iSLS12

5—8 March 2024
Lipidomic Technologies
and Applications

National University of Singapore
Centre for Life Sciences

SLING
Singapore Lipidomics Incubator

NUS
National University
of Singapore

12th International Singapore Lipid Symposium

5th March – 8th March 2024

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

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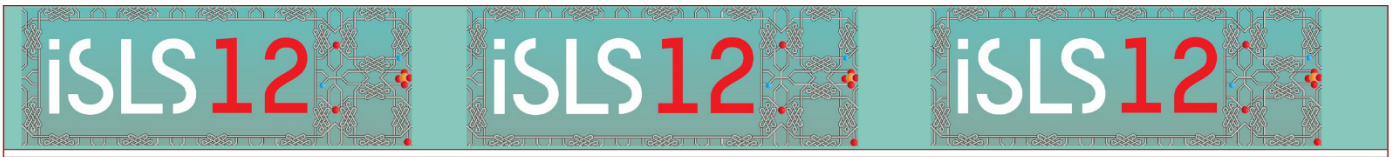

Time (UTC+8)	TUESDAY 5th MARCH	WEDNESDAY 6th MARCH	THURSDAY 7th MARCH	FRIDAY 8th MARCH
09:00 - 09:10	**Workshop 1** **Bibliometrics for Lipidomics - Part 1** Magnus Palmblad 9:00-10:00	**Precision medicine** Peter Meikle 9:00-9:30	**Precision oncology** Michal Holčapek 9:00-9:30	**Deep Targeted Plasma Lipidomics** Julijana Ivanisevic 9:00-9:30
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09:20 - 09:30				
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10:00 - 10:10	*Break* *10:00-10:30*	*Break* *10:00-10:30*	*Break* *10:00-10:30*	*Break* *10:00-10:30*
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11:00 - 11:10		**Lipidomics in Cell Biology** Noemi Jimenez-Rojo (11:00-11:20)	**Diet and microbiome-derived metabolites** Dorrain Low (11:00-11:20) Naazneen Sofeo (11:20-11:40)	**Bioactive lipids in human diseases** Anna Nicolaou 11:00-11:30
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11:20 - 11:30		**Lipid homeostasis alterations in diseases** Charna Dibner (11:20-11:40)		
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12:40 - 12:50				
12:50 - 13:00		**Sponsored talk - Sciex** Paul Baker 12:40-13:30	**Sponsored talk - Agilent** Shane Snyder 12:40-13:30	**Sponsored talk - Thermo Scientific** Daniel Hermanson 12:40-13:30
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14:00 - 14:10	*Break* *14:00-14:30*	**Imaging MS** Shane Ellis 14:00-14:30	**Microbial lipidome and inflammation** Xueli Guan (14:00-14:20) Hong Yong Peh (14:20-14:40)	**Targeting ceramide double bond** Scott Summers 14:00-14:30
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17:40 - 17:50				**Register at:**
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This schedule is subject to change without prior notice
All timings are correct for Singapore time zone (UTC+8).

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Workshops

Workshop 1 - Bibliometrics for Lipidomics

Tuesday 5th March – 9h00-11h50

Magnus PALMBLAD

Leiden University Medical Centre, The Netherlands

Bibliometrics is the use of computational methods to analyze bodies of text, such as scientific articles. This iSLS workshop will introduce two bibliometrics resources and demonstrate how to use them to study lipid research. However, the tools are not specific to lipids and are equally useful to study other fields of science. In this workshop, we will specifically cover how to perform advanced literature searches in Europe PMC and visualize the results as networks using VOSviewer.

Europe PMC provides access to life sciences literature, metadata and annotations. It is available for anyone, anywhere, for free. With Europe PMC you can search and read 43.5 million publications, including all those in PubMed and PubMed Central, preprints, patents and other documents enriched with links to other databases and repositories. Europe PMC also provides an interface to text mining results, including for organisms, diseases, genes, proteins and small(er) molecules such as lipids.

Complementing Europe PMC, the VOSviewer is a software tool for building and visualizing networks from a body of documents such as scientific papers. The nodes in the network can represent (key)words, authors, journals or institutions, while the edges typically represent co-occurrences, co-authorships or citations. Information about publication date can be used to visualize trends in research topics or methodology. The VOSviewer and Europe PMC work well together, with the VOSviewer being able to search Europe PMC directly, as well as take Europe PMC results as input.

Europe PMC and the VOSviewer are a powerful combination for generating overviews of a field of research, addressing questions such as who does research on what and with whom, and when and where this research was performed or published. This is equally useful for PhD students starting out in a field as for senior researchers writing grant applications or reviews.

Workshop 2 - How to be a good reviewer?

Tuesday 5th March – 13h00-14h00

Jerzy ADAMSKI

Editor in Chief, Journal of Steroid Biochemistry and Molecular Biology, Elsevier

The peer review process is an indispensable tool in modern scientific communication. The peer review process ensures that communications are new, of high quality and rigor, follow standardization rules, contribute to democratic exchange, and exclude plagiarism. Reviewers who participate in a peer review process may be interested in improving communication in academia, increasing their visibility, and enhancing their own professional skills. Reviewers can be identified through a variety of approaches, including personal invitations or registration in specialized portals. Most large publishing houses rely heavily on honest, fair, and well-organized reviewers. There are a number of rules about what should be included in a good review and what should be avoided. The essential elements are discussed interactively.

Workshop 3 - Clinical Data Analytics Workshop

Tuesday 5th March – 13h00-14h00

Hyung Won CHOI

National University of Singapore, Singapore

In this workshop, we work with the data published in *Tan et al., Variability of the Plasma Lipidome and Subclinical Coronary Atherosclerosis, Atheroscler Thromb Vasc Biol, 2021* (DOI: 10.1161/atvbaha.121.316847).

The paper describes a lipidomics study of ~80 individuals at a high risk of coronary artery disease (CAD). The participants were invited monthly for blood sampling up to five times and had coronary artery plaque burden assessed using computed tomography coronary angiography (CTCA) at the end of the follow-up.

Using high-quality quantitative lipidomics data and other meta data of the participants, we will first inspect overall data trends via visualization and dimension reduction. Using custom R scripts, we synchronize the quantitative lipidomic data and the plaque burden data (outcome) for the same subjects and practice clustering of the subjects by different plaque subtypes. We fit linear mixed effects models on repeated measure data (lipids) and compute population-level properties of lipid species such as within-individual and between-individual variability (coefficients of variation). Finally, we identify lipid species in which the visit-to-visit variability is significantly associated with different plaque types.

Please ensure the latest versions of R and RStudio are installed on your computer. We will install R packages **before the start of the workshop** with assistance by teaching assistants.

- R (version 4.3.2. Download from https://cloud.r-project.org/.
- RStudio (newest version, at least 2022.02). Download from https://posit.co/download/rstudio-desktop/.

R markdown document and the data sets will be distributed in the beginning of the workshop.

Abstracts of Oral Presentations

List of Speakers (Alphabetical)

List of Titles (Alphabetical by speaker)

Interlaboratory comparison of metabolomics analyses of human and rodent blood using Biocrates MxP Quant 500

Jerzy ADAMSKI

Institute of Experimental Genetics, Helmholtz Zentrum München, German Research Center for Environmental Health, Germany
Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Slovenia

Metabolomics provides valuable insights into the molecular underpinnings of phenotypes. To identify reliable metabolomic biomarkers of phenotype and possible companion diagnostics for human diseases validation of studies are required. In this project we evaluated the analytical performance of the MxP® Quant 500 kit from Biocrates life science ag (Innsbruck, Austria) among 14 international laboratories. Laboratories were provided with identical sets of human serum and plasma (female and male), human plasma NIST SRM 1950, lipaemic human plasma, and rat and mouse plasma samples and quality control samples (QC2) as well. Pre-processed data (µmol/L) were collected from the participating laboratories and submitted to joint statistical analyses. The MxP® Quant 500 kit from Biocrates life sciences ag can be applied for the determination of more than 600 metabolites in 10 µL liquid sample (e.g. plasma and serum). The kit enables the quantification of selected metabolites belonging to following compound classes: alkaloids, amine oxides, amino acids, amino acid related, bile acids, biogenic amines, carbohydrates and related, carboxylic acids, cresols, fatty acids, hormones and related, indoles and derivatives, nucleobases and related, vitamins and cofactors, acylcarnitines, lysophosphatidylcholines, phosphatidylcholines, sphingomyelins, ceramides, dihydroceramides, hexosylceramides, dihexosylceramides, trihexosylceramides, cholesteryl esters, diglycerides, and triglycerides. Detection and quantification are carried out by triple quad mass spectrometry (MS/MS). One part of the metabolites (alkaloids – vitamins and cofactors) is separated by ultra-high-performance chromatography (UHPLC) before entering the MS (LC-MS/MS). The other part of the metabolites (acylcarnitines – triglycerides) is directly infused into the mass spectrometer and thus determined via flow injection analysis (FIA). Electrospray ionization was used for all modes of MS-analysis. The principal workflow includes a derivatization step, the extraction of the analytes and the LC-MS/MS or FIA determination.

All labs have observed the same metabolic signatures. Different matrices revealed distinct metabolic signatures. Some metabolites were present only in human samples whereas additional few were identified in the rodent plasma. Out of the 634 targeted metabolites, 505 were detected by all 14 laboratories, i.e., a concentration above the calculated limit of detection (LOD) was reported for at least one project sample. To provide an overview of the variation observed across all 504 measured project samples (14 laboratories x 12 project sample types in triplicates), we performed a principal component analysis (PCA) of the (unnormalized) measured concentrations and mapped information about samples type and laboratory. This analysis showed that the largest variation in the data was linked to the lipid content of the samples, separating lipemic samples from the res. The highest number of metabolites was quantified in the NIST SRM 1950 sample. We have calculated the CV values for the NIST SRM 1950 sample to facilitate comparison of the CV with publicly certified or laboratory data and observed significant similarities.

Our observations demonstrate that large cohorts can be analysed in parallel in distinct labs applying the same targeted metabolomics technology. Large scale or replication studies can be based on data determined by distinct labs worldwide.

Wednesday 6th March – 9h30-10h00

Lipidomics of host-microbiome interactions

Makoto ARITA

Division of Physiological Chemistry and Metabolism, Graduate School of Pharmaceutical Sciences, Keio University, Japan
WPI Bio2Q, Keio University, Japan
Laboratory of Metabolomics, RIKEN Center for Integrative Medical Sciences, Japan
Graduate School of Medical Life Science, Yokohama City University, Japan

Abnormal lipid metabolism is often a background factor of diseases, which may lead to the discovery of new seeds for drug discovery and medical applications such as early diagnosis and treatment. Recent advances in mass spectrometry have provided a major impact on lipid biology, suggesting that the lipid molecules analyzed in the past are only the tip of the iceberg. Host-microbiota interactions create a unique metabolic milieu that modulates intestinal environments. By combining non-targeted mass spectrometry with feature-based molecular spectrum networking technology, we revealed that lipids with complex structures are produced by gut microbiota. Correctly capturing such molecular groups that have been overlooked by conventional targeted analysis will lead to understanding the significance of bacterial species that correlate with various diseases such as cancer, obesity, and allergy. The method developed in this study is a technology that enables us to view such changes in the bacterial flora as changes in the lipidome environment from a bird's eye view and is expected to contribute to the identification of functional metabolites that mediate the host-microbiome interactions.

JST-ERATO Grant number JPMJER2101
https://www.jst.go.jp/erato/en/research_area/ongoing/jpmjer2101.html

WPI Bio2Q
https://bio2q.keio.ac.jp

Thursday 7th March – 13h30-14h00

Live single-cell lipidomics using capillary sampling

Melanie BAILEY

University of Surrey, UK

The SEISMIC facility for spatially resolved single and sub-cellular “omics” is a UK national facility that is developing new technology for automated single and sub-cellular sampling. This enables single living cells, or parts of cells to be captured into capillaries under confocal microscopy. The cell material can be transferred to vials for LC-MS, simply sprayed into a mass spectrometer for analysis. In this presentation we will show the progress we have made to develop methodology for lipidomics analysis of extracted material and show the observed variation in single cell lipid profiles in response to biological stimuli such as irradiation, peroxidising agents and drugs.

Sponsored talk – SCIEX

Quantitation and structural characterization of lipid mediators by high-resolution mass spectrometry

Paul R.S. BAKER

SCIEX, USA

Lipid mediators are cell-derived signaling molecules that modulate numerous biological functions. Of note, they play a significant role in all stages of inflammation, including its initiation, propagation, and resolution. Consequently, this class of molecules and their biochemical pathways are attractive candidates for anti-inflammatory drug development. Lipid mediators are potent signaling molecules whose endogenous concentrations are in the pM to nM range making them a challenge to analyze. Typically, these molecules are measured using highly sensitive triple quadrupole mass spectrometers using multiple reaction monitoring (MRM) scan modes. However, the recent advent of an ultra-sensitive high-resolution mass spectrometer (HRMS) now enables accurate and precise measurements of lipid mediators *in vivo* while simultaneously providing critical structural characterization.

The ZenoTOF 7600 system is an HRMS instrument capable of measuring the low endogenous concentrations of many lipid mediators with a sensitivity level rivaling high-end triple quadrupole instruments. In addition to traditional collision-induced dissociation (CID)-based fragmentation, the instrument has a complimentary fragmentation mode, electron-activated dissociation (EAD), that provides diagnostic fragment ions for the structural characterization of singly-charged small molecules. Lipid mediators are derived from a limited number of precursor fatty acids and are distinguished not necessarily by mass but by the location of their hydroxyl functional group(s), double bonds and potentially carbon rings. Consequently, many of these compounds are isomeric at the precursor and the CID-based product ion levels; hence, the identification and quantitation of these compounds have relied heavily on careful chromatographic separation to achieve isomeric resolution. However, the ZenoTOF 7600 instrument has a tunable electron beam to produce EAD-based fragments that can distinguish lipid mediator isomers.

This seminar will demonstrate the use of the ZenoTOF 7600 system to quantitate and fully characterize lipid mediators using both CID- and EAD-based fragmentation. The results presented will demonstrate that this level of molecular characterization can be achieved on a liquid chromatography (LC) time scale, which enables high-throughput data acquisition in samples of diverse origins. The data presented will show that the ZenoTOF 7600 system with EAD is uniquely capable of the specific structural identification of lipid molecular species in simple and complex matrices.

