different stages of CRC. 9 Meanwhile, the use of blood metabolites for CRC diagnosis is also increasingly acknowledged in studies with relatively small sample size. 6 Notably, there is still lack of investigation comparing the diagnostic performance between fecal and blood metabolites. Here, we collected samples from 1,251 individuals of CRC patients, CRA patients, and healthy subjects including 373 fecal and plasma samples from the discovery cohort, and 878 plasma samples from three independent validation cohorts. By metabolomic profiling, we identified fecal and plasma metabolite signatures across NC, CRA, and CRC respectively, especially for oleic acid enrichment and allocholic acid depletion along the adenoma-carcinoma sequence. The role of these key metabolites was confirmed using CRC cell lines, patient derived organoids (PDOs), and mouse models, with subsequent investigations on their underlying mechanisms. Moreover, we compared the clinical potential of fecal and plasma metabolites, while the latter had better diagnostic performance. Upon confirmation by targeted metabolomics and validation cohorts, we developed a diagnostic panel with 17 plasma metabolite biomarkers that accurately discriminated patients with different stages of CRC.

Fully Automated Sample Preparation and Untargeted 2D-LC-MassSpectrometry Workflow for Glycerophospholipid Analysis in PlasmaRelative

Chiew Mei CHONG[1], Laura G. Gonzalez Iglesias[1], Guenter Boehm[2], Renzo Picenoni[2], Gérard Hopfgartner[2]

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Analyzing glycerophospholipids (GPLs) in chronic kidney disease of unknown etiology (CKDu) presents significant challenges due to the vast diversity of these molecules. Metabolomics analysis using liquid chromatography-mass spectrometry (LC-MS) has already revealed distinct differences in urine samples between control subjects and patients at various stages of chronic kidney disease (CKD). This study employs two primary approaches: relative quantification of glycerophospholipids and untargeted lipid profile analysis, alongside extensive characterization of lipid species. In our methodology, we utilized an adjusted Matyash method for automated lipid extraction, which involves a biphasic extraction process using methanol and methyl tert-butyl ether (MTBE). During the extraction, a standard mix from SPLASH Lipidomix (Avanti) is added to ensure consistency and accuracy. The lipids are then partitioned into the organic phase and collected for subsequent analysis by LC-MS. We developed an integrated workflow that combines hydrophilic interaction liquid chromatography (HILIC) with sequential window acquisition of all theoretical mass spectra (SWATH-MS) for quantitative analysis. For qualitative characterization of complex lipidomes, particularly glycerophospholipids, we employed a two-dimensional liquid chromatography (2D-LC) approach, combining HILIC with reversed-phase liquid chromatography (RPLC) and data-dependent acquisition (DDA) enhanced with electron-activated dissociation (EAD) and multiple reaction monitoring high-resolution (MRMHR) EAD. Robotic automation played a crucial role in streamlining sample preparation and enabling efficient fractionation for analysis. The use of EAD was particularly valuable for determining the positions of double bonds within lipid molecules, especially when used in conjunction with 2D-LC. This comprehensive and powerful workflow not only enhances the accuracy and depth of lipid analysis but also provides a robust platform for extending these techniques to other lipid categories.

Illuminating host-mycobacterial interactions with functional genomic screening

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Existing antibiotics are inadequate to defeat tuberculosis (TB), a leading cause of death worldwide. We sought potential targets for host-directed therapies (HDTs) by investigating the host immune response to mycobacterial infection. High-throughput CRISPR knockout and CRISPR interference (CRISPRi) screens were used to identify perturbations that improve the survival of human phagocytic cells infected with Mycobacterium bovis BCG (Bacillus Calmette-Guérin), as a proxy for Mycobacterium tuberculosis (Mtb). Many of these perturbations could constrain the growth of intracellular mycobacteria. We identified over 100 genes associated with diverse biological pathways as potential HDT targets. We proved that the inhibition of hydrocarbon receptor signaling pathway blocks bacterial pathogenesis via oxylipin metabolism and its inhibitor CH223191 is a potential HDT for TB by enhancing human macrophage survival and limiting the intracellular growth of Mtb. In summary, high-throughput functional genomic screens, by elucidating highly complex host-pathogen interactions, can serve to identify HDTs to potentially improve TB treatment.

Semi-quantification of triacylglycerols with resolved fatty acid composition using a targeted MS3 approach

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Triacylglycerols (TGs) are the most abundant lipids in human plasma made up of a glycerol backbone esterified with three fatty acids. The measure of their total content in plasma has been a key clinical biomarker of cardiometabolic diseases, however, this lipid class comprises a wide variety of chemically diverse species. Their individual measurement with higher selectivity would lead to a better understanding of their role in health and disease. Triacylglycerol profiling and structural characterization is typically performed by LC-HRMS followed by their quantification using a Selected Reaction Monitoring (SRM)-based approach. In SRM-based methods, TG transitions correspond to a molecular ion ammonium adduct, as a precursor, with the neutral loss of one of the three fatty acids residues (and the ammonium ion) as a product ion. Further fragmentation of these product ions allows for the determination of the complete fatty acid composition of TGs. In this work, LipidSearch software was used to match the experimentally acquired MS2 and MS3 spectra of potential TG species detected in NIST plasma against its in silico predicted spectral library. More than 100 TAGs annotated with high confidence (“grade A”) were the fed into a targeted MS3 assay on the new hybrid nominal mass instrument for their routine quantification in human plasma. Thus, a targeted MS3 method enabled the quantification of individual TG species with high selectivity resolving their specific fatty acid composition.