Wednesday 6th March – 12h40-13h30

A renaissance of radical ions for de novo structure elucidation of lipids by mass spectrometry

Steve BLANKSBY

Queensland University of Technology, Australia

Recent discoveries of lipids derived from across the kingdoms of life that possess unexpected molecular structures suggest the existence of significant gaps in current knowledge of lipid biosynthesis and function in nature. To bridge this gap new technologies are required that can both (i) enhance separation of lipid extracts into individual components and (ii) can achieve complete elucidation of the molecular structure without reliance of reference standards. While contemporary liquid chromatography-mass spectrometry is a powerful approach toward both these goals its reliance on low energy collision-induced dissociation for structure elucidation leaves significant portions of lipid molecular structure unassigned and ambiguous assignment of lipid isomers. Significantly, much of the structural diversity across the lipidome arises from distinctive patterns of carbon-carbon bonding (e.g., unsaturation, branched-chain and carbocyclic motifs) that cannot be assigned from conventional tandem mass spectrometry of even electron ions. This presentation will focus on renewed efforts to generate and fragment radical ions to enhance cleavage of carbon-carbon bonds in ionised lipids. Integration of these advances with current approaches will be demonstrated as a potential pathway towards complete *de novo* structural identification of individual lipids in complex biological matrices.

Wednesday 6th March – 15h00-15h30

Identification of isomeric and enantiomeric lipids by cold ion spectroscopy of non-covalent complexes

Oleg BOYARKINE

EPFL, Switzerland

The tremendous structural and isomeric diversity of lipids enables a wide range oftheir functions in nature, but makes identification of these biomoleculeschallenging. We distinguish and quantify isomeric lipids using cold-ion UVfragmentation spectroscopy of their non-covalent complexes with aromatic aminoacids and dipeptides. Based on structural simulations, particularlyisomer-sensitive aromatic “sensors” have been pre-selected for lipids of eachstudied class. Tyrosine appeared to be a good “sensor” to distinguish steroidsand prostaglandins, which are rich in functional groups, while diphenylalanineis a better choice for sensing largely hydrophobic phospholipids. With thissensor, the relative concentrations of two mixed in solution isomericglycerophospholipids have been determined with 3.3% accuracy, which shoulddegrade only to 3.7% for a 14 s express-measurement. Enantiomers of many chiralbiomolecules and drugs exhibit quite different functionality in vivo. In anon-chiral environment these isomers have identical physicochemical properties,which often makes analytical identification of enantiomers challenging. We test IR and UV cold ion spectroscopy for identification andquantification of enantiomers, taking a difficult case of a large lipidmolecule erythro-sphingosine. The lipid was non-covalently tagged insolution with a 2-butanol solvent or an aromatic chiral reporter molecule forIR and UV gas-phase spectroscopy of the formed protonated complexes,respectively. While IR spectroscopy failed to distinguish the two enantiomers, thedifference in the UV spectra of the complexes with two enantiomerically purestandards of the lipid enabled quantification of the test mixtures with a fewpercent accuracy. Moreover, the diastereomeric configuration of such complexesallowed for the enantiomeric quantifications using just a single analyticalstandard but both enantiomers of the reporter molecule instead. Furthermore, this property of the complexes enablesspectroscopic identification of non-racemic analytical solutions without havingany standards of the mixed molecules at all.

Wednesday 6th March – 16h00-16h20

Arachidonic acid, the FADS Indel, and colorectal cancer

J.T. BRENNA

Dell Pediatric Research Institute, University of Texas at Austin, USA

Specialized mass spectrometry based on covalent adduct chemical ionization of unsaturated fatty acids enables routine high sensitivity analysis of fatty acid double bond structure. This method has been applied to characterize the substrate specificity of the three genes sharing similar genetic structure collocated at the fatty acid desaturase (FADS) gene cluster on chromosome 11. FADS3 is not a fatty acid desaturase. FADS2 has 6, 4, and 8 desaturase activity toward at least 16 fatty acids. In contrast, FADS1 has only 5-desaturase activity toward C20 PUFA; among these the major activity is against the major eicosanoid/lipid mediator precursor arachidonic acid (AA, 20:4n-6).

In separate experiments in human cells and with human cohorts, we showed that a specific functional polymorphism (rs66698963) causes differences in circulating arachidonic acid and its precursor dihomogamma linolenic acid (DGLA, 20:3n-6). Rs66698963 is an insertion-deletion polymorphism of 22 bp (deletion allele, D) or a repeat 44 bp (insertion allele, I) in intron 1 of FADS2, that affects expression of FADS1. In six free living populations with over 4,000 individuals, circulating 20:4 concentration rises in the order of genotypes DD<ID<II while for 20:3 the pattern is opposite, consistent with cell culture studies indicating that the FADS indel controls FADS1 catalysis of 20:3→20:4.

Arachidonic acid is precursor for eicosanoids that signal for a wide range of physiological processes, including inflammation, thrombosis, and parturition. The abundance of free AA limits synthesis of some eicosanoids which in turn is limited by the abundance of AA in membrane phospholipids. We hypothesized that the FADS Indel genotype would stratify participants in randomized trials targeting AA-derived eicosanoids. The seAFOod trial (Hull et al., Lancet 2018) randomized high risk colorectal cancer patients to 2 grams/d of EPA and/or aspirin for one year when follow-up rectal polyps were assessed. The original trial showed no differences in polyp number due to EPA. We stratified the same participants by FADS Indel genotype. We found a non-significant trend to higher polyp number, and a significant 52% reduction in polyp number in genotypes with the I allele (ID and II), as well as increased ratio of circulating 20:4 to 18:2 (linoleic acid) (Hull et al., medRxiv 2023).

This precision oncology/nutrition approach was enabled by our lipidomic technology for detailed characterization of the FADS gene product specificity, followed by their application to human therapeutics. Follow-ups in other populations with other conditions is in progress.

A lipidomics-based method to eliminate negative urine culture in general population

Michael CHEN

University of British Columbia, Canada

Background

Urinary tract infections (UTIs) pose a significant challenge to human health. Accurate and timely detection remains pivotal for effective intervention. Current urine culture techniques, while essential, often encounter challenges where urinalysis yields positive results, but subsequent culture testing returns negative. This highlights potential discrepancies between the two methods and emphasizes the need for improved correlation in urinary tract infection detection. A faster workflow that maintains accuracy of current methods while reducing costs and time to identification is essential.

Objectives

The overall aim of this study was to evaluate the use of a fast lipid analysis technique (FLAT) to eliminate the need for urine culture in the general population.

Methods

A total of 435 urine samples were collected from outpatients suspected to have UTIs from Victoria General and Royal Jubilee Hospital, British Columbia and analyzed using our novel FLAT method. The FLAT method allows direct lipid extraction on a MALDI plate for pathogen identification by MALDI-TOF MS. Briefly, 1 µL of urine was spotted on an MFX µFocus MALDI plate. Acidified and incubated in a humidified chamber at 110°C for 30 min. The plate was rinsed with distilled water and then 1 µL of Norharmane (10mg/mL) was added to each spot. After drying, samples were analyzed using a Bruker Microflex in negative ion, linear mode with automated laser operation. Results were compared against the hospital's standard protein biotyper identification.

Results

Lipid fingerprinting by MALDI-TOF MS directly from urine samples correctly identified the most significant uropathogens within a 1-hour timeframe when compared to urine culture. We identified clinically significant pathogens such as *E. coli*, *P. aeruginosa, Proteus* sp, *Klebsiella sp, S. epidermidis, A. urinae* and even as polymicrobial directly from 1 µL of urine. The FLAT analysis ruled out negative UTIs with 99% agreement, whereas urinalysis only showed 37% agreement with urine culture as gold standard. The FLAT data showed an overall sensitivity of 70% and specificity of 99% with positive and negative predictive values of 95 and 92%, respectively for both Gram positive and negatives.

Conclusions

The ability to identify pathogens without need for culture can allow for faster pathogen identification, reduced time to appropriate antimicrobial therapy and improved patient outcomes. Eliminating culture-negative urines can save healthcare resources and improve healthcare by redirecting physicians more quickly to other causes of patient symptoms. The potential impact of this innovative lipidomic-based approach extends beyond conventional diagnostic limitations, offering new avenues for early detection and targeted management of urinary tract infections. This research marks a paradigm shift in urine culture methodology, paving the way for improved clinical outcomes and public health interventions.

Friday 8th March – 13h30-14h00

Temporal and absolute lipid homeostasis alterations associated with metabolic diseases in humans

Charna DIBNER

Division of Thoracic and Endocrine Surgery, Department of Surgery, University Hospital of Geneva, Switzerland
Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva, Switzerland
Diabetes Center, Faculty of Medicine, University of Geneva, Switzerland
Institute of Genetics and Genomics in Geneva (iGE3), Switzerland

Lipid homeostasis in humans follows a diurnal pattern in muscle andpancreatic islets, altered upon metabolic dysregulation. Recently, we employed tandem and liquid chromatography mass spectrometry to investigate daily regulation of lipid metabolism in subcutaneous adipose tissue (SAT) and serum of type 2 diabetic (T2D) and non-diabetic (ND) human volunteers. Around 8% of ≈440 lipid metabolites exhibited diurnal rhythmicity in serum and SAT from ND and T2D subjects. The spectrum of rhythmic lipids differed between ND and T2D individuals, with the most substantial changes observed early morning, as confirmed by lipidomics in an independent cohort of ND and T2D subjects at single morning time-point. Strikingly, metabolites identified as daily rhythmic in both serum and SAT from T2D subjects exhibited tissue-specific phase differences. Gastric Bypass surgery (GBS) represents a well-established approach to counteract human morbid obesity. Emerging evidence suggests that GBS impacts on the circulating phospho- and sphingolipids. However, long-term effects of GBS on lipid metabolism have not been explored. Thereby, we aimed to unveil to what extent GBS improves lipid homeostasis in serum and tissues from morbid obese individuals. To investigate alterations in lipidomic signatures associated with massive weight loss following GBS in morbid obese patients, we employed direct infusion tandem mass spectrometry (MS) allowing to quantify awide range of lipid metabolites in serum and subcutaneous adipose tissue (SAT) samples. Systematic lipidomic analyses were conducted in samples collected in a longitudinal cohort of patients (cohort 1, n = 11) prior to GBS, and one year following the surgery. Over 400 phospholipid and sphingolipid species have been quantified in serum and SAT, allowing to establish detailed lipidomic signatures associated with morbid obesity in a tissue-specific manner. Concomitant with weight loss and improvement of metabolic parameters, a massive rearrangement of lipid metabolites was observed one year following GBS. Strikingly, a substantial reduction of ceramide levels and increased amount of hexosylceramides were detected in both serum and SAT. These novel data were cross-compared with our recent lipidomic analyses conducted by the same approach in an independent cohort of morbid obese patients and lean controls, where serum and visceral adipose tissue (VAT) lipids were analysed (cohort 2, n = 39), revealing that GBS partly restored the lipid alterations associated with morbid obesity. Our study reports massive temporal and tissue-specific alterations of human lipid homeostasis in T2D, paving the way for the development of lipid biomarkers in a temporal manner. Moreover, it provides the first systematic analysis of the long-term lipid homeostasis modifications upon GBS in humans SAT and serum and demonstrates that lipid metabolism alterations associated with morbid obesity might be partly reversed by GBS.

The research protocol was registered with the Protocol Registration and Results System at ClinicalTrial.gov [NCT03029572].

Wednesday 6th March – 11h20-11h40

Mass spectrometry imaging

Shane ELLIS

University of Wollongong, Australia

Mass spectrometry imaging (MSI) is a powerful and unique technology for the label-free imaging of lipids within tissues, providing insights into lipid composition and metabolism throughout heterogeneous biological environments. However, conventional MSI techniques encounter limitations in detecting numerous lipid species owing to their low ionisation efficiencies, consequently also limiting the achievable spatial resolution. Our research seeks to address these challenges by leveraging laser post-ionisation (MALDI-2) coupled with an Orbitrap mass spectrometer and developing a prototype timsTOF mass spectrometer equipped with plasma post-ionisation. In both cases, post-ionisation significantly enhances the detection of numerous lipid species by up to 100-fold, including for neutral glycosphingolipids, phosphatidylethanolamine, and sterols. Furthermore, the sensitivity gains offered by post-ionisation also facilitates rich imaging of lipids at spatial resolution as low as 5 µm.

This presentation will also outline the development of an internal standard mixture tailored for MSI analysis of brain tissue. This approach addresses variations in lipid class and region-specific ionisation efficiencies, enabling per-pixel quantitation (in pmol/mm^2) of over 200 lipid species across numerous phospholipid and sphingolipid classes from brain tissue utilising both MALDI and MALDI-2. Finally, recent work addressing the challenge of lipid isomers, in particular *sn*-isomers, using a shotgun lipidomics approach combined with DIA and ozone-induced dissociation will be discussed. This workflow enables the characterisation of >500 *sn*-resolved PC and PE lipid species in <20 minutes and reveals how *sn*-isomer populations vary across cell lines.

Wednesday 6th March – 14h00-14h30

Revisiting the lipidome of *Escherichia coli*, a man's friend and foe

Xue Li GUAN

Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.

Escherichia coli is a commensal residing in the gut and used as probiotics with benefits to human health. *E. coli* can on the other hand be a deadly pathogen causing a broad spectrum of human diseases including diarrhoeal disease, urinary tract infections, sepsis and meningitis. As a Gram- negative bacteria (GNB), *E. coli* is enveloped by two lipid bilayers which protect the bacterium from its external environment, and the outer membrane lipid, lipopolysaccharide is an endotoxin. For long, *E. coli* had served as a model organism for studies on bacterial lipid metabolism, with its lipid biosynthetic and transport machinery extensively characterized. Despite its remarkable history in lipid biology and numerous reports available, there lacked a coherent view of the true lipidome of *E. coli*. The advent of increasingly sensitive and high-resolution technology had enabled the discovery of new lipids and intermediates. Here, I will present the application of a combinatorial approach involving mass spectrometry and metabolic labelling and incorporation to revisit the lipidome of *E. coli*. I will further share the ongoing international inter-laboratory efforts to define the reference lipidome of this model bacterium.

Thursday 7th March – 14h00-14h20

Advancing lipidomics and metabolomics clinical research: optimizing pre-analytical protocols for investigating inflammatory skin diseases

Lisa Hahnefeld

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

Considering the stability of samples during the pre-analytical phase is crucial for obtaining reliable results in clinical investigations using lipidomics and metabolomics approaches. However, there is often a conflict between the strict protocols recommended by most studies on sample stability and their practicality in clinical settings. Comprehensive analyses in metabolomics and lipidomics cover a broad range of analytes, including both stable compounds (e.g., ceramides or triglycerides) and unstable compounds (e.g., sphingosine-phosphates, lysophosphatidic acids, and endocannabinoids). This presents a challenge in selecting a sampling protocol that is both rigorous and feasible. Additionally, assessing the quality of already collected samples can be difficult as they may not conform to protocol recommendations.