Overcoming Speed and Sensitivity Barriers in Lipidomics with the Orbitrap Astral Mass Spectrometer

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Introduction

Untargeted lipidomics offers a powerful tool for detailed characterization of a sample's lipid profile. However, limitations in sensitivity and scan speed can hamper in-depth and confident analysis. This study investigates the potential of a mass spectrometer with MS2 scan speeds of up to 200 Hz to improve depth of coverage, confident annotation, and analysis throughput for high-resolution lipidomics studies.

Methods

Lipids were extracted from diverse sources including liver, plasma, cells and plants using the Bligh and Dyer method. Separations were achieved on a C30 column using a Thermo Scientific™ Vanquish™ Horizon UHPLC system and analyzed on a Orbitrap™ Astral™ mass spectrometer. MS1 scans were acquired in the Orbitrap analyzer at 240,000 resolution and fast, sensitive MS2 was acquired in the Astral analyzer at 80,000 resolution to facilitate in-depth fragmentation and confident annotation of lipid species. Data analysis was performed using various software platforms.

Preliminary Data

Several parameters were optimized to ensure efficient transmission and detection in the Astral analyzer. This included radio frequency (RF) lens voltage, injection time, automatic gain control (AGC) target and collision energy. Notably, low AGC targets allowed the instrument to operate at its maximum speed of 200 Hz while maintaining spectral quality. This resulted in a significant number of MS2 spectra (over 250,000) within a 30-minute run. Further analysis with various sample types confirmed the instrument's reliability and robustness, indicating its potential for advancing lipidomics research.

Novel Component

This study presents a high-throughput lipidomics workflow that achieves deep coverage and confident annotation in a single injection.

Deciphering Spatial Variation in Lipid Profiles of X-Ray Irradiated Single Cells using nLC-MS lipidomics

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Lipidomics is of significant interest in cell biology because cells contain lipid droplets, which play an important role in managing cell stress in response to infection, irradiation and drug treatment. Herein, we describe an optimized workflow for single cell lipidomics utilizing HRAM and ddMS2 fragmentation of lipids from a single cell in both positive and negative mode on the Orbitrap Exploris 240 using nano-LC based separation. It is known that cells irradiated by photons can instigate bystander effects in neighboring cells leading to DNA damage, apoptosis and even secondary cancers. After live spatial sampling of irradiated cells and their unirradiated neighbors, we apply this workflow to characterize the lipidomic impact of X-ray radiation and its bystander effect.Adherent PANC-1 cells were irradiated with X-Ray (6 Gy) prior to sampling using an irradiation cabinet (Xstrahl), select areas of the dish were shielded with lead. Following radiation, live cell imaging and spatial sampling of single cells was performed on a Single Cellome™ SS2000. For nanoLC-MS, lipids were separated on a Thermo Scientific PepMap™ - column connected to a Thermo Scientific Vanquish™ Neo UHPLC system. Data were acquired on a Thermo Scientific Orbitrap Exploris™ 240 Mass Spectrometer, both in positive as well as negative mode ionization using polarity switching. Thermo Scientific LipidSearch™ 5.0 and Compound Discoverer™ 3.3 software was used for lipid annotation and data processing. Lipid standards and bovine liver lipid extracts were analyzed using nano-LC-MS. The flow rates and run time for the nLC were optimized for greater separation and resolution of the eluted lipid species. Dilution series of cells were run to benchmark the reproducibility, robustness and the sensitivity of the workflow.

For sampling single cells, the Single Cellome™ System SS2000 (Yogokawa) was used. The system utilizes high-resolution images captured with a confocal microscope to automatically and accurately collect single cells. For cells irradiated with X-Ray, the system was used to collect single cells spatially, based on their distance from irradiated areas. The cells collected were immediately transferred to dry ice, before transfer to LC-MS vials using the starting mobile phase spiked with internal standard (EquiSPLASH©, 16 ng/mL, Avanti). The SS2000 can be used to isolate single adherent cells under incubation, preserving metabolic activity and reducing the need to flow cells prior to sampling.Single cells lipid extracts were run using the optimized nLC-MS workflow run with polarity switching to yield comprehensive lipid species coverage. Differences in cellular lipid profiles were observed based on the spatial location of the cells. Major differences were seen between cells on the periphery and cells in the middle of the plate.

Heureka Labs

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At Heureka Labs, we are building research tools and systems to help scientists make more efficient and impactful discoveries. Our approach combines automated scientific workflows with deep domain knowledge to actively drive research projects from hypothesis to publication. Unlike traditional tools that simply respond to queries or follow templates, our platform proactively identifies research opportunities, designs experiments, analyzes results, and generates insights, using validated data to support each step of the research process. At the core of our offering is a network of specialized scientific systems and autonomous agents that work together to tackle complex research challenges – from data-driven scientific exploration and literature synthesis to experimental design optimization and automated multi-omic analysis. These systems leverage our proprietary knowledge base of experimental protocols and outcomes – including negative results – to make informed decisions and navigate away from redundant research paths. Heureka is already being used by researchers at biotech companies and leading institutions like NUS, Harvard, Duke, INSERM, and more. Our innovative tools have demonstrated significant improvements in research efficiency and success rates. By streamlining routine analytical and administrative tasks while providing dynamic, personalized strategic scientific guidance, we enable researchers to focus on breakthrough discoveries rather than procedural overhead – accelerating workflows, reducing waste, and maximizing impact.