Therefore, our study aimed to establish pre-analytical protocols for both prospective sampling and retrospective evaluation of sample quality. We focused on various metabolite and lipid classes in different clinical settings. To achieve this, we consolidated our findings with eight previous publications and created a comprehensive database. This database contains over 23,000-fold changes for more than 1,000 metabolites, along with 110 pre-analytical conditions specifically for EDTA plasma and serum samples. To facilitate easy access and utilization of this extensive dataset, we developed an interactive R shiny app. This app allows users to explore the database and receive personalized recommendations for one of six different sampling protocols based on their specific compounds of interest. Moreover, users can input the actual pre-analytical conditions of their existing samples to estimate compound stability under those conditions.

Based on the insights gained regarding pre-analytical factors, we conducted a prospective study to investigate polar metabolites, lipids, and lipid mediators in the context of inflammatory skin diseases. Specifically, we collected plasma samples (both EDTA and fluoride/citrate) from 58 patients with atopic dermatitis, plaque-type psoriasis, hidradenitis suppurativa, as well as healthy control subjects. The primary objective of our study was to identify commonalities and differences in the systemic inflammation and metabolite profiles among these diseases. The findings indicate alterations in cysteine metabolism across all three diseases, suggesting a shift in non-enzymatic antioxidant defense. Additionally, a lipidomic network enrichment analysis reveals both similarities and differences in local subnetworks when compared to healthy controls.

In conclusion, our study highlights the importance of considering sample stability in pre-analytical phases of lipidomics and metabolomics investigations, despite the challenges posed by conflicting protocols and sample quality assessment. By establishing comprehensive pre-analytical protocols and consolidating a pre-analytical database, we developed an interactive tool that provides personalized sampling recommendations. Using this knowledge on appropriate pre-analytical sample handling, we successfully identified commonalities and differences in inflammatory skin diseases.

Sponsored talk – Thermo Scientific

Comprehensive data acquisition workflow on Orbitrap Astral MS for untargeted lipidomics to achieve deep lipidome coverage with high confidence annotations

Daniel HERMANSON

Thermo Fisher Scientific, USA

Introduction

Discovery lipidomics using mass spectrometry aims to gather comprehensive insights into the lipid diversity of a sample. The presence of large number of isobaric and isomeric species in the lipidome necessitates the use of fragmentation for confident annotation. Nevertheless, challenges such as mass spectrometer speed, sensitivity, and accuracy may result in suboptimal production of high-quality MS^2 spectra, leading to reduced annotation percentage and confidence. To address this, a thorough untargeted lipidomics approach was established using Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer, known for its capability of faster MS2 scanning in concert with high resolution accurate mass orbitrap detection.

Methods

Lipid standards and bovine liver lipid extracts were purchased from Avanti Lipids. Animal and plant-based milk samples were obtained from local markets (San Jose, California). Lipids were extracted from the biological matrices using Folch method. The extracted milk lipids were spiked with equal amounts of liver lipid extract to create a complex matrix sample set. Lipids were separated on a Thermo Scientific™ Accucore™ C30 column connected to a Thermo Scientific™ Vanquish™ Horizon system. Data were acquired (both positive and negative polarities) on the Orbitrap Astral, which allows faster scanning on the MS^2 level for a higher annotation rate. Thermo Scientific™ Compound Discoverer™ 3.3 and MS-Dial software was used for data processing, statistical analysis, and unknown annotation.

Preliminary Data

In this study, the complex lipid samples were analyzed on the Orbitrap Astral, with a full-scan Orbitrap HRAM MS1 analysis at 120Kresolution and a data dependent fast and sensitive Astral HRAM MS^2 with a resolution on 80K. The30-minute LC-MS method recorded around 250000 MS2 events leading to very high percentage of detected compound being fragmented (i.e., >90%). MS2 spectral quality from Astral was assessed by comparing to data from traditional detectors. High quality MS2 fragmentation was observed for very low abundant lipids. The improved ratio of MS^2 fragmentation is a result of the faster scanning rates of the Astral mass spectrometer which therefore allows a higher and more confident annotation of unknown lipids. Around2700 lipids were annotated on Orbitrap Astral compared to 800 lipids on traditional detectors. Reducing the LC separation to 15 minutes gave around 1500lipids with confident annotations. The assessment of isotopically labeled internal standards revealed high data quality, reliability, and measurement robustness. Instrument performance, evaluated through metrics like retention time, mass accuracy, and signal response, demonstrated minimal chromatographic shift and consistent signal responses, as indicated by low % CV in quality control and sample replicates.

For Research Use – Not for Diagnostic Procedures.

Novel Aspect: A thorough untargeted LC-MS lipidomics workflow to facilitate deeper coverage and confident annotation of lipids.

Friday 8th March – 12h40-13h30

Proposition of a scoring system for data quality assessment in mass spectrometry-based lipidomics

Nils HOFFMANN

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Institute for Analytical Chemistry, Universitaet Wien, Austria

The increasing frequency of lipidomics publications in scientific literature demands robust quality standards to ensure comparability.The official shorthand nomenclature for lipids is a significant step towards reporting quality, but there's a need for a more granular approach. To address this, we propose a lipidomics scoring system that integrates mass spectrometry, chromatography, and ion mobility spectrometry data, assigning scores to different analytical layers. This scoring is also linked to annotation levels following the official shorthand nomenclature, aiding in assessing the reported compound details. Lipids, vital in cellular processes and life's biophysical foundation, have been studied through mass spectrometry since the 1990s, evolving into lipidomics for comprehensive lipid analysis. The lipid classification system by LIPID MAPS and subsequent shorthand nomenclature have contributed to standardization. However, a globally accepted scoring system for identification quality remains absent. Unlike proteomics, where relative scores assess database search quality, lipidomics lacks such a standard. As Lipidomics attracts researchers beyond mass spectrometry, the necessity for a transparent data quality assessment tool grows. Addressing this gap, the authors, as part of the International Lipidomics Society and the Lipidomics Standards Initiative, propose an additive lipidomics scoring system. This system aims to enhance transparency for non-mass spectrometry lipid researchers, reviewers, and editors, becoming a vital tool for data quality judgment. The scoring, expressed numerically, offers a standardized measure across platforms, experiments, and publications, while at the same time providing an unambiguous scoring code that allows to assess, which features where used for identification, and subsequently, score calculation. Even with the shorthand nomenclature providing insights into structural determination, variations in identification certainty persist, as demonstrated by examples. The proposed quality score provides a systematically defined and comparable metric, facilitating easy interpretation for non-experts and ensuring a standardized assessment of published data. It is immediately comparable within the same lipid class, and interpretable across classes, while allowing future expansion, once additional analytical methods may enhance the level of structural resolution. In conclusion, the lipidomics scoring scheme emerges as a versatile tool for researchers, reviewers, and editors, whether for self-control or peer-review processes. It complements the shorthand nomenclature, offering a more nuanced understanding of a compound's identification status. Its numerical expression enhances comparability and transparency, empowering non-experts to confidently interpret and draw conclusions from published data.

Lipidomic diagnostics of pancreatic cancer (LDPC): clinical validation of screening test for early detection of pancreatic cancer

Michal HOLČAPEK

University of Pardubice, Department of Analytical Chemistry, Czech Republic

Pancreatic cancer has the worst prognosis among all cancers. The main reason is that most patients are diagnosed too late in an incurable stage because pancreatic cancer does not have symptoms at an early stage, and no screening test exists so far. We have demonstrated in a cohort of 830 human blood samples that lipidomic analysis enables the differentiation of patients with pancreatic cancer from healthy controls with an accuracy of more than 90%, including the early stage [1], and is applicable for high-throughput analysis. Based on the patent [2] granted in EU, Japan, and Singapore, the University of Pardubice and FONS company established in 2022 spin-off company Lipidica with the goal of translating the screening methodology into real clinical practice. The OPLS-DA statistical models include concentrations of ca. 150 lipid species measured by ultrahigh-performance supercritical fluid chromatography-mass spectrometry method, which provides high robustness and short analysis time (<5 min). The major dysregulations are observed for very long chain sphingomyelins, ceramides, and some lysophosphatidylcholines. Now Lipidica, together with the Masaryk Memorial Cancer Institute and many cooperating clinics, is preparing the clinical validation of LDPC (Lipidomic Diagnostics of Pancreatic Cancer) test for high-risk groups according to the IVDR EU Regulation, which should start in the Czech Republic after the approval of the State Institute for Drug Control as the local authority. The clinical study includes high-risk subjects for pancreatic cancer due to a family history of pancreatic cancer (≥ 2 first- or second-degree relatives), selected genetic mutations (STK11, CDKN2A, APC, ATM, BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, EPCAM, PALB2, or TP53), or hereditary pancreatitis in agreement with the recommendations of the American Society for Gastrointestinal Endoscopy (ASGE), the Cancer of the Pancreas Screening Study (CAPS) guidelines, and the national consensus of the Czech professional societies. Our new data indicate that the LDPC test does not decrease accuracy even for very early stages of pancreatic cancer. Measurements from patients with other types of cancer show that similar lipidomic dysregulations are observed for multiple types of cancer [3]. The goal of our ERC Advanced project is the investigation of the biological mechanism of these dysregulations. The interesting observation is that altered lipidomic profiles do not return to normal even for cured patients with no symptoms for >5 years.

This work was supported by grant projects NU21-03-00499 from the Czech Health Research Council and ERC Advanced grant No. 101095860.

[1] D. Wolrab et al., Nat. Com. 13 (2022) 124.

[2] M. Holčapek et al., A method of diagnosing pancreatic cancer based on lipidomic analysis of a body fluid, European patent EP 3514545.

[3] D. Wolrab et al., Sci. Rep. 11 (2021) 20322.

Advancing targeted lipidomics for population profiling: insights and hindsights

Kevin HUYNH

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

The plasma lipidome is a complex mixture of diverse individual species and broad classes that exist in concentrations that span several orders of magnitude. Dysregulation of lipid metabolism has been identified in many diseases. For biomarker discovery and biological interpretation, substantial sample numbers are required to observe underlying lipid signatures that are often masked or confounded by natural inter-individual variation. Advancements to chromatographic separation approaches along with mass spectrometry technology has enabled comprehensive screening of lipids at run times suitable for large cohort studies. Over the last decade, Iterative development of our platform has resulted in a refined assay which has been applied to profile population and clinical studies including the Australian Diabetes, Obesity and Lifestyle Study (n=10,300) and the Busselton Health Study (n=4,492), the Australian Imaging Biomarkers and Lifestyle (n = 1,112 individuals with >4000 samples), the Alzheimer's Disease Neuroimaging Initiative study (n = 1,300 baseline, > 4000 samples).

Here we report our current lipidomics platform, and the lessons learnt from its development. Our current platform covers 45 lipid classes and subclasses, measuring 820 species using a targeted approach with reverse phase chromatography (ZORBAX Eclipse+ C18 RRHD, 100 x 2.1 mm, 1.8 µm with Solvent A; 50% H_2O / 30% ACN / 20% IPA with 10mM ammonium formate + 5µM medronic acid and Solvent B, 1% H_2O / 9% ACN / 90% IPA with 10mM ammonium formate. Samples consists of 10µl of plasma extracted into 100µl of 1:1 butanol / methanol.

Over the last decade, we've identified critical factors that have enabled large cohort analysis, notably the utilisation of single-phase lipid extraction, standardisation of sample processing, identifying and correcting sources of signal contamination and regulation of various HPLC conditions. Expansion and validation of new lipid classes and species (including many atypical species) resulted from several complementary approaches including the systematic generation of targeted lipid reference libraries from plasma, leveraging orthogonal mass spectrometry conditions, chemical synthesis / modification, and applying statistical approaches that capitalise on unique genetic and biological lipid signatures.

Deep targeted plasma lipidomics conveys sex-specificity and individuality of lipid signatures: a longitudinal study of apparently healthy population

Julijana IVANISEVIC

University of Lausanne, Switzerland

Lipid metabolism and circulatory lipid levels are tightly associated with the (cardio)metabolic health. Consequently, mass spectrometry (MS)-based lipidomics has emerged as a powerful phenotyping tool in epidemiological, human population, and in clinical intervention studies. However, ensuring high throughput and reproducible measurement of a wide panel of circulatory lipid species in large-scale studies poses a significant challenge. Even more, epidemiological studies require the acquisition of multiple sample cohorts over extended periods of time and are, therefore, subject to inherent variation in MS detection. In this talk, I will present our recently developed quantitative LC-MS/MS lipidomics platform and its application to the first subset of 1092 fasted plasma samples belonging to apparently healthy participants from prospective Lausanne population study. This high-coverage and high-throughput HILIC-based approach allowed for the robust measurement of 790 circulatory lipid species in each individuals' sample. Measured lipids spanned 22 different lipid classes and concentration range of six orders of magnitude. Optimal robustness results were achieved by combining automated sample preparation using stable isotope dilution approach (with 75 isotopically labeled standards) and the alternate analysis of NIST plasma reference material, as external quality control. The significantly lower intraindividual (compared to inter-individual) variability, and unsupervised sample clustering demonstrated the high individuality and sex-specificity of acquired lipid signatures. The most prominent sex differences were reported for sphingomyelins and ether-linked phospholipids which were found to be present in significantly higher concentrations in female plasma.

Friday 8th March – 9h00-9h30

Lipidomics in cell biology

Noemi JIMENEZ-ROJO

University of Geneva, Switzerland

Why has nature acquired such a huge lipid *repertoire*? Although it would be theoretically possible to make a lipid bilayer fulfilling barrier functions with only one glycerophospholipid, there are diverse and numerous different lipid species. Lipids are heterogeneously distributed across the evolutionary tree with lipidomes evolving in parallel to organismal complexity. At the cellular level, lipids are differentially distributed across organelles and membranes are asymmetric and laterally heterogeneous. This lipid asymmetry at different scales indicates that these molecules may play very specific molecular functions in biology, many of which remain still unknown. In this talk I will show how lipidomics combined with quantitative cell and chemical biology approaches can help us understand better membrane biology and uncover new lipid functions in cells.