Towards targeted and quantitative 4D Lipidomics - From PASEF to prm-PASEF

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Bruker

Parallel accumulation–serial fragmentation (PASEF) in combination with trapped ion mobility spectrometry (TIMS) enables mobility-resolved fragmentation and a higher number of targeted precursors per time unit compared to conventional MS/MS experiments. PASEF is proven for 4D-Lipidomics profiling experiments and screening of unknown samples. High confidence lipid annotations derived from 4D-Lipidomics data in MetaboScape can be utilized as basis for targeted screening and quantitation methods. For targeted lipidomics, the highest data quality could be achieved using a parallel reaction monitoring (prm-PASEF) data acquisition(1) offering potential for clinical lipidomics applications(2). Yet, the creation of prm-PASEF methods requires concerted acquisition and data processing methods. We present the integration of MetaboScape and TASQ software to offer a seamless workflow for high confidence in lipid quantitation.

Methods: For benchmarking, phospholipids of the well-characterized standard reference plasma from the National Institute of Standards & Technology (NIST SRM 1950) was quantified by HILIC-TIMS-MS/MS using prm-PASEF acquisition with timsControl 5.0 on a timsTOF fleX instrument. For lipid quantification, coelution of the isotope-labeled internal standards with the corresponding lipid class was achieved by hydrophilic interaction liquid chromatography (HILIC). MetaboScape 2024b conducts untargeted analysis on the PASEF lipidomics dataset, including data processing and rule-based lipid annotation. Lipids of interest including species-dependent characteristic fragment ions are selected and transferred to TASQ's targeted analysis. TASQ 2024b curates a transition list for prm-PASEF acquisition and subsequent quantitative data analysis.

Preliminary Data: By phospholipid profiling of NIST SRM 1950 plasma lipids, a deep lipidome coverage with high confidence annotation based on retention time, isotope pattern, collisional cross section and fragmentation behavior, i.e. the 4D lipidomics approach, was obtained in MetaboScape. The transfer of the identified lipids including lipid species-dependent fragment ions to the targeted/quantitative software solution TASQ facilitates the seamless generation of a prmPASEF MS/MS transition list and the generation of a TASQ method. MS/MS transitions were used to generate indicative qualifier ions as an additional tool for identification to support reliability in lipid quantitation on species or molecular species level.

Using HILIC-MS/MS, a lipid class separation was achieved which favors quantitation by using one isotope-labeled internal standard per lipid class. However, this coelution also favors isobaric type-II overlaps that result from the natural isotopic pattern of lipid species with an additional double bond ($\Delta m/z$ = 2 Da). Through the additional TIMS dimension, a separation of the isobaric overlap could be achieved, highlighting the benefits of the multidimensional HILIC-TIMS-MS/MS approach for lipid quantitation. For accurate quantification of non-baseline separated overlaps in TIMS, we recommend the use of the M+1 signal for quantitation, which is also implemented in the TASQ software. All in all, the combination of MetaboScape and TASQ software solutions for targeted Lipidomics by HILIC TIMS MS/MS and prmPASEF served for a precise and user-friendly lipid quantitation with high confidence, offering potential for applications in clinical lipidomics.

Novel Aspect: Demonstration of a HILIC-TIMS-MS/MS workflow with high confidence in lipid quantitation by prm-PASEF and combination of MetaboScape and TASQ software.

1) E. Rudt, M. Feldhaus, C. G. Margraf, S. Schlehuber, A. Schubert, S. Heuckeroth, U. Karst, V. Jeck, S. W. Meyer, A. Korf, and H. Hayen, Analytical Chemistry 95 (2023) , 9488-9496.

2) A. Lesur, and G.. "The clinical potential of prm-PASEF mass spectrometry." Expert Review of Proteomics 18 (2021) 75-82.

Mass spectrometry imaging reveals region-specific alterations of brain lipids induced by parkinsonism and L-DOPA-induced dyskinesia

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We employed matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) to comprehensively map different molecular species(spatial omics), such as neurotransmitters, neuropeptides, and lipids in specific brain regions. Using brain samples from an experimental Parkinson'sdisease (PD) model (MPTP, Macaca mulatta) with L-DOPA-induced dyskinesia (LID), we previously observed abnormal elevations of L-DOPA and itsmetabolite, 3-O-methyldopa, in the whole brain of LID animals. This resulted in increased dopamine and downstream metabolites in all brain regions,except putamen and caudate. Furthermore, we found that the abundance of selected neuropeptides was associated with L-DOPA concentrations in theputamen, emphasizing their sensitivity to L-DOPA.In the present study, we conducted extensive imaging of various lipid species, enabling the detection of specific distributions of hydroxylated and non-hydroxylated sulfatide lipids within the same individual brains. Hydroxylated sulfatides were abundant within several basal ganglia brain regions,whereas long-chain hydroxylated sulfatides were depleted in motor-related regions, with non-hydroxylated showing an elevation. When comparingindividuals with LID to those without dyskinesia, we observed a decrease in plasmalogen phosphatidylcholines and an increase in polyunsaturated fatty acid-containing glycerophospholipids specifically in the internal segment of globus pallidus. These changes were significantly correlated with the LIDscores, L-DOPA and dopamine levels within the specific brain regions of the animals. This MALDI-MSI study provides valuable insights into signalingsystem dynamics during PD and its treatment.

LC-MSn analysis of FAHFAs and TG(O)s in breast milk

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Breast milk is recognized as the optimal form of nutrition for infants and studies on their major lipid components are well-documented. Characterizations of minor lipids with anti-inflammatory and anti-diabetic properties such alkyl-diacylglycerols (TG(O)s) (a neutral lipid), and fatty acid esters of hydroxy fatty acids (FAHFAs) (a polar lipid), however, have been lacking. Difficulties in recovering minor lipids from complex matrices are well-recognized, but accurate characterizations of FAHFAs and TG(O)s are further complicated by the need to develop a reproducible method for the extraction of minor lipids with contrasting chemistries from the same sample. This project aims to develop and validate methods for the reliable identification and measurement of FAHFAs and TG(O)s in breast milk using the Orbitrap™ IQ-X™ Tribrid™ Mass Spectrometer. Designed for the analysis of small molecules, a combination of the quadrupole, ion trap, and high resolution Orbitrap mass analyzers are used for data acquisition. Lipid extracts are first obtained by modification of the established Folch method of extraction and solid phase extraction. Semi-automated analysis is then conducted following data acquisition and the development of lipid libraries for the identification of FAHFAs and TG(O)s. Systematic validation of the extraction and analytical methods have been completed using pooled milk samples from the Singapore PREconception Study of long-Term maternal and child Outcomes (S-PRESTO) cohort. These parameters include analysis of calibration curves, determination of the lower limit of quantitation, carryover, selectivity, accuracy, precision, recovery, and matrix effects.

Employing Ballistic Gradients, Vacuum Jacketed Columns and a Novel MRT to Increase Lipidomic Throughput Whilst Maintaining Highly Confident Identifications

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One common theme between all omics application is the ever-increasing size of patient cohorts, driven by the need for identification of novel disease biomarkers and an increase in the power of the studies. Sample throughput becomes a limiting factor. This limitation can be overcome by faster chromatography that maintains narrow peak widths, and this can be achieved through the use of vacuum jacketed columns (VJC) in combination with the Xevo MRT. We applied this approach for lipidomics analysis of a study cohort and we observed that the decrease in gradient time did not impact the ability to sufficiently profile the chromatographic peak due to fast scanning ability of the Xevo MRT, without any impact on mass resolution. Transferring a standard method to VJC led to 3.5x more features identified and a ~37% decrease in solvent consumption. Halving the gradient time while maintaining flow rate identified more features than a longer conventional method while also using ~70% less solvent. The speed, resolution, and sensitivity of the Xevo MRT in combination with VJC-enabled ballistic gradients makes the perfect combination for high-throughput cohort studies.

Simultaneous Quantitation and Discovery (SQUAD) Analysis of Triglycerides in Commercial Vegetable Oils using LC-HRAM-Tribrid platforms

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Vegetable oil is primarily comprised of triglycerides (TAG) and diglycerides (DAG). The analyses of these species are traditionally done by chemical hydrolysis followed by an evaluation of the fatty acid profile by GC-MS. This approach provides an indirect analysis of the TAG and DAG species, and it is not able to provide details into their composition in the vegetable oils. In this study, we applied simultaneous Quantitation and Discovery (SQUAD) method that combines targeted and untargeted workflows for the analyses of lipids in commercial vegetable oils samples using a Thermo Scientific™ Orbitrap™ LC-HRAM-Tribrid mass spectrometer platform. The instrument acquisition versatility allowed simultaneous targeted lipid quantification and unknown lipid characterization with a single HPLC-MS run. Six different types of commercial vegetable oil samples were analyzed using reversed phase chromatography on a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Thermo Scientific™ Orbitrap Ascend™ Mass Spectrometer. A Tribrid-based mass spectrometer equipped with a high-resolution Orbitrap and sensitive linear ion trap mass analyzers which allows for high confidence in untargeted analysis using the orbitrap as well as a high dynamic range tSIM quantitation utilizing the linear ion trap. FullScan-data dependent MSn approach with the combination of HCD MS2 and CID MSn, and UVPD MSn was employed for structural determination of triglyceride species in the vegetable oils. Thermo Scientific™ TraceFinder™ and Compound Discoverer™ software were used for data processing, compound annotation, structural elucidation, and statistically analysis. The results presented herein serve as a proof of principle of using Simultaneous Quantitation and Analysis (SQUAD) method for lipidomic analysis in any biological matrix. In a single LC-MS run, both targeted ion trap data (for targeted quantitation) and high resolution Orbitrap data (for unknown annotation) were collected. The FullScan and MSn data generated by Orbitrap Tribid mass spectrometer with high resolution and sub-ppm mass accuracy enables confident lipid annotation and structural characterization. Data was analyzed using Compound DiscovererTM for unknown characterization and TraceFinderTM for quantitation. Compound DiscovererTM software offers a comprehensive approach for comparative analyses, annotation, and data visualization, allowing for the identification of dozens of lipid species in this study. The ion trap quantitative results showed remarkable sensitivity and a great dynamic range for the lipid standard compounds, with limit of quantitation (LOQ) reaching low ppb levels.

Improving Selectivity of Lipid Mediator Analyses by Coupling Nanoflow Chromatography with the Thermo Scientific Stellar Mass Spectrometer