Wednesday 6th March – 11h00-11h20

Sphingolipid analysis reveals metabolic differences in obese and lean asthmatic children from MENA region

Shana JACOB

Analytical Chemistry Core Facility, Sidra Medicine, Qatar

Asthma stands as a prevalent chronic ailment among children globally, with an elevated susceptibility in those with obesity. Research indicates that obesity not only impacts early-onset atopic asthma but also contributes to the emergence of late-onset non-atopic asthma. Disruptions in sphingolipid metabolism have been linked to airway hyperreactivity and asthma. This study aimed to explore potential alterations in sphingolipid metabolism in childhood obesity-related atopic and non-atopic asthma.

Blood samples were obtained from five distinct groups (lean-atopic asthmatic, obese-atopic asthmatic, obese non-atopic asthmatic, obese non-asthmatic, and healthy controls), each consisting of 10 individuals aged 6-18 years, residing in Qatar. Erythrocytes from these samples underwent analysis to assess the concentration of 26 specific sphingolipids, including sphingomyelins (SM), ceramides (Cer), and dihydroceramides (DiCer), using liquid-chromatography-triple quadrupole mass spectrometry.

The sphingolipid concentration values underwent single-factor ANOVA, and lipids with a p-value less than 0.05 were subjected to further t-test analysis. Significant variations in specific sphingolipids were observed in atopic-obese asthma compared to healthy controls (24:1SM, 24Cer, 24DiCer, and 24:1Cer, $p < 0.05$; 16:1SM and 24:1DiCer, $p < 0.005$). Conversely, no significant differences were noted between non-atopic obese asthma and healthy controls. Furthermore, several noteworthy differences were identified between obese-atopic asthma and lean-atopic asthma, with particular emphasis on 24Cer and 24:1DiCer ($p < 0.0005$). Notably, these two lipids did not exhibit significant differences when comparing obese non-asthmatic individuals with healthy controls.

The outcomes of this study suggest that sphingolipid metabolism undergoes alterations in various paediatric asthma types. Atopic-obese asthma and non-atopic obese asthma exhibit distinct sphingolipid profiles, with 24Cer and 24:1DiCer levels influenced by the combination of obesity and atopic asthma. These modified sphingolipid profiles suggest the potential of these lipids as biomarkers and therapeutic targets for tailoring personalized medicine for different asthma endotypes.

Multi-platform lipidomic profiling reveals age-related changes to skin lipids, impacting the epidermal barrier

Alexandra C. KENDALL

Faculty of Biology, Medicine and Health, The University of Manchester, UK

The lipid profile of human skin is crucial for an effective epidermal barrier, maintaining the structure of the skin and keeping water in, and chemicals, irritants and pathogens out. As we age, skin becomes fragile, and more vulnerable to damage, irritation and infection. We sought to investigate how the cutaneous lipid profile changes with age, and whether these changes differ in males and females. Adhesive tapes (D-SquameTM, Clinical and Derm) were used to sample lipids from the skin of male (n=46) and female (n=52) healthy volunteers aged 18-40yrs and 70+yrs. Samples were taken from three anatomical sites: forehead (a sebaceous site), armpit (moist) and buttock (dry, photoprotected). Photoprotected (buttock) skin biopsies were also taken from a subset of volunteers, in order to assess epidermal morphology and markers of intrinsic ageing. Ultra-high performance liquid chromatography coupled to tandem mass spectrometry with electrospray ionisation (Acquity UPLC and Xevo TQ-S,Waters) was used to analyse epidermal ceramides. Ultra-high performance supercritical fluid chromatography coupled to quadrupole time-of-flight mass spectrometry with electrospray ionisation (AcquityUPC^2 and Synapt G2, Waters) was used to analyse free fatty acids (FFA), triacylglycerols (TG) and diacylglycerols (DG). Gas chromatography with flame-ionisation detection (6850 system, Agilent) was used to analyse cholesterol. Epidermal morphology was assessed by immunohistochemistry. Principal component analysis (PCA) and bootstrapped LASSO regression modelling were used to assess associations between epidermal lipids, body site and age, in males and females. Using mass spectrometry lipidomics, 902 species of ceramides, 60 species of FFA, 97 species of TG and 51 species of DG were identified and semi-quantitated using class-specific deuterated internal standards. PCA of all lipids revealed separation of the sebaceous forehead site from the non-sebaceous armpit and buttock sites. Abundance of FFA, TG and DG was higher in forehead samples, likely reflecting the contribution of sebum, whilst the level of ceramides was similar at the three anatomical sites. Comparison of young and aged skin revealed decreased levels of FFA, TG, DG and ceramides in older subjects, with more pronounced changes in females. Ceramides were also shorter in the older cohort. Bootstrapped LASSO regression modelling revealed that the epidermal ceramide profile was a strong predictor of age and body site, in both males and females. The lipid changes observed in aged individuals reflected increased skin surface pH and transepidermal water loss, and altered epidermal morphology, demonstrating the impact of ageing on epidermal barrier function. Analysis of a wide range of lipid classes and species has revealed age-related changes to the epidermal lipidome, which may contribute to the impaired barrier function of aged skin. Epidermal lipids showing strong associations with ageing are potential targets for nutritional and topical interventions to enhance skin health into later life.

Exploration for the personalized combinatorial chemotherapies for neuroblastoma

Hsinyu LEE

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Neuroblastoma is the most common extracranial solid tumor of childhood. In our previous efforts, we have demonstrated the involvement of aryl hydrocarbon receptor (AHR) during the tumorigenesis process of neuroblastoma. Through metabolomic strategies, novel endogenous ligands for AHR were identified. Activation of AHR by its ligand leads to the differentiation of tumor suggested it may develop into a treatment strategy. Due to different mutation profiles in patients, personalized medicine is the most desirable future for cancer therapy. However, a comprehensive combinatory effect of chemotherapy treatments is difficult to achieve due to limited tumor tissue obtained from biopsy. Microinjection and fabrication procedures were developed to assess for effectiveness of combinatorial effect of chemotherapies using limited number of cells. We have tested the combinatorial effect of chemo drugs on our system to find out the best treatment. The results were evaluated in different cancer cell lines and confirmed in xenograft tumor models. We are currently testing the effectiveness of our system using genetically modify neuroblastoma animal model. The activation of AHR and combinatory chemotherapy strategy may improve the effectiveness of current neuroblastoma treatments.

Using the food metabolome to assess dietary intakes in a multi-ethnic Asian population

Dorrain LOW

Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

Dietary biomarkers reflecting habitual diet are explored largely in European and American populations. However, the "food metabolome" is highly complex, with its composition varying to region and culture. Here, by assessing 1,055 plasma metabolites and 169 foods/beverages in 8,391 comprehensively phenotyped individuals from the multi-ethnic Asian HELIOS cohort (69% Chinese, 12% Malay, 19% South Asian), we report novel observations for ethnic-relevant and common foods. Using machine-learning feature selection approach, we developed dietary multi-biomarker panels (3-39 metabolites each) for key foods and beverages in respective training sets.

These panels comprised distinct and shared metabolite networks, and captured variances in intake prediction models in test sets better than single biomarkers. Composite metabolite scores, derived from the biomarker panels, associated significantly and more strongly with clinical phenotypes (HOMA-IR, type 2 diabetes, BMI, fat mass index, carotid intima-media thickness and hypertension), compared to self-reported intakes. Lastly, in 235 individuals that returned for a repeat visit (averaged 322 days apart), diet-metabolite relationships were robust over time, with predicted intakes, derived from biomarker panels and metabolite scores, showing better reproducibility than self- reported intakes. Altogether, our findings show new insights into multi-ethnic diet-related metabolic variations and new opportunity to link exposure to health outcomes in Asian populations.

Precision medicine: development and translation of metabolic and cardiovascular risk scores.

Peter J. MEIKLE

Baker Heart and Diabetes Institute, Australia
Baker Department of Cardiovascular Research Translation and Implementation, La Trobe University, Australia

Dysregulation of lipid metabolism underpins multiple diseases, including type 2 diabetes (T2D), cardiovascular disease and age-related dementia. While the metabolic pathways of lipids are well characterised, dysregulation of the same pathways due to environmental and genetic influences is not well understood. We have developed a high throughput lipidomics platform that can analyse over 750 individual lipid species from 10 µL plasma in 15 minutes. Application of this platform to large clinical and population studies is providing new insight into the role of lipid metabolism in chronic disease, new markers for risk assessment and the opportunity for integration with genomic datasets for a systems approach to identify causal pathways with the potential for therapeutic intervention.

Our recent analyses of two large population cohorts; the Australian Diabetes, Obesity and Lifestyle Study (AusDiab, n=10,300) and the Busselton Health Study (BHS, n=4,492), have provided an opportunity to investigate new concepts in population lipidomics. These include the use ridge and LASSO regression models for the development of metabolic BMI and metabolic AGE scores, that can provide new, easily interpretable, measures of metabolic health. Using an analogous strategy, we have developed a lipidomic risk score (LRS) for cardiovascular disease by using the lipidome (predictor) to model the Framingham risk score (FRS, outcome). The LRS provided an improvement, over the FRS, with an increase in the AUC of 0.11 (95% CI 0.04-0.19) and 0.08 (95% CI 0.01-0.14) in the intermediate-risk group (0.1 ≤ calibrated FRS < 0.2) in AusDiab (cross-validation) and BHS (external validation), respectively. In AusDiab, the LRS yielded a continuous net reclassification improvement (NRI) of 0.20 (95% CI 0.01-0.39) among cases and 0.19 (95% CI 0.11-0.28) among non-cases. In BHS, the NRI was 0.25 (95% CI 0.03-0.46) among cases and 0.12 (95% CI 0.03-0.22) among non-cases.

When we combined the lipidomic risk score with a polygenic risk score (based on DNA variants) the predictive ability of the combined score was further improved. Focusing on people classified as intermediate risk, the combined score was able to reclassify more than half of those who went on to have a cardiovascular event as high risk. When used in clinical practice this would allow more effective, targeted treatment of these high-risk individuals to prevent future cardiovascular events.

We have recently established a clinical lipidomics platform (CLP) than can measure 250 lipid species in a 5 min run time and are working with our partners Trajan Scientific and Medical to translate these metabolic health, and cardiovascular risk, scores into clinical and population health services.

Deuterated lipids from the National Deuteration Facility, ANSTO, Australia

Michael MOIR

National Deuteration Facility, ANSTO, Australia

The National Deuteration Facility (NDF), ANSTO is internationally known for the provision of a wide variety of deuterium labelled molecules. Facility users from academic institutions and industry can access deuterated compounds via the user merit scheme or commercially. Our deuterated molecules are used in a diverse range of experiments spanning neutron scattering and reflectometry, NMR spectroscopy, mass spectrometry, IR spectroscopy and drug development. One of the key capabilities of the NDF is the production of a large catalogue of custom deuterated lipids, many which are not commercially available. Synthetic and biosynthetic expertise in lipid production allows for the provision of different classes of lipids with deuteration levels and regiospecific deuteration tailored to suit the needs of our users. The talk will showcase the diverse range of deuterated lipids the NDF can produce, including unsaturated and polyunsaturated fatty acids, phospholipids and glycerides and sterols and highlight the impact of deuteration on research outcomes.

Wednesday 6th March – 16h20-16h40

LPPtiger 2 enhanced epilipid-centric workflow

Zhixu NI

Center of Membrane Biochemistry and Lipid Research, University Hospital Carl Gustav Carus and Faculty of Medicine of TU Dresden, Germany

Epilipidome, a set of lipids formed by enzymatic and non-enzymatic modifications, significantly enhance the complexity of native lipidomes. Among epilipids, products of lipid (per)oxidation and oxygenation have been actively studied over the last decades with numerous excellent examples illustrating their roles in directing cell fates and signalling events. Oxidized lipids play significant part in regulating immune responses during tissue injuries and development of multiple metabolic pathologies. With the key role of lipid peroxidation in the execution of ferroptosis, the link between lipid metabolism and cell death pathways emerged. Thus, detection, identification, and quantification of oxidized glycerophospho- and neutral lipids are of significant interest for understanding pathomechanisms of metabolic, malignant, degenerative and inflammatory conditions. However, integrated workflows for detailed molecular characterization, and identification of oxidized lipids from high-throughput LC-MS data are largely missing. Lack of the suitable computational tools capable to address accurate annotation of modified lipids is among the main bottleneck in high-throughput screening for this class of epilipids.

LPPtiger software established a novel workflow based on the *in silico* prediction of customized epilipidome from defined lists of unmodified phospholipids, flowed by their *in silico* fragmentation and identification using multiple scoring algorithms. Here, we present LPPtiger 2 which extends the epilipid-centric workflow by covering oxidized neutral lipids (oxTG, oxDG, and oxCE), with improved *in silico* prediction and fragmentation analysis. LPPtiger 2 also enables users to export customized *in silico* predicted epilipidome, including elemental compositions, fragmentation patterns, corresponding structures in SMILES format, and fingerprint *m/z*, in a form of reusable library as JSON files. These JSON files provide complete open access to all predictions and identification details, making it possible for data scientists to conduct further advanced post-processing. Among other new features of LPPtiger 2 is a new “Inclusion List Generator” module to support direct design of targeted (PRM/MRM) acquisition methods with automatically generated *m/z* lists of epilipids precursor and corresponding essential fragment ion associated with modifications. LPPtiger 2 is an open-source software released under AGPL license and is cross-platform freely available for academic users (GitHub repository: https://github.com/LMAI-TUD/lpptiger2).

Bioactive lipids mediating the functionality of the epidermal barrier

Anna NICOLAOU

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The skin presents a multifaceted barrier that protects the body from external threats including exposure to ultraviolet radiation, pathogens and chemicals, and prevents excessive trans-epidermal water loss. These varied activities are reliant on a vast array of lipids, many of which are unique to skin. Skin lipids support the structure and function of the epidermal permeability barrier as well as the associated chemical, microbiological and immunological barriers.

The epidermal physical barrier is dependent on a specific mixture of free fatty acids, cholesterol and ceramides, that surround the terminally-differentiated keratinocytes found in the outmost layer of the epidermis, whilst sebum- and keratinocyte-derived acylglycerols, fatty acids and oxylipins cover the skin's surface, controlling the pH of the chemical barrier, and supporting and regulating the skin microbiome. Fatty acid-derived lipid mediators including eicosanoids, octadecanoids and endocannabinoids, and various sphingolipids signal between resident and infiltrating cutaneous immune cells, driving numerous cutaneous processes, including cutaneous inflammation and its resolution. These diverse and complex lipids are necessary for maintaining homeostasis in healthy skin, and are implicated in many cutaneous diseases, systemic conditions with cutaneous involvement, and natural processes including ageing. Lipids also contribute to the gut-skin axis, signalling between these two important barrier sites.