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Signaling lipids play a central role in biology where they mediate a wide range of cellular processes. One of the emerging roles of lipids over the last decades is as mediators of inflammation, a process that involves biochemical pathways that enable inflamed tissues to return to homeostasis. From an analytical perspective, it is challenging to reliably detect and quantitate these species in biological matrices, as they are chemically unstable, structurally similar, and exist at low concentrations (typically from ppt to ppb). The analysis of lipid mediators is typically done by LC-MS/MS utilizing SRM/MRM methods with triple quadrupole mass spectrometers and high-flow UHPLC systems. Although very sensitive, these analyses are cumbersome, as they require optimization of SRM transitions for each individual lipid prior to their detection. To overcome these challenges, we present herein a sensitive targeted approach utilizing a ThermoTM ScientificTM VanquishTM NeoTM UHPLC system operated in the nanoflow regime (0.5 µL/min) coupled with a ThermoTM ScientificTM StellarTM mass spectrometer equipped with a sensitive dual pressure linear ion trap detector. One of the key benefits of this approach is that the Stellar mass spectrometer uses parallel reaction monitoring (PRM) to monitor many product ions simultaneously and has the capability to perform both HCD and CID fragmentation with fast acquisition speeds (up to 140 Hz). Additionally, it utilizes MSn acquisition to selectively analyze individual isomeric species, and employs an Adaptive RT feature for dynamic retention time adjustments, ensuring reproducible and specific measurements. This approach allowed us to reliably detect and quantitate dozens of lipid species in less than 30 min, using less than 20 µL of solvent per sample. Noteworthy, the analysis of the same panel of lipid mediators using a ThermoTM ScientificTM TSQ AltisTM Plus triple quadrupole mass spectrometer reached similar sensitivity levels, highlighting the great performance of the Stellar platform. Finally, the nanoflow method presented here showed higher sensitivity than an analogous method using an analytical flow regime (0.5 mL/min), showcasing another benefit of this approach. Overall, our preliminary results indicate that the nanoflow-Stellar-MSn method presented herein is remarkably selective and sensitive, with limits of quantitation (LOQ) reaching low ppt levels.

Development of an Extensive LC-MS Fecal Library for Advancing Microbiome Metabolomics

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Introduction: Recent studies underline the crucial role of the human gut microbiome in maintaining a healthy gastrointestinal tract and influencing pathological processes. The fecal metabolome is also increasingly studied for clinical diagnosis potential, revealing insights into the microbiome's impact on health. Targeted MS-metabolomics identifies and quantifies fecal metabolites such as amino acids, fatty acids, and bile acids. However, limitations including instrument speed and selectivity can lead to lowering the number of targets. A comprehensive fecal metabolites library was built on the novel mass spectrometer; the Stellar™ MS, combining fast scan speed, high selectivity, and detection sensitivity. The developed library was then used to evaluate metabolic variations in mice fecal samples collected from hypoxia- and dietary-based interventions.

Methods: A Fecal Metabolites Library from MetaSci was utilized for the library development. Fecal samples were collected from 8-week-old mice in either room air (21% FiO2) or normobaric hypoxia chambers (8% FiO2). Within each oxygen treatment, mice were fed one of three diets, each with 15% fat kcal%. Diets differed by fat source: standard (soybean oil), SFA-rich (90% cocoa butter, 10% soybean oil), and MUFA-rich (90% olive oil, 10% soybean oil). After 29 days in each condition, fresh fecal samples were collected. Metabolites were extracted using 80% methanol and separated via Thermo Scientific™ reversed-phase and HILIC columns. The novel mass spectrometer, the Stellar™ MS, provided fast scanning MS/MS and MSn levels for precise annotation and extended quantitation used for data acquisition.

Preliminary Data: The new mass analyzer's high scan speed reduced LC acquisition time, crucial for high-throughput analyses with sample stability challenges. In addition, the MSn-based quantitation provided the selectivity necessary for detecting and accurately quantifying co-eluting isomers and isobars, improving discrimination between analyte signals and those from matrix interferences. The established library allowed reliable quantitation over a broad dynamic range (5 orders of magnitude) in fecal components, with an improved sensitivity reflected in a low limit of quantification of 10 femtomoles and a low limit of detection of 0.25 femtomoles for most targets. Assessment of isotopically labeled internal standards demonstrated high data quality, reliability, and robust measurement, evidenced by minimal chromatographic shift and consistent signal responses, as indicated by low % CV for quality control and sample replicates. This study enables the assessment of metabolic profile differences in the mice fecal samples across various groups. For instance, variations in bile acids were noted among the different dietary samples, indicating potential metabolic impacts of diet on the studied parameters.

Novel Aspect: A comprehensive fecal library using the fast, sensitive, and selective Stellar™ mass spectrometer enabling high throughput and accurate microbiome metabolomics.

Automated and detailed structural elucidation of lipids in biological matrix

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Introduction: While at the forefront of biology and analytical chemistry, lipidomics is still emerging as it works to decode the complex language of lipids and their multifaceted roles. Driven by cutting-edge technologies, like mass spectrometry and separation techniques, lipidomics enables the mapping of the lipid landscape with unprecedented precision. Innovations such as electron-activated dissociation (EAD) have eased the challenges of analyzing the detailed structure of lipids. Use of informatics for qualitative lipidomics is growing, and there are several approaches, such as lipid databases that match mass spectra with reference databases from known analytes, in silico spectral libraries and fragmentation pattern-based tools. Here, we introduce a research-grade software module for analyzing lipids and its application to whole lipidome profiling in biological samples.

Methods: Data were acquired using Avanti polar lipid standards and NIST SRM 1950 human plasma. Plasma were extracted by the methyl-tert-butyl ether (MTBE) method. Liquid chromatography (LC) and MS analyses were performed using an Agilent 1290 LC coupled to a SCIEX ZenoTOF 7600 system. LC separation was achieved with an Eclipse+ C18 column (2.1 x 50 mm,1.8 µm). MS data were collected in positive ion mode with data-dependent acquisition (DDA) either collision-induced dissociation (CID) or (EAD) fragmentation.