The properties of the cutaneous lipidome clearly show that skin lipids can provide a valuable resource for exploration of healthy cutaneous processes, local and systemic disease development and progression, and accessible biomarker discovery, as well as an opportunity to fully understand the relationship between the host and the skin microbiome.

Friday 8th March – 11h00-11h30

Visualizing the lipid literature by integrating text mining and computational chemistry

Magnus PALMBLAD

Leiden University Medical Centre, The Netherlands

The open-access scientific literature contains a wealth of information that can be retrieved through text mining. However, it is not always trivial to analyze and visualize this information. In Monday's workshop, we learned how to build networks from the literature by combining Europe PMC and the VOSviewer. Here I will discuss how to extend these tools by combining Europe PMC queries, the Europe PMC Annotations API, computational chemistry and machine learning for large-scale visualization of properties of small molecules, including lipids, appearing in specific methodological or biological contexts. The workflow is demonstrated on comparisons of analytical chemistry techniques to study lipids and lipids appearing in different biomedical (disease) research, but the same workflow may also be used to aid experimental design, drug discovery, chemical syntheses, business intelligence, and studies of the history of science. The workflow is implemented in the SCOPE platform, which is entirely coded in Python and open source, and uses only freely available data, services, and software that scale to millions of publications and named chemical entities in the literature.

Thursday 7th March – 15h00-15h30

Specialized pro-resolving mediator 15-epi-lipoxin A_5 promotes tissue exudate neutrophil clearance

Hong Yong PEH

Department of Pharmacology, National University of Singapore, Singapore

Specialized proresolving mediators (SPMs) promote macrophage efferocytosis and induction of apoptosis but excess leukocytes early in inflammation require additional leukocyte clearance mechanisms for resolution. Here, neutrophil clearance from localized acute inflammation was investigated in mouse dorsal air pouches. Using LC-MS/MS to profile the exudates, levels of 15-hydroxyeicosa-5Z,8Z,11Z,13E,15S,17Z-pentaenoic acid (15-HEPE) were increased. Activated human neutrophils converted 15-HEPE to 5R,6R,15R-trihydroxy-eicosapentaenoic acid (15-epi-lipoxin A_5) and 5S,15S-dihydroxy-eicosapentaenoic acid (resolvin E4). Exogenous SPM analogs were effective in resolving neutrophilia through the known routes of apoptosis and local tissue macrophage efferocytosis, however, the data suggested another route of clearance. We employed CD45 variant animals to track the fate of the neutrophils in the air pouch. Not only did the lipoxin analog augmented exit of the adoptively transferred neutrophils into the systemic circulation, but it also activated splenic $SIRPa^+$ and $MARCO^+$ macrophages to efferocytose these neutrophils. These findings demonstrate new systemic resolution mechanisms for localized tissue inflammation and SPMs play an active role in accelerating the resolution of inflammation.

Thursday 7th March – 14h20-14h40

Control of intracellular cholesterol distribution via non-vesicular lipid transport at membrane contact sites

Yasunori SAHEKI

Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
Institute of Resource Development and Analysis, Kumamoto University, Japan

The distribution of cellular cholesterol between various organelles and membranes is precisely controlled, and its dysregulation is associated with numerous diseases, including cardiovascular and neurodegenerative diseases. Regulated transport of cellular cholesterol is essential for maintaining its proper distribution, yet the underlying mechanisms remain incompletely understood. Cells either synthesize cholesterol in the endoplasmic reticulum (ER) or acquire it from the extracellular environment primarily via uptake of lipoproteins. While the regulatory network that controls cholesterol production and uptake is located in the ER, cholesterol is more enriched in other cellular compartments with highest enrichment in the plasma membrane (PM). Therefore, the ER needs to sense the levels of cholesterol in various other cellular membranes and adjusts its production and uptake. Such homeostatic regulation is facilitated, at least in part, through non-vesicular transport of a specific pool of cholesterol, termed accessible cholesterol, by lipid transfer proteins (LTPs). We previously showed that evolutionarily conserved and ER-anchored LTPs, namely the GRAMD1s/Asters (GRAMD1a/1b/1c) (Lam/Ltc proteins in yeast), contribute to this process by transporting accessible cholesterol from the PM to the ER at the sites of contacts formed between these cellular compartments[1-3]. Importantly, GRAMD1b dysfunction is associated with various neurological disorders, including schizophrenia, intellectual disability, and multiple sclerosis, suggesting critical roles of GRAMD1b (and potentially other GRAMD1s) in maintaining neuronal development and function. In my presentation, I will describe the molecular basis of cholesterol sensing and transport mediated by GRAMD1s. I will also describe our characterization of the interplays between GRAMD1s and oxysterol-binding protein (OSBP)-related proteins (ORPs), which collectively contribute to the maintenance of cellular cholesterol distribution[4]. Finally, I will discuss our recent development of a novel cholesterol biosensor based on the cholesterol sensing element (GRAM domain) of GRAMD1b and visualization of the distribution of accessible cholesterol in real-time in various cell types, including iPSC-derived neurons[5]. Our study provides deeper insights into the molecular mechanisms of intracellular cholesterol distribution and reveals a new link between dysregulation of intracellular cholesterol transport and abnormal brain function.

1. Naito T, Ercan B, Krshnan L, Triebl A, Koh DHZ, Wei FY, Tomizawa K, Torta FT, Wenk MR, and Saheki Y (2019). Movement of accessible plasma membrane cholesterol by the GRAMD1 lipid transfer protein complex. *eLife*. 8: e51401.
2. Ercan B*, Naito T*, Koh DHZ, Dharmawan D, and Saheki Y (2021). Molecular basis of accessible plasma membrane cholesterol recognition by the GRAM domain of GRAMD1b. *EMBO J.* 40: e106524. *Co-first authors.
3. Naito T and Saheki Y (2021). GRAMD1-mediated accessible cholesterol sensing and transport. *BBA - Molecular and Cell Biology of Lipids*. 1866: 158957.
4. Naito T, Yang H, Koh DHZ, Mahajan D, Lu L, and Saheki Y (2023). Regulation of cellular cholesterol distribution via non-vesicular lipid transport at ER-Golgi contact sites. *Nature Communications*. 14: 5867.
5. Koh DHZ, Naito T, Na M, Yeap YJ, Rozario P, Zhong FL, Lim KL, and Saheki Y (2023). Visualization of accessible cholesterol using a GRAM domain-based biosensor. *Nature Communications*. 14: 6773.

Friday 8th March – 9h30-10h00

Skin sphingolipids in health and disease

Roger SANDHOFF

German Cancer research center, Germany

The epidermis provides terrestrial vertebrates with a pivotal defensive barrier against water loss and harmful pathogens. The lipid-enriched lamellar matrix that embeds the enucleated corneocytes of the stratum corneum is a vital demand for this epidermal permeability barrier. Ceramides are major components of these highly ordered intercellular lamellar structures, in which linoleic acid- and protein-esterified ceramides are crucial for structuring and maintaining skin barrier integrity. These special ceramide structures and topology demand adaptation of ceramide metabolism during keratinocyte maturation and the expression of unique gene combinations. Therefore, genetic mutations of genes involved in ceramide metabolism often result especially in skin barrier deficiencies although some of these genes are ubiquitously expressed in our body. Moreover, the complex ceramide metabolism leads in human epidermis to the most manifold ceramide pattern by that still challenging modern lipid analytics.

Friday 8th March – 10h30-11h00

Ultra-high-resolution shotgun mass spectrometry for the organism-wide assessment of lipids turnover kinetics

Andrej SHEVCHENKO

Max Planck Institute of Molecular Cell Biology and Genetics, Germany

Lipidome is dynamic. Stable steady-state concentration of individual lipids is maintained by permanent lipids remodelling orchestrated by multiple interconnected biosynthesis pathways. While dynamics of lipids remodelling is the critical factor for sustainable lipid homeostasis *in vivo*, there is no generic analytical method for providing the quantitative assessment of how lipids turnover differs between lipid classes and molecular species in different organs, and how it is changing with organism age. Feeding animals with common lipid precursors (*e.g.* $^{13}C_6$-glucose) they produce cumbersome isotopic patterns influenced by both fatty acid synthesis and lipids turnover. In contrast,^{15}N labeling yields simpler isotopic profiles of major classes of membrane lipids and is arguably more suited to full organism labelling.

We combined *in vivo* ^{15}N-metabolic labelling using isotopically enriched food with shotgun ultra-high-resolution mass spectrometry and determined the absolute (molar) abundance and ^{15}N incorporation rate for 120 species from major classes of glycerophospholipids, sphingolipids and glycosphingolipids in mouse plasma; whole blood; four distinct regions of brain and liver obtained from the animals of different age. We achieved ultra-high mass resolution (~1.5M @ *m/z* 200) by coupling Q Exactive Orbitrap mass spectrometer with Booster X2 data acquisition system and quantified lipids by LipidXplorer software. Within the first isotope cluster of unlabelled lipid precursors, we baseline resolved peak of ^{13}C-isotope of unlabelled and monoisotopic peak of ^{15}N-labelled lipid species that are spaced by 0.00633 Da. The kinetics of ^{15}N-incorporation was independently validated by analysing phosphatidyl-containing lipids by MS/MS and reading out the peak intensity of ^{15}N-containing head group.

In this way, we produced a comprehensive and expandable resource that delineates the organ- and age-specific turnover rate of major membrane lipid species together with their absolute (molar) abundance. We report on significant differences in lipid class-dependent kinetics and ^{15}N-incorporation rates across different organs that provided direct, quantitative, and comprehensive assessment of lipid homeostasis *in vivo*.

Wednesday 6th March – 15h30-16h00

Sponsored talk – Agilent Technologies

Exploring the great unknown: new tools to assess complex environmental mixtures

Shane A. SNYDER

Nanyang Environment and Water Research Institute (NEWRI); Nanyang Technological University, Singapore
Civil & Environmental Engineering; Nanyang Technological University, Singapore

Pollution never occurs as a few or even several discrete substances within the environment. Conversly, environmental contaminants occur within extremely complex mixtures of anthropogenic and endogenous substances. Monitoring programs often focus on a limited subset of contaminants based on previously reported or known propesity for occurrence and/or harm. However, many pollutants are not identified and realized until damage has already occurred. Synthetic chemicals were not part of the human experience until the late 19th century with the accidental discovery of the synthetic dye, mauve. Today, more than 65 million chemicals are commercially available, many of which enter the environment both knowingly and unknowingly. From a water exposure standpoint, not only are the chemicals themselves a potential risk, but also the innumerable transformation products formed during water treatment processes. Historically, analytical techniques to measure trace levels of environmental pollutants were time-consuming, labour-intensive, and technically-sophisticated. New techniques are rapidly evolving to measure ultra-trace levels of chemicals with automated and/or minimum sample preparation and detection by tandem and/or high-resolution mass spectrometry. Monitoring indicator compounds that provide specific information regarding watershed contamination and treatment process efficacy by high-resolution quadrupole mass spectrometry (QTOF) coupled to both GC and LC interfaces provides both targeted analytic information while simultaneously acquiring full mass spectra that allows a more comprehensive view of the complex chemical mixtures and results transformation products in water. Coupled with high-throughput bioassays, these techniques provide a new view as to the complex mixtures occuring in the environment and their potential impacts to health. Recently, high performance analytical platforms are coupled with advanced genomics tools which can even encompas pathogen monitoring in environmental samples, such as monitoring of the Sars-CoV-2 virus in municipal sewer systems, which can be normalized to chemical markers to provide a wide-population level evaluation of disease occurrence and transmission. Another recent discovery is that vulcanization agents which leach from tires are among the most toxic substances ever evaluated on salmonid fish. In our work, we chlorinate on of the common vulcanization substances known to occur in surface waters, and demonstrate that the resulting chlorination byproducts are more toxic than the parent compound, as evaluated through several in vitro methods. This presentation will demonstrate some of the latest findings and provide a view for the future of addressing the unknown world of chemical and biological mixtures in the environment.

Thursday 7th March – 12h40-13h30

Production of microbial lipids sustainably through adaptive evolution and side stream valorization

Naazneen SOFEO

*Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology, and Research (A*STAR), Singapore*

Most food oil and fat are currently obtained from plants and animals. However, pollution caused by livestock farming and limited availability of land for agriculture has led to global efforts for sustainable production of fat. Single cell oil using oleaginous yeasts is a promising alternative to animal and plant-derived lipids. But substrate costs for microbial fermentation are a major bottleneck. Using side streams as alternative to substrates like glucose, for growing yeast, is a potential cost-effective solution. By combining our recently reported process of growing yeasts on a solid Cocoa Fatty Acid Distillate (CFAD) side stream (DOI:10.1016/j.biortech.2023.129630) with adaptive evolution, we improved the growth of Yarrowia lipolytica by 2-fold and boosted lipid yield by more than 3-fold (DOI:10.1016/j.biortech.2024.130302). We were also able to generate lipid compositions that closely matched the profile of pork fat. By optimizing the fermentation conditions, we successfully produced lipids with more than 90% total unsaturation and improved biomass production in the wild type strain by more than 2-fold. Using transcriptomics, we identified key genes that is involved in adaptation to side stream and enhancement of lipid titre. We also identified candidate genes that enable efficient growth and utilization of fatty acids from the CFAD side stream. In summary, our research has laid a foundation for a sustainable microbial lipid production platform using oleaginous yeast.

The ceramide double bond that causes cardiometabolic disease

Scott Summers

Department of Nutrition and Integrative Physiology, University of Utah, USA

Overnutrition, physicalinactivity, and genetic aberrations promote the accumulation of fat-derivedmolecules in tissues not suited for lipid storage, leading to tissuedysfunction that underlies diabetes and cardiovascular disease. Of the myriad of lipids that accumulate, sphingolipids such as ceramides may be amongst themost deleterious, as they alter metabolic programs and induce apoptosis andfibrosis. In humans, serum ceramides are strong biomarkers of diabetes andmajor adverse cardiac events, and clinics have started measuring circulatingceramides as markers of disease risk. In rodents, inhibiting ceramidebiosynthesis ameliorates diabetes, steatohepatitis, and heart failure. The author will discuss the therapeutic potentialof a new approach to inactivate ceramides and combat these metabolic pathologies.

Friday 8th March – 14h00-14h30

Concordant inter-laboratory derived concentrations of ceramides in human plasma reference materials via authentic standards

Markus WENK

College of Health & Life Sciences, Hamad Bin Khalifa University, Qatar

The inventory of lipid molecules found in blood plasma (plasma lipidome) offers insights into individual metabolism and physiology in health and disease. Plasma lipidomics based on mass spectrometry has become a powerful tool to support clinical research towards innovative approaches in precision health and medicine. However, the translation of laboratory-developed tests towards robust, rapid and quantitative tests that deliver concordant results across different analytical platforms and sites does require considerable efforts. Specifically, I will present results on behalf of an extensive community effort hosted by the International Lipidomics Society (ILS). We compared measurements between 34 laboratories from 19 countries, utilizing mixtures of stable isotope labelled authentic synthetic standards, to quantify by mass spectrometry four clinically used ceramide species in the NIST (National Institute of Standards and Technology) human blood plasma Standard Reference Material (SRM) 1950, as well as new suite of candidate plasma reference materials (RM 8231).

Novel insights into the regulating of cellular lipid homeostasis

Christian WOLFRUM

ETH Zürich, Switzerland

Metabolic crosstalk of the major nutrients, glucose, amino acids and fatty acids ensures systemic metabolic homeostasis. The coordination between the supply of glucose and fatty acids to meet various physiological demands is especially important as improper nutrient levels lead to metabolic disorders, such as diabetes and nonalcoholic steatohepatitis (NASH). In response to the oscillations in blood glucose levels, lipolysis is thought to be mainly hormonally regulated to control fatty acid liberation by insulin, catecholamine and glucagon. Our current work suggests that this concept might be wrong, and that glucose and fatty acids are indeed direct effectors of this process. The presentation will focus on newly identified mechanisms of fatty acid and glucose sensing in liver and adipose tissue, which are important regulatory pathways determining the rate of fatty acid release from lipid droplets by post-transcriptional regulation of ATGL stability through different ubiquitin ligases. Targeting of these pathways by genetic and pharmacological approaches leads to the amelioration of ectopic lipid deposition in liver and might thus serve as a new therapeutic angle for NALFD/NASH.

Wednesday 6th March – 10h30-11h00

Abstracts of Poster Presentations

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* Location is subject to last minute changes. Please check information in the poster room or at the registration desk.

Size Exclusion Chromatography - Static Dual-Angle Light Scattering and Refractive Index Detection Method for Analysis of Stability of mRNA-Lipid Nanoparticles

Suresh BABU C.V.[1], Ravindra Gudihal[1], Erhan Simsek[1], Li Zhang[2], Melgious Jin Yan Ang[2], Yi Yan Yang[2]

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2 Bioprocessing Technology Institute, Agency for Science, Technology and Research, Singapore

Lipid nanoparticles (LNPs) have been used very effectively for the delivery of nucleic acid payloads. The stability of mRNA loaded LNPs (mRNA-LNPs) is of widespread interest for the successful development of mRNA vaccines. The stability of mRNA-LNPs in physiological fluids such as blood plasma is an equally important consideration when developing new therapeutics because it strongly influences the drug's efficacy and pharmacokinetics. In this study, we use size exclusion chromatography (SEC) with on-line static dual-angle light scattering and differential refractive index detection to characterize the reaction kinetics of mRNA-LNPs in human plasma. We show that the degradation kinetics of a widely used mRNA-LNPs composition can be measured in real-time by changes in hydrodynamic radius (R_h) and molecular weight (Mw). The results show that having an on-line, high-resolution SEC step greatly increases the power of light scattering analysis as it eliminates interference from plasma components that typically confound bulk dynamic light scattering (DLS) analyses.

MiDAR: Reproducible Data Processing, Quality Control, and Reporting of Large-Scale Targeted Lipidomics Analyses

Bo BURLA

Singapore Lipidomics Incubator, Life Sciences Institute, National University of Singapore, Singapore

Data processing steps in published lipidomics studies are often inadequately documented, limiting the evaluation of data quality and the potential for reuse of datasets. The R package {midar} aims to provide a toolset for structured, documented, sharable, and reproducible data processing of targeted small molecule mass spectrometry analyses. {midar} offers flexible functions for importing data and metadata, normalizing and quantifying analytes, correcting for drift and batch effects, conducting quality control assessment and filtering, as well as exporting and sharing the processed data. The utility of these tools is demonstrated through their application in method development and in the processing and quality control of large-scale lipidomics analysis.

Identification of lipid biomarkers for risk stratification of vascular conditions in the emergency department

Jing Kai CHANG[1], Markus R. Wenk[1], Federico Torta[1], Hyungwon Choi[1], Win Khaing Nwe[2], Yvette Jee[2], Win Sen Kuan[2]

1 Department of Biochemistry, National University of Singapore, Singapore
2 National University Hospital, Emergency Medicine Department, Singapore

Vascular conditions possess strong links with inflammation and are the leading global cause of morbidity and mortality, but existing methods in the Emergency Department (ED) for assessment of patient prognosis are lacking in sensitivity and objectivity. Early risk stratification of vascular conditions is crucial for management and intervention in the early pathophysiological stages so as to reduce disease burden, by ensuring that medical resources, manpower and attention are appropriately focused on patients that require them the most. Therefore,we aim to identify lipid biomarkers for risk stratification of these conditions through a lipidomics prospective cohort study of 500 ED patients at National University Hospital. Using targeted lipidomic analysis, candidate lipid biomarkers such as Ceramide d17:1, LPC and Thromboxane species have been identified in elastic net prediction models for clinical outcomes of interest such as hospital readmissions and transfer from general ward to ICU. Future efforts will involve longitudinal analysis of identified markers and confirmation of these findings, which may then be translated and utilized by healthcare professionals to improve decision making processes in healthcare facilities.

Fast and precise lipidomic analysis using ultra-high performance supercritical fluid chromatography coupled to mass spectrometry

Michaela CHOCHOLOUSKOVA[1,2], Federico Torta[1,3]

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2 Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
3 Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Recently, lipidomic analyses are led by fast and precise analytical methods. Ultra-High Performance Supercritical Fluid Chromatography coupled to Mass Spectrometry (UHPSFC/MS) belongs to the fastest analytical approaches suited for high-throughput analysis of both non polar and polar lipid classes. UHPSFC/MS methods use a supercritical fluid (mainly carbon dioxide) as a mobile phase. This offers significant advantages, such as low viscosity, high compressibility, and high diffusion. So far, we have optimized the UHPSFC/MS method for separation of 33 lipid classes/subclasses from 6 lipid categories within 5 min with excellent precision. More than 90% of the lipid species showed a CV below 20% (when using a triple quadrupole). UHPSFC separation connected to high resolution mass spectrometry (Q-TOF) has shown even better results. Our UHPSFC method is based on lipid class separation, leading to the co-ionization of internal standards and analytes from the same class. This approach brings advantages to quantitation and data processing. Finally, we show how to quickly determine with a single UHPSFC/MS method and single run the levels of free, esterified and total cholesterol for clinical research applications based on the application of in-source fragmentation. The determination of different levels of cholesterol was applied to Standard Reference Material 1950 – Metabolites in Frozen Human plasma (NIST) and compared to certified values with high accuracy (<8.2%).

Investigating the chronometabolome: untangling the alterations with semi-targeted metabolomics

Jiangwen DONG, Yi Ning Yong, Leroy Sivappiragasam Pakkiri, Christiani Jeyakumar Henry, Sumanto Haldar, Maxine Bonham, Chester Lee Drum

National University of Singapore, Singapore

Circadian rhythms in humans are driven exogenously by zeitgebers including food intake. It firmly regulates the metabolism process in tissues and organs. The disturbing of circadian rhythms induced by unfavorable food intake conditions such as late eating and high glycemic index (GI) diet will cause the metabolomic dysregulation and lead to severe diseases like cardiometabolic disease. With the power to phenotype body metabolism, the field of metabolomics is developing rapidly on its clinical application for circadian rhythms, chrono-nutrition, and metabolomic dysregulation related research. This study aims to reveal the effects of different food intake conditions - including meal timing and dietary composition - on body metabolome, which manifest as the alterations of key metabolites and pathways. LC-MS/MS based semi-targeted metabolomics approach is employed, together with a medium throughput panel of more than 300 monitored transitions and metabolites. Preliminary investigation has been performed on the post-prandial metabolome of high or low GI meal taken as dinner or breakfast. The findings indicate that meal timing exhibits stronger effects on metabolome than dietary composition, and that late eating relates closely to increasing metabolomic dysregulation and disease risk. Evidences are shown as the significant alterations of disease related metabolites and pathways such as the Arginine-NO pathway. Follow-up investigation will be conducted on post-prandial metabolome of low-GI meal consumed at night, mid-night, and morning for validation and further exploration based on preliminary findings. This study may provide insights for developing health strategies to improve chrono-specific health and to mitigate the metabolic disease risk evoked by food intake-induced circadian perturbation/misalignment.

LICAR: automated isotopic correction tool for targeted lipidomic data acquired with class-based chromatographic separations using multiple reaction monitoring

Liang GAO[1,2], Shanshan Ji[2], Bo Burla[2], Amaury Cazenave-Gassiot[1,2]

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2 Singapore Lipidomics Incubator, Life Sciences Institute, National University of Singapore

LICAR2 is an open-source software package designed for automated isotopic correction for targeted lipidomic data acquired with class-based chromatographic separations using multiple reaction monitoring. We developed a systematic MRM pattern recognition algorithm. Based on harmonized lipid names, *m/z* of precursor and product ions, this algorithm could automatically divide the MRM transitions into corresponding MRM patterns. Users can simply submit a single data file including all the measured MRM transitions and MS intensity data without pre-splitting it into multiple files for each MRM pattern. The pre-set MRM patterns were also extended to cover more lipid classes, ion adducts and product ions. In addition, isotope-labelled species from either internal standards or specific experimental designs were also taken into consideration. With user input of the number and location of heavy isotopes, the algorithm could perform isotopic correction in the same way for these species. The common M+2 isotope correction hence was extended to cover M+1 and M+3 for more accurate quantification. The R package could be easily incorporated into other lipidomic software packages. In summary, LICAR2 offers fully automatic and highly comprehensive isotopic correction for MS raw peak data before any other data processes, which enables more accurate quantification in lipidomic studies.

Determination of plasma cholesteryl ester levels in preadolescent children by LC/MS

Divyavani GOWDA[1], Siddabasave Gowda B. Gowda[1], Atsuko Ikeda[1,2], Rahel Mesfin Ketema[1,2], Yu Ait Bamai[2], Reiko Kishi[2], Hitoshi Chiba[3], Shu-Ping Hui[1]

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3 Department of Nutrition, Sapporo University of Health Sciences, Japan

Cholesteryl esters (CE) are sterols composed of various acyl chains attached to the hydroxyl moiety of cholesterol. Abnormalities in CE metabolism have been associated with numerous diseases, including fatty liver disease and chronic kidney disease. CEs are often considered plasma biomarkers of liver function, but their absolute concentrations in plasma of preadolescent children were not well-explored. This study aimed to determine the plasma CE levels in preadolescent children using targeted liquid chromatography/mass spectrometry. Analysis was performed on the non-fasting plasma of preadolescent children ages 9 - 12 (n = 342 volunteers, boys =181, and girls =161) from Sapporo, Hokkaido. Eight CEs were analyzed using a bi-phase extraction followed by highly sensitive targeted quantification. The analysis showed that the total CE levels in boys and girls were 886 ± 104 nmol/mL and 862 ± 96 nmol/mL, respectively. The participants were further classified into three groups based on BMI underweight (n = 239), normal range (n = 95), and overweight (n = 8). CE 18:2 is the most abundant species followed by CE16:0 in all three groups. Interestingly, the CE 16:0, CE 18:2, and CE 22:6 levels were significantly higher in overweight children (132 ± 17 nmol/mL, 389 ± 31 nmol/mL, and 126 ± 26 nmol/mL) compared to normal-weight children (117 ± 17 nmol/mL, 361 ± 34 nmol/mL, and 112 ± 21 nmol/mL) respectively. This study marks the initial investigation of absolute CE levels in children's plasma and could help to elucidate the relationship between CEs and childhood obesity.

A lipidome aging clock shows age acceleration in individuals with autism

Djakim LATUMALEA[1-3], Maximilian Unfried[1,3], Diogo Barardo[1,3], Jan Gruber[1,3,6], Brian Kennedy[1,3-5]

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Recent advances in lipidomics and machine learning have been harnessed to explore biological age prediction in individuals. This study delves into age acceleration patterns, entropy, and the role of dolichol as a potential aging biomarker. We present a novel aging clock in combination with explainable AI that utilizes lipid composition of the prefrontal cortex to predict biological age of individuals without known neurological conditions, as well as in those with autism, schizophrenia, or Down syndrome. Significant age acceleration was observed in individuals with autism, with a higher acceleration after the age of 40. In addition, entropy increases significantly around the age of 40, indicative of mevalonate pathway dysregulation. These findings underscore the feasibility of predicting biological age using lipidomics data, opening avenues for feature research into the intricate relationship between lipid alterations and aging of the prefrontal cortex, while providing valuable insights into the associated molecular mechanisms.

Method development for 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 as alkyl-diacylglycerols (TG(O)s) and fatty acid esters of hydroxy fatty acids (FAHFAs), however, have been lacking. Accurate characterizations are further complicated by the shortage of existing validated methods for studies of these components from breast milk matrices. This project focuses on the development and validation of analytical procedures for reliable identification and measurement of FAHFAs and TG(O)s in breast milk. Lipid extracts are obtained by modification of the established Folch method of extraction and solid phase extraction. Semi-automated analysis is further aided by the development of lipid libraries for the identification of FAHFAs and TG(O)s. Liquid chromatography mass spectrometry techniques for the identification and quantitation of FAHFAs and TG(O)s have also been optimized. Preliminary studies have identified 18 FAHFA precursors and 23 TG(O) precursors, along with their corresponding molecular species, from pooled milk samples from the Singapore PREconception Study of long-Term maternal and child Outcomes (S-PRESTO) cohort. Putative regioisomer identifications have been conducted on FAHFA with the use of MS3 techniques, and further work is in progress for a systematic validation of the methods that have been developed through the course of this project.