Preliminary data: Using the research-grade software module, we were able to identify the relevant LC-MS peaks and then searched for the MS/MS signals of all major classes of lipids in glycerophospholids, Glycerolipids and Sphingolipids in human plasma. For identified candidate lipids, detailed MS/MS information led to their structural determination. Coverage for candidate lipids was up to three orders of magnitude in the EAD mode yielding over 500 lipid identifications. In the CID mode, coverage extended to more than five orders of magnitude, yielding over 700 identifications. The difference in the number of identifications was due to the number of precursor candidates set in the respective acquisition methods. We validated our data set with published work with more than 85% overlap of identification. The software module was able to identify deep structure information from the EAD data beyond headgroup, bond type and chain length, such as the attachment of acyl, alkyl and alkenyl chains to the backbone, unsaturation and locations of double bond positions. We demonstrated resolving structural isomers in plasma samples that were co-eluting or closely eluting. The software module identified a few species that were not previously identified in human plasma, for example, Cer class: Cer t18:0/24:0, Cer t18:0/24:1.We found that for the investigated lipid classes, the lipid fingerprint in positive ion mode EAD MS/MS data provided insight on structure that surpassed the information in the lipid fingerprint from the combined positive and negative modes of the CID MS/MS data.

Alternative Enriched Source of (E)-β-Farnesene: Preliminary Volatile Profiling of the Rare Ethnobotanical Herb Curcuma candida (Zingiberaceae) Using SPME-GC/MS-TOF Approach

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Curcuma is a well-known medicinal genus in the ginger family (Zingiberaceae) and is primarily distributed throughout the Indo-Malayan ecoregion. Several herbal species in this genus, including turmeric (C. longa), white turmeric (C. zedoaria) and wild turmeric (C. aromatica), have been widely studied for their pharmacological effects such as treatments against biliary diseases, hepatic disorders, carminative and rubefacient. A number of bioactive chemical constituents found in this genus have been previously reported, comprising flavonoids, terpenoids and phenolic compounds. Particularly, the key phytochemicals, including curcuminoids and volatile oils in the terpenoids groups, namely curcumin, ar-turmerone and eucalyptol, were considerably detected in Curcuma. Although some Curcuma species have been phytochemically examined, many others with ethnobotanical uses remains unexplored. In this study, C. candida, a rare and endemic species found in the Tenasserim Range along Thailand-Myanmar border, was selected for investigation due to its long-term ethnobotanical use as both an edible and medicinal plant. This study therefore aims to identify and compare the potential volatile compounds in C. candida collected from two disjunct populations in different provinces of Thailand (N = 30), Kanchanaburi (n = 15) and Tak (n = 15), using untargeted metabolomic approach. Fresh rhizomes of C. candida were freeze-dried and powdered by a ball mill machine. The 100 µm PDMS solid-phase microextraction (SPME) fibre was used to extract volatile constituents from 0.1 g powder before analysing by GC/MS-TOF for compound separation and detection. Over 200 phytochemical compounds were identified from both C. candida populations. For the major compounds, six key derivatives of sesquiterpene hydrocarbons were significantly reported in both populations, namely (E)-β-farnesene, longiverbenone, mustakone, a-farnesene, copaene and a-cubebene, respectively. Notably, (E)-β-farnesene displayed on the highest peak on the chromatogram, followed by a secondary peak representing the combination of longiverbenone and mustakone. All the three volatile components have been pharmacologically reported to exhibit antineoplastic effects (against breast cancer) and antieczematic properties. The chemical difference between two populations were found as the minor compounds. Subsequently, this preliminary study provides important information on phytochemicals found in C. candida, highlighting its potential to serve as an alternative source of key bioactive compounds for future clinical and medicinal applications.

Producing tailored lipids through enzyme engineering of proteins in the triacylglyceride biosynthetic pathway

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Current food oils and fats are derived from animal and plant sources. The increase in global demand for food is threatened with the issues of climate change and limited land and water resources. Hence, we need an alternative platform for lipid production like microbial lipids. Microorganisms like oleaginous yeasts can accumulate high amounts of lipids naturally. Triacylglycerols (TAGs) are the main component of these lipids. The composition of the fatty acid in TAGs vary, for different kinds of oils and animal fats. There are three distinct enzymes which add the three acyl chains to the glycerol backbone forming TAGs. Enzymes that add the fatty acids to the glycerol backbone of TAGs namely, glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidyl acyltransferase (LPAT) and diacylglycerol O-acyltransferase (DGAT) can be engineered for efficient activity, stability and positional selectivity. This would generate customized lipids thatcontain short, medium and long-chain fatty acids at desired positions of glycerol backbone. Careful selection of fatty acids at the different sn-1/2/3 positions of TAG can lead to different kinds of fat products. Hence, it is important that we understand the substrate specificity of enzymes involved in TAG biosynthesis pathway.DGAT and LPAT enzyme structures were modelled using AlphaFold and the putative acyl binding pocket was identified through in silico analysis. Site saturation mutagenesis was performed for 10 different amino acids in the binding pocket of the DGAT enzyme that adds the fatty acid at the third position of the TAG backbone. Automation was used to clone the mutants, grow them in a high throughput manner, screen active mutants using nile red staining and extract lipids for mass spectrometry analysis. As a result, DGAT mutants that could incorporate medium and longer chain fatty acids to the TAG backbone were identified. Mutant with higher activity compared to the wild type was also identified. LPAT adds the fatty acid at the second position of the TAG backbone. LPAT site saturation mutagenesis was performed for two amino acids in its binding pocket. Shift in substrate specificity for LPAT was also identified. In summary, we harness the power of enzyme engineering to modify the fatty acyl makeup of the triglyceride. Thus, creating a sustainable platform for production of custom alternative lipids.