Probing the environmental fortitude of mRNA lipid nanoparticles: a TIMS-TOF insights into stress-induced degradation

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Forced degradation studies on mRNA lipid nanoparticles (LNPs) can provide valuable insights into how environmental stress affects their stability and durability for various applications. In this study, LNPs are typically subjected to common strenuous conditions such as heat, UV light, oxidation, and repeated freeze-thaw cycles. These harsh circumstances can adversely impact one or more components of the LNPs. Here, we utilized Trapped Ion Mobility Spectrometry Time-Of-Flight (TIMS-TOF) Mass Spectrometry (MS) to investigate the degradation products of LNPs formulated by various ionizable lipids to gain a deeper understanding of their limitations and identify strategies for improving their stability. Luciferase-encoding mRNA LNPs comprised of three structurally distinct ionizable lipids were formulated using Spark NanoAssemblr(TM) (Precision NanoSystems) with a standard formulation technique. An equal amount of LNPs was exposed to an individual stress condition for 72 hours. These conditions were heating at 28 ºC, exposure to UV light (wavelength = 360 nm), mixing with 100 mM hydrogen peroxide, or freeze/thaw cycles of -20ºC for 12 hours followed by 22ºC for 12 hours (ambient temperature). Samples were acquired on a Bruker timsTOF Pro 2 coupled with a Bruker Elute UHPLC and a VIP-HESI source. A full scan MS and MS/MS (PASEF) were implemented in both positive and negative modes. Data were analyzed by Metaboscape 2023 and Data Analysis 6.0. The encapsulation efficiency was determined by the Quant-IT Ribogreen assay to be 70-90%. The nanoparticle average diameter was 55-70 nm as determined by dynamic light scattering using a Zetasizer Nano ZS. HEK293 cells were transfected with the LNPs at a 200-ng dose to evaluate in vitro expression and confirm the successful formulations. While heat and the freeze/thaw alteration seem to have minimal effect on ionizable lipids in mRNA LNPs, UV light and oxygen was shown to pose the most detrimental effect on all tested ionizable lipids. Interestingly, the degradation products produced by oxidation condition and UV light were very similar. Our results suggest the occurrence of a free radical mechanism initiated by a reactive oxygen species.

Multimodal quantification of lipid metabolism in the Asian phenotype of insulin resistance

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Insulin resistance (IR) is fundamental to the development of type 2 diabetes mellitus (T2DM) and usually precedes the disease by several years. Although obesity is a well-known risk factor for IR, it is not the soledeterminant. Further, body mass index (BMI) fails to fully explain the variance of IR in the general population. People from Asian ethnicities experience high rates of T2DM compared to people in other regions of the world despite relatively low BMI. It has been shown that insulin sensitivity index (ISI), steady state insulin response, and waist/hip ratio are significantly different among various ethnic groups including Caucasians, Asian-Americans, African-Americans, and Mexican-Americans. Within Asia, different ethnic groups have varying degrees of IR. By understanding the molecular pathogenesis of insulin resistance and deployment of timely intervention strategies for early course correction, the natural progression into pre-diabetes and full-blown T2DM and NAFLD can be modulated. Here we describe an integrated approach of anthropometric and biochemical measures in conjunction with serum lipidomics for metabolic phenotyping of multi-ethnic male subjects with varied insulin sensitivity. By using insulin sensitivity index (ISI) derived from the gold standard hyperinsulemic-euglycemic clamp (HIEC) procedure, we stratified the study subjects (n=250) into tertiles of ISI and found that Asian-Indians were predominantly insulin resistant compared to Chinese and Malay ethnicities within Singapore population. The dyslipidemia associated with insulin resistance (IR) in these subjects showed a decrease in HDL-C and an increase in total triglycerides with decreasing ISI. These lipid abnormalities are the classic features of IR and subsequently T2DM and NAFLD, however, these measures alone fail to explain the complex molecular pathogenesis of these metabolic diseases. By detailed characterization of the serum lipidomic profiles in the fasting state as well as temporal changes during HIEC procedure, we identified a lipidomic signature of insulin resistance and metabolic plasticity in these non-obese and metabolically healthy individuals. We also identified the dynamic changes in serum lipids in response to HIEC procedure represented by acylcarnitines, non-esterified fatty acids, lysophospholipids, sphingosine-1-phosphate and phosphatidylserine lipid classes. By using the association studies, we identified lipid signatures of IR and liver fat. We demonstrate that a shared lipid signature between IR and liver fat shows a gradual increase with increasing liver fat percentage. This sharedlipid signature outperforms traditional lipid indices in predicting IR measuredby the HIEC procedure. In summary, by stratifying non-obese and healthy subjects according to the insulin sensitivity index derived from HIEC procedures, we identified a circulating lipidomic signature of insulin resistance and liver fat with a potential for prediction and management of metabolic health before the development of T2DM and other metabolic diseases.

Untargeted lipidomics of gut microbiome-derived sulfonolipids

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The gut microbiota produces a huge number of complex metabolites, and many of them are unknown. Therefore, their structural elucidation and characterization of their biosynthesis and bioactivities is an important challenge to understand the mode of gut microbiome-host interaction at the molecular levels. Here, we applied LC-MS/MS-based untargeted lipidomics, which can comprehensively measure lipids including unknowns, to the gut microbiota to search for unique lipids and estimate their structures. As a result, a unique cluster of lipids, namely sulfonolipids, was found in mouse feces. Sulfonolipids are distinct class of sphingolipids with a sulfonic group in the sphingoid base. We identified the producing bacteria based on untargeted lipidomics of isolated gut bacterial cultures. Sulfonolipids were distributed in host organs such as liver and kidney in bacteria-dependent manner. MALDI-based mass spectrometry imaging revealed their distribution through the lymphatics and mesenteric lymph nodes. These results suggest that gut microbiome-derived sulfonolipids may exert their bioacitivity locally in the host tissues.

Sphingolipid inventory and variability in human platelets

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Sphingolipids play essential roles as membrane lipids and as bioactive molecules that are involved in cellular regulations. Platelets are important blood cells in hemostasis and wound healing. So far, most published work on the platelet lipidome covered only few sphingolipid classes and species. Given the interest on sphingolipids, we aimed to establish an in-depth human platelet sphingolipidome profile and to determine the biological variability of sphingolipids in platelets. We isolated platelets from whole blood of healthy individuals and validated purity and integrity of the platelet isolates via flow cytometry. Targeted sphingolipid profiling from human platelets and autologous plasma was conducted using liquid chromatography mass spectrometry with multiple reaction monitoring (LC-MS-MRM). Around 200 platelet sphingolipids were quantified with a low analytical coefficient of variability (<20% CV), including ceramides, sphingomyelins, mono- and dihexosylceramides, globosides, monosialogangliosides (GM3) and sphingosine-1-phosphates (S1P). We also report for the first time to our knowledge the presence of deoxyceramides in platelets. The platelet sphingolipidome revealed a high degree of sphingoid bases and fatty acyl chain diversity. Concentrations of platelet sphingolipids showed an overall significant positive correlation within platelets and with autologous plasma, but also some specific negative correlations. We furthermore determined initial estimates of biological variabilities of the platelet sphingolipids and compared them between lipid classes and between male and female subjects. This study presents a targeted platelet sphingolipidomics workflow, and a detailed map of the platelet sphingolipidome, its biological variability, and its association with other lipid classes and with the plasma lipidome. We hope these results may be valuable for future studies of platelet biology and functions.

Multi-tissue profiling of oxylipins reveal a conserved up-regulation of epoxide:diol ratio that associates with white adipose tissue inflammation and liver steatosis in obesity

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Background. Obesity drives maladaptive changes in the white adipose tissue (WAT) which can progressively cause insulin resistance, type 2 diabetes mellitus (T2DM) and metabolic dysfunction-associated liver disease (MASLD). Obesity-mediated loss of WAT homeostasis can trigger liver steatosis through dysregulated lipid pathways such as those related to polyunsaturated fatty acid (PUFA)-derived oxylipins. However, the exact relationship between oxylipins and metabolic syndrome remain elusive and cross-tissue dynamics of oxylipins remains ill-defined.

Methods. We quantified PUFA-related oxylipin species in the omental WAT, liver biopsies and plasma of 88 patients undergoing bariatric surgery (female N=79) and 9 patients (female N=4) undergoing upper gastrointestinal surgery, using UPLC-MS/MS. We integrated oxylipin abundance with WAT phenotypes (adipogenesis, adipocyte hypertrophy, macrophage infiltration, type I and VI collagen remodelling) and the severity of MASLD (steatosis, inflammation, fibrosis) quantified in each biopsy. The integrative analysis was subjected to (i) adjustment for known risk factors and, (ii) control for potential drug-effects through UPLC-MS/MS analysis of metformin-treated fat explants ex vivo.

Findings.We reveal a generalized down-regulation of cytochrome P450 (CYP)-derived diols during obesity conserved between the WAT and plasma. Notably, epoxide:diol ratio, indicative of soluble epoxide hydrolyse (sEH) activity, increases with WAT inflammation/fibrosis and hepatic steatosis and T2DM. Increased 12,13-EpOME:DiHOME in WAT and liver is a marker of worsening metabolic syndrome in patients with obesity.

Interpretation. These findings suggest a dampened sEH activity and a possible role of fatty acid diols during metabolic syndrome in major metabolic organs such as WAT and liver. They also have implications in view of the clinical trials based on sEH inhibition for metabolic syndrome.

A fast UHPLC-DDA-HRMS using HILIC separation for untargeted lipidomics

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Untargeted lipidomics aims to screen as many detectable lipids as possible and provides comprehensive information of the lipidome. Ultra-High Performance Liquid-Chromatography High Resolution Mass Spectrometry (UHPLC-HRMS) coupled with data-dependent acquisition (DDA) is a widely used methodology for untargeted lipidomics. Two commonly used chromatographic methods are reverse-phase (RP) and Hydrophilic Interaction Liquid-Chromatography (HILIC) separation. RP separates lipid species by acyl-chain composition while HILIC separates lipids by class. HILIC separation, while enabling a better quantitation, produces chimeric spectra of isomeric and isobaric lipids within each class in UHPLC-DDA-HRMS methods. Data processing toolkits for DDA cannot deconvolute chimeric spectra and often only report the top scoring ID, resulting in loss of annotation of co-eluting isomeric and isobaric lipids. However, despite the complexity in the acquired data, HILIC separation offers some advantages over RP in untargeted analyses, such as the use of a less extensive panel of internal standards and the ease of shortening the runtime without sacrificing chromatographic separation between lipid classes. To increase the throughput, we have developed a 6.5 minutes HILIC-DDA-HRMS method in positive polarity on the SCIEX ZenoTOF 7600 system. We used commercially available human plasma for method development. Data processing was done using our in-house toolkit called MetaboKit. We adapted the original toolkit by adding additional matching and filtering functionalities and thereby enabling deconvolution of chimeric spectra produced by HILIC separation. With the new method and software adaptation, we were able to annotate more than 500 lipids at the molecular or sum composition level in positive mode, while we only annotated around 200 lipids with the original version, developed for generic DDA analysis. The number of lipids annotated in a single polarity shows that the proposed method is promising for high-throughput lipidomic analyses. Future work will entail development of a DIA method based on the fast HILIC separation and establishment of a tandem DDA/DIA workflow to improve coverage and allow for semi-quantitation at the MS/MS level.

Ultraviolet photodissociation (UVPD) mass spectrometry for structural characterization of lipids in biological matrices on chromatographic time scales

Dhaval PATEL

Thermo Fisher Scientific

Introduction. Complete structural characterization of lipids is an essential component of lipidomics but remains challenging as commonly used activation methods, such as HCD and CID, do not generate fragments to discern regioisomers and pinpoint the double bond positions within the acyl chains. Ultraviolet photodissociation (UVPD) energizes ions via absorption of high-energy photons generated from an UV laser source. This increases the internal energy of a selected precursor ion (electronic excitation), until there is sufficient internal energy present to overcome the barrier for dissociation, therefore generating fragments. Here we show the use of liquid chromatography-based separation of lipids with the use of hybrid dissociation strategies using conventional and UVPD MSn fragmentation for structural characterization of specific classes of lipids in biological matrices. Preliminary Data UVPD allows access to new dissociation pathways complementary to more conventional ion-activation methods and has been shown to generate fragments useful for complete structural elucidation of lipids. In this study, initially lipid standards were infused into the mass spectrometer using hybrid strategy for fragmentation utilizing HCD/CID for MS2 followed by UVPD based MS3 fragmentation of specific productions generated by the neutral loss of head group. The optimal activation time required for UVPD to generate diagnostic fragments for regioisomers and doublebond positions of acyl chains was determined for lipid class phosphatidylcholines (PC) and triacylglyceride (TAG). 150 ms-300 ms activation time was found to be sufficient for characterization of regioisomers.The lipid standards and extracts were separated using LC. The data was acquired using mass spectrometer with optimized parameters for fragmentation. Thermo Scientific AcquireXTM strategy, with iterative precursor workflow, was used for analyzing the samples. This workflow automatically generates an exclusion list for background compounds to focus on the fragmentation of extracted lipids. Iterative injections of the sample update the exclusion list and hence even low intensity lipid ions can be fragmented. The use of AcquireXTM allows efficient use of the mass spectrometer duty cycle for fragmentation of ions in LC timescale. Lipids present in the biological matrices were annotated for lipid class and the acyl chain composition using LipidSearch™ 5.0 software. Manual annotation as well as using Mass Frontier™ was done on the UVPD fragmentation data of specific PC and TAG lipids to determine the regioisomers as well as the double bond positions on the acylchains. Using the hybrid dissociation strategy, we can do complete structural characterizations of lipids in biological matrices on LC time scale.

Novel Aspect. Structurally defined molecular lipid annotations in biological matrices using LC-MS with a hybrid dissociation strategy.

Simultaneous Quantitation and Discovery (SQUAD) metabolomics: an intelligent combination of targeted and untargeted workflows in a single injection

Hai Ning PEE

Thermo Fisher Scientific

Without internal standards and libraries, untargeted metabolomics lacks accurate metabolite quantitation and identification that are needed to study biological systems. These steps can complicate data processing; thus, researchers prefer to target a few analytes and risk missing significant unknown compounds with potential biological significance. Therefore, we developed a single-injection simultaneous quantitation and discovery (SQUAD) metabolomics workflow that provides confident identification and/or accurate quantitation of preselected metabolites, without compromising the untargeted analysis. When available, analysing authentic standards of target compounds, provides absolute quantitation, standardizing response across instrument platforms and laboratories providing additional QC and QA to the study. The simultaneous acquisition of HRAM full scan data, future proofs the discovery of metabolites through data retro-mining as new targets are discovered.

Methods and Results: NIST SRM 1950 plasma was spiked with a dilution series of isotope-labeled compounds and extracted with 80% methanol. Data were acquired on multiple Thermo Scientific orbitrap-based platforms including the novel Orbitrap™ Astral™ mass spectrometer to evaluate the SQUAD metabolomics workflow. SQUAD analysis on Orbitrap IQ-X™ Tribrid instrument, for example, utilized the sensitive and wide dynamic range linear Ion-Trap for PRM quantitation of low-abundant endocannabinoids involved in the etiology of human pain sensation. This was done in parallel with Orbitrap untargeted screening without compromising its resolution or mass accuracy. The novel mass spectrometer (Orbitrap Astral) was also used to evaluate the SQUAD workflow. The novel Astral analyzer allows faster scanning on the MS2 level for a higher annotation rate and enough points across the peak on the Orbitrap MS1 level for accurate and extended quantitation of the analytes (i.e., 4–5 orders of magnitudes dynamic range, with LLOQ of 10 femtomoles and LLOD of 5 femtomoles). A high percentage of compound fragmentation (~90%) with the Astral analyzer secures the fragmentation of lower-abundance compounds in a complex matrix like plasma. SQUAD analysis data was also collected on other orbitrap-based platforms showing promising capabilities of the new metabolomics workflow.

Multi-cohort validation of blood lipid markers of psychiatric disorders and their application to the prediction of post-traumatic stress disorder

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Currently, no clinically useful diagnostic test exists that could support psychiatric disorder diagnosis. While molecular signatures of psychiatric disorders are still undefined, lipidomics methods offer promise in uncovering new type of biomarkers. In our previous study (Tkachev et al., 2023), we identified a consistent signature of lipidome alterations in the blood plasma of individuals with psychiatric disorders such as schizophrenia, depression, and bipolar disorders using liquid chromatography coupled to mass spectrometry. Building on this work, we adapted a high-throughput direct-infusion lipidomic method to assess blood lipid profiles in more than 1500 individuals with schizophrenia, depression, post-traumatic stress disorder (PTSD), as well as control unaffected with mental disorders. Our results showed not only considerable statistical differences in the blood lipid abundances between healthy controls and psychiatric patients, but we also found that amultivariate model was quite successful in separating the two groups of individuals. Moreover, our study design included three independent sample cohorts including individuals from three different cities, which enabled us to assess the robustness and general ability of the proposed mathematical model. In particular, we found a very robust signature of lipid alterations in schizophrenia compared to healthy controls that was well reproduced across the cohorts. Model performance for other psychiatric disorders, depression and PTSD, indicated that many of the lipidomic alterations were common across disorders, as well. Our results suggest the potential of development of a lipid-based diagnostic test for mental health assessment. Future directions of investigation would comprise the possibility of using blood lipids for more detailed diagnosis differentiation between the psychiatric disorders. This study was sponsored by the Moscow Center for Innovative Technologies in Healthcare. Research was funded by grant from the Moscow government, No. 2102-11.

Tkachev A, Stekolshchikova E, Vanyushkina A, et al. Lipid Alteration Signature in the Blood Plasma of Individuals WithSchizophrenia, Depression, and Bipolar Disorder. JAMA Psychiatry.2023;80(3):250-259. doi:10.1001/jamapsychiatry.2022.4350

Illuminating the cellular and molecular response to drug treatment by combining bioenergetic measurements with untargeted metabolomics

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Introduction: To better understand the biology of cancer cells and their dynamic metabolic response to therapeutic compounds, advanced methodologies are required. We combined results from two technologies that measured metabolic pathway utilization at two scales, cellular and molecular. An acute monocytic leukemia cell line was treated with the drugs SU1498 and AG-879, which were selected from a library of 80 kinase inhibitors based on their modulation of mitoATP production rates. Separately, cells treated with the drugs were lysed, metabolism was quenched, and metabolites and lipids were extracted with an automated sample preparation method. Extract were directly analyzed with LC/Q-TOF and discovery-based MS software. The combined results provided deeper insight into the cellular and molecular metabolic response to drug treatment.

Conclusion: Combining cell analysis and LC/MS technologies provided deeper insight into the cellular and molecular metabolic response to drug treatment for cancer research. Seahorse (XF) cell analysis of AG-879 and SU-1498 corroborated previous results and newly demonstrated that both drugs cause mitochondrial uncoupling. Untargeted Mx identified changes in key metabolites affected in glycolysis and mitochondrial respiration that correlate with XF results. Untargeted Lx showed an increase in TG content with SU-1948 treatment which may be related to build up of energy precursors for the TCA cycle, shown to be reduced with XF. Future directions could include 1) further mining of the unidentified significant features, 2) qualitative flux analysis, and/or 3) the Seahorse XF Substrate Oxidation Stress Test to assess fuel dependencies with drug treatment.

Variability of lipids in human milk

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Background and Objectives: Human milk (HM) is recognised as the gold standard for infant nutrition due to its nutritional and protective properties. However, lipid proportions in HM are highly skewed, with triacylglycerols (TGs) comprising 99% of total lipids, while bioactive phospholipids and sphingolipids represent only 1-2%. Consequently, much of the literature oversimplifies fat analysis. This study focused on investigating the variability of both the major and minor lipids associated with a number of maternal and pregnancy-related characteristics in Singapore.

Methods: An efficient workflow for a comprehensive lipidomic analysis was first established and validated. The workflow allowed quantification of 237 lipids, encompassing 12-13 lipid sub-classes. Exploratory linear regression analysis was then conducted to examine the associations between the HM lipidome and several maternal factors, including ethnicity, parity, breastfeeding practices, education and maternal mental health.

Results: Ethnicity emerged as a key factor and was associated with 146 lipids at 3 weeks and 81 lipids at 3 months. Asian confinement diets and the FADS2 rs174583 variant were also found to be associated with ethnicity, potentially driving some of the observed ethnic-based differences. STAI scores, parity, exclusive breastfeeding and education were also found to be significantly associated with the HM lipidome at 3 weeks but not at 3 months.

Conclusions: The data primarily suggests that there is a presence of inherent differences in the milk lipidome associated with modifiable (confinement diets) and unmodifiable (genetics) factors that may have implications in terms of variable infant growth trajectories and developmental outcomes for the different ethnicities in Singapore. By considering the unique needs and characteristics of individual ethnic groups, we can design interventions that address specific dietary and lifestyle factors, ultimately enhancing infant outcomes. More public health awareness regarding antenatal mental health is also required to support mothers during this vulnerable period.

Leveraging untargeted metabolomics to increase diagnostic yield in pediatric rare diseases

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Background: Diagnosing rare diseases is challenging due to their complexity and heterogeneity. Multi-omics approaches have emerged as powerful diagnostic tools for addressing these challenges. Untargeted Metabolomics involves the broad characterization of small biochemical molecules in biofluids. This offers a unique advantage by enabling the exploration of downstream effects across diverse metabolic pathways. As part of our Mendelian program, we integrated the metabolomic profile and whole-genome sequencing (WGS) data from a diverse cohort of 37 subjects with a spectrum of metabolic diseases spanning neurological (54%), endocrine disorders (27%), and metabolic-related symptoms (19%). Our study aims to delineate the underlying pathophysiology of these diseases and proves the utility of untargeted metabolomics in solving rare disease cases.

Methods: WGS was performed on patients and their parents using Illumina HiSeq X platform. Trio WGS analysis identified a short list of rare, predicted damaging variants. Clinical-grade untargeted metabolomics was performed by Baylor Genetics and Metabolon, Inc using Liquid-Chromatography coupled with Mass-spectrometry (LC-MS). We then conducted a comparative analysis, examining the relationship between the metabolic profile, the patient's phenotype, and the function of relevant genes using mGWAS, KEGG, and HMDB datasets.

Results: Of the total number of 728 metabolites per patient, on average 63 metabolites were significantly dysregulated. Almost all cases, even those lacking a genetic candidate, showed a metabolic profile consistent with their disease phenotypes. Out of the total subjects enrolled (37 subjects from 29 families), 31 carry a genetic candidate variant. Within this group, we discovered the genetic cause for 17 cases, and strong genetic candidates for 14 cases. Metabolomics data provided support for 31.25% (5/16) of cases with a discovered genetic cause and 21.43% (3/14) of cases with a genetic candidate. The cases lacking a genetic candidate remained unsolved even with the utilization of metabolic data to guide the identification of genetic candidates.

Conclusion: Overall, our study demonstrates the potential of metabolomics to improve diagnostic accuracy by 21% (8/37), substantiated by its ability to provide evidence in validating significance of candidate genes where metabolites do correlate with the genetic candidate. Furthermore, metabolomics provided valuable insights into the underlying molecular pathways of rare diseases, even in the cases where it does not correlate with the candidate gene.

Conserved and unique mammalian responses to TLR7 stimulation in primary macrophages

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Macrophages sense viruses, and mediate inflammation through the biosynthesis of proinflammatory proteins, metabolites, and lipids. Recent research has demonstrated the importance of networks including genes and metabolites in modulating the macrophage immune response. However, the immunometabolism of macrophages in response to viruses remains unclear, and there is still confusion about which responses are conserved between mammalian species and which are unique to certain species. In this study we profiled the transcriptomes, metabolomes, and lipidomes of bats, mice, rats, and human primary macrophages stimulated with a TLR7-specific agonist after early (3h) and late (18h) incubation. We chose bats as an atypical mammalian species having a resilient immunity against viruses. Overall, transcriptional responses following TLR7 stimulation occurred both early and late, while changes in metabolites and lipids were only detected at late incubation. We observe a common upregulation of glycolysis genes including those encoding GLUT1 (SLC2A1) and rate-limiting hexokinases (HK1, HK2, and HK3), and an increase in the abundance of metabolites within the glycolytic pathway. Across multiple species, metabolites of the pentose phosphate pathway and citric acid cycle also increased in general abundance, as did phosphatidyinositols, though there was a marked specific decrease in phosphatidyinositol 38:4, a precursor of arachidonic acid-derived lipid proinflammatory mediators. We also find a common increase in the expression of proteasome subunits and ubiquitin conjugating enzymes. Unlike metabolites and transcripts, changes in lipids showed more species-specific signatures: Following TLR7-stimulation, the abundance of ether phosphatidylcholines uniquely decrease in mice, cholesterol esters uniquely increase in rats, and acylcarnitines uniquely increase in bats. Concomitantly, bats uniquely increase expression of PDK1 (pyruvate dehydrogenase kinase 1, which functions to inhibit the conversion of pyruvate into acetyl-CoA by inactivating phosphorylation of pyruvate dehydrogenase) and accumulate pyruvate. Furthermore, we observe a bat-unique increase in expression of GPT2 (glutamic–pyruvate transaminase 2, which catalyzes the conversion of pyruvate to alanine) and increased abundance of alanine in bats' macrophages relative to any other species. These results provide a comprehensive overview of conserved and unique immunometabolic macrophage response to TLR7 stimulation in mammals. Furthermore, they pave the way for investigations into the unique metabolic adaptations in bats that may explain their enhanced immunity against viruses.

A three-in-one end-to-end automated sample preparation and LC/MS metabolomics, lipidomics, and proteomics workflow for plasma

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Introduction: LC/MS omics analyses, comprising metabolomics, lipidomics, and proteomics, offer a way to measure metabolism. This includes measuring components of the three main metabolic branches: (1) conversion ofnutrients into energy, predominantly ATP, (2) conversion of nutrients intobuilding blocks, including those used to build proteins and nucleic acids, and (3) conversion of nutrients into waste products.We developed a novel automated workflow for extraction of metabolites, lipids and proteins from 20 µl plasma samples and analyzed each extracted fraction with a unique LC/MS method. The coverage and reproducibility across metabolites, lipids, and proteins for this three-in-one omics workflow were assessed. Methods: Metabolite, lipid and protein fractions were extracted from 20 µl pooled mouse plasma samples using a liquid handler with commercial accessories. Proteins were precipitated and collected as a pellet that was prepared via tryptic digestion. Metabolites and lipids were separated by passing the supernatant through a solid phase extraction plate, metabolites being collected in the flow-through and lipids being captured by the sorbent and subsequently eluted. Each separate fraction was analyzed using targeted or untargeted LC/MS analysis. Polar metabolite analysis used HILIC chromatography and an LC/TQ. Lipid analysis used RP C18 chromatography and a LC/TQ. Protein analysis used RP chromatography and an LC/Q-TOF. Data analysis utilized commercially available quantitative software and Spectrum Millsoftware. Preliminary data: Targeted metabolomics analysis detected 437metabolites, covering major metabolite classes and metabolic pathways. A subset of 68 metabolites were selected for reproducibility analysis. Results indicated excellent reproducibility across the end-to-end workflow, including automated sample preparation and LC/MS analysis. Targeted lipidomics analysis detected 615 lipids across commonly analyzed lipid classes. Percent RSDs were typically between 10 and 15% for these lipid classes but uniquely higher for cholesterol esters (less than <50%RSD). Untargeted protein analysis detected anaverage of 1479 proteins from each 20 µl plasma sample when using a 2 peptide per protein guidance and 1% FDR rate. The average number of protein identifications from the automated protein extraction was not statistically different to the average protein identifications, 1493, detected in a side-by-side manual protein extraction. For the automated protein extraction, the RSD for number of proteins identified was 3.5%. Overall, this three-in-one LC/MS omics workflow offers great coverage and reproducibility for metabolites, lipids and proteins. This end-to-end workflow achieves reliable results with small plasma volumes and is a fast and efficient solution for multi-omics analyses. Finally, the workflow offers flexibility for those who may only want to collect and monitor a single type of biomolecule, e.g., metabolite, lipid or protein, or those who are interested in measuring two of the three types of biomolecules.

Deciphering shared and distinct lipidomic signatures in schizophrenia and major depressive disorder across human brain regions

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Schizophrenia and majordepressive disorder (MDD), affecting approximately 3% of the global population,present complex challenges in understanding their molecular mechanisms. Despitethe efficacy of genetic studies, the highly polygenic nature of these disorders hinders detailed molecular insights. Genetic research reveals a notable overlapof loci associated with both conditions, mirroring their common clinical manifestations. To explore potentially shared molecular mechanisms, wecarried out lipidome measurements of post-mortem brain samples, taken from 36 different regions of 21 donors (7 controls, 7 schizophrenia patients and 7 depression patients). For each region, we systematically assessed the abundance of 616 lipids, covering more than 20 different lipid classes, which represent all themajor lipid groups found in the brain. In our analysis, we investigated global lipid changes associated with major depressive disorder and schizophrenia, as well asdifferences between the two conditions. Results indicated that 125 lipids weresignificantly changed in major depressive disorder compared to healthy controls,while in schizophrenia, 104 lipids showed a significant alteration. Notably, certain classes, including PE, PG BMP, CAR, were significantly decreased in bothdepression and schizophrenia, whereas LPC, Cer, CE exhibited increased levels in major depressive disorder and were not significantly changed inschizophrenia.Bothdisorders demonstrated increased free fatty acids, while lipid alterations in cerebellum suggested compromised myelination. Brain region analysis pinpointed disorder-specific patterns: schizophrenia affects white matter, depression has more pronounced effects in the frontal cortex. Upon closer examination, our data revealed two distinct trends