Omics profiling of iPSC-derived motor neuron with nuclear loss of TDP-43 reveals early pathological phenotype for ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease primarily characterized by the degeneration of motor neurons (MN) in the brain and spinal cord, leading to muscular weakness and atrophy. Given the limited effective treatments and unclear pathophysiological mechanisms, a multifaceted approach is essential to explore the diverse genetic, metabolic, and functional factors contributing to ALS. TAR DNA-binding protein 43 (TDP-43) is known to play a key role in the pathophysiology of ALS and other neurodegenerative disorders. In this study, we established and characterized human-induced pluripotent stem cell (iPSC)-derived motor neurons (iMN) expressing wild type (TDP-43WT) or mutant form of TDP-43 with dysfunctional nuclear localization signal (TDP-43ΔNLS) to investigate the impact of TDP-43 nuclear depletion in ALS. Our results demonstrate that the iMN TDP-43 ΔNLS model effectively reproduces pathological features of ALS such as impaired neurite outgrowth, axonal regeneration, and hyperexcitability. By employing proteomics and metabolomics approaches, we assessed the functional significance of these features and observed that TDP-43 nuclear depletion in MN induces dysregulation of oxidative stress pathways and affects neurotransmitter release. We utilized both untargeted and targeted methods to profile and quantify various lipids and metabolites playing important role in ALS-related pathways. Comparative analysis revealed critical metabolic alterations, including amino acid metabolism, energy homeostasis, and lipid metabolism in TDP-43 ΔNLS iMN compared to TDP-43WT iMN. This work provides valuable insights into the molecular and biochemical landscape of ALS, particularly in understanding the role of TDP-43 nuclear depletion and offers potential targets for therapeutic intervention. Furthermore, the findings may facilitate the identification of early diagnostic markers, ultimately advancing development of precision medicine approaches for ALS management.

Introducing Clinical Grade Mass Spectrometry-based Steroid Panel Profiling in Singapore

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Background: Sex steroid hormones, including testosterone, estradiol, and progesterone, are essential in metabolism, reproduction, and disease progression. Accurate measurement is crucial for hormone-related research, yet traditional immunoassays suffer from cross-reactivity and limited specificity, leading to inconsistent results. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the gold standard for steroid hormone analysis, offering superior specificity, sensitivity, and the ability to measure multiple hormones simultaneously.

Methods: We validated an LC-MS/MS method for 18 steroids hormone quantification using a commercial TECAN steroid analysis kit. The method was assessed for accuracy, precision, linearity, and lower limit of quantification to ensure analytical robustness. Stringent quality control measures were implemented to align with international standards, supporting our ongoing efforts toward ISO 15189 accreditation.

Results: Our validated method demonstrated high specificity and sensitivity, with good precision and reproducibility across a broad concentration range. The analytical performance meets international standards (FDA guidelines), ensuring reliability for research applications.

Conclusion & Translational Value: This validated LC-MS/MS method enables high-quality steroid hormone analysis, supporting cohort studies and research collaborations. The pursuit of **ISO 15189 accreditation** reinforces our commitment to quality and standardization, enhancing the credibility of our research services. By establishing a reliable analytical platform, we contribute to advancements in endocrinology, biomarker discovery, and hormone-related disease research.

High-Throughput and Highly Selective Quantitative Lipidomics with the Stellar Mass Spectrometer – a Novel Hybrid Nominal Mass Instrument

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The increasing prevalence of metabolic disorders underscores the need for robust, high-throughput clinical lipid panels and lipidomics methods to quantify lipid species comprehensively, with high precision and selectivity[JI1]. Structurally related lipids, with varying fatty acid composition, were associated with distinct metabolic consequences and proven to have different biological roles. Thereby, measuring individual lipid species with highest selectivity possible is crucial for corroboration of clinical utility including the improved risk prediction and diagnostics. We present an advanced methodology leveraging the unique capabilities of the new Thermo Scientific Stellar mass spectrometer. This nominal mass instrument addresses current limitations in specificity and throughput by enabling fast acquisition parallel reaction monitoring, and revolutionizing lipidomics workflows by delivering unprecedented specificity and quantitation. This system combines a quadrupole and a dual cell linear ion trap that significantly improves the ion accumulation efficiency and scan speed. This allows for the acquisition of full MS2 spectra including multiple transitions, thus improving the annotation specificity by resolving isomeric lipids and structurally similar species. This unique capacity of Stellar positions it as a practical solution for translational research, biomarker discovery and clinical lipidomics where rapid, sensitive and selective lipid quantitation is essential.

Building on a widely adopted lipid panel, we evaluated the performance of the Stellar MS using a HILIC-MS/MS platform, originally measuring over 1000 lipids in the 1950 NIST plasma reference material in 12 minutes (combining positive and negative modes) in a TSQ Altis mass spectrometer. The data acquired with Stellar MS along with the LipidCreator tool allowed us to create a targeted list including multiple transitions per lipid species. This approach resulted in an average of 3.5 transitions per analyte, thus increasing for a 50% the diversity of screened lipid species, while simultaneously improving for at least 20% the peak definition or a number Of scans across a peak. This work highlights a significant advancement in lipidomics technology. By integrating the speed and sensitivity of the Stellar MS, we deliver a method that improves both specificity and quantitative reliability. These enhancements not only address key limitations of the previous approach but also establish a robust platform for advancing lipid-based research and precision medicine applications. This novel method represents a critical step forward in lipidomics providing researchers and clinical labs alike, with a powerful tool to explore the complexity of lipid biology with unprecedented precision and speed.

Simultaneous quantitation and discovery (SQUAD) metabolomics workflow for the analysis of fecal bile acids

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Introduction: Bile acids (BA) are synthesized from cholesterol in the liver and play a crucial role in lipid digestion and absorption. Fecal bile acids are known to serve as biomarkers and signaling molecules due to their intricate interplay with gut microbiota. Disordered microbiomes can alter the composition and size of the bile acid pool, producing a variety of conjugated bile acids and structurally similar metabolites. These changes may indirectly confer disease states. Here an LC-MS metabolomics workflow was developed for the simultaneous quantitation and discovery (SQUAD) of fecal BA and BA conjugates. The workflow utilizes Real-Time Library Search (RTLS) for spectral similarity measures and heightened identification confidence for molecular species during method execution.

Methods: Human stool material and BA standards (unlabeled and isotopically labeled) were obtained from NIST and CIL, respectively. Feces were spiked with the labeled standards and extracted with 80% methanol. Metabolites were separated on a reversed-phase column contained within the UHPLC system. Data were acquired on an Orbitrap IQ-X™ Tribrid™ mass spectrometer, which facilitates sensitive and highly dynamic PRM-quantitation utilizing the linear ion trap. It also allows orbitrap MS1 scanning for higher annotation rates. AcquireX™ and RTLS workflows were used to maximize the number of relevant compounds interrogated by MS2 and MS3, for confident annotation.

Preliminary Data: Unlabeled and labeled BA standards were used to generate calibration curves for absolute quantitation. This workflow facilitates a reliable ion trap quantitation over a wide dynamic range (i.e., 5 – 6 orders of magnitudes) of the targeted BA in feces. A lower limit of quantification (LLOQ) of 10 femtomoles and a lower limit of detection (LLOD) of 0.25 femtomoles was observed for most of the targets. Simultaneously, the HRAM Orbitrap data and the increased percentage of fragmented compounds using the advanced deep scan AcquireX workflow resulted in improved annotation capability on a wider dynamic range of fecal compounds. In addition, Real-Time Library Search provided spectral similarity measures and identification confidence scores for BA species upon which acquisition decision-making can be based during method execution. This study enables the assessment of bile acids and other metabolic profile differences in mice fecal samples collected from a dietary-based intervention (i.e., soy oil, soy and olive oils, and coconut butter and soy oil) due to improved annotation capabilities of the HRAM-data. Finally, confidence in the targeted and untargeted MS-based workflow's quality was obtained by regularly monitoring the spiked IS. High data quality, reliability, and robustness of measurement were observed by evaluating the performance of isotopically labeled compounds over time through metric tracking (e.g., retention time, mass accuracy, signal response).

Novel Aspect: SQUAD metabolomics workflow for parallel targeted quantitation, deep coverage, and confident annotation of fecal bile acids.

Advancing Lipoprotein Measurements: Evaluating the Accuracy of High-Throughput Nuclear Magnetic Resonance Spectroscopy Compared to Standard Methods

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Lipoproteins are supramolecular protein-lipid aggregates routinely used to assess cardiovascular disease risk. Nuclear Magnetic Resonance (NMR) spectroscopy is a high-throughput, cost-effective alternative for lipoprotein profiling. Proponents argue that NMR surpasses the resolution of standard clinical assays and is cheaper than ultra-centrifugation methods. The implementation of proton NMR methods for measuring lipoproteins in commercial platforms relies on cheminformatics and experimental parameters to resolve overlapping peaks. However, distinguishing resonances of interest from overlapping signals remains a challenge in NMR lipoprotein profiling, prompting calls for independent accuracy evaluations.The primary objective of this study was to evaluate the accuracy of NMR lipoprotein measurements derived from the standard one-dimensional proton experiment with solvent pre-saturation (herein known as 1DSTD) using the Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA) method, compared to standard techniques for assessing lipoprotein parameters. Specifically, NMR-B.I.LISA measurements of Total Cholesterol (TC), Total Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDLC) and Low-Density Lipoprotein Cholesterol (LDLC) in plasma samples (N=620) from a randomized dietary intervention trial were compared to those obtained using the precipitation method (HDLC), ultracentrifugation (LDLC), and enzymatic assays (TC and TG). The second objective was to compare lipoprotein modelling based on the 1DSTD spectra to another 1D spectra data based on a newer pulse sequence, known as JEDI-PGPE, which removes interfering resonances by combining J-editing, relaxation, and diffusion pulse sequences. The lipoprotein modelling involved Orthogonal Partial Least Squares (OPLS) models on the methyl (0.6 – 1.04 ppm) and methylene (1.2 – 1.4 ppm) resonances of lipoproteins, using the standard lipoprotein measurements as responses. All models were cross-validated and subjected to 20 permutation tests. All NMR experiments were acquired on a 600 MHz Bruker Avance III HD spectrometer.Lipoproteins measurements using the NMR-B.I.LISA method showed high consistency with standard measurements. The highest correlations were observed for HDLC (r = 0.92), followed by TG (r = 0.91), TC (r = 0.85) and LDLC (r = 0.81). However, Bland-Altman analysis revealed systematically lower values from NMR-B.I.LISA method, with the lowest median deviation of 4% for HDLC and the highest deviation of 16% for TG. OPLS models indicated superior accuracy of the 1DSTD method over the JEDI-PGPE method for all four lipoproteins. The Q2Y values for 1DSTD versus JEDI-PGPE were 0.907 versus 0.875 for HDLC; 0.874 versus 0.836 for TG; 0.774 versus 0.648 for LDLC; and 0.769 versus 0.634 for TC.In conclusion, the NMR-B.I.LISA method shows comparable results to standard methods for lipoprotein quantification. The OPLS model proved to be effective at distinguishing lipoprotein signals from overlapped peaks, outperforming the JEDI-PGPE method where filtering is accompanied by distortion and loss of signal on the key lipoprotein resonances. Thus, the 1DSTD NMR approach offers a high-throughput and more cost-effective alternative to standard methods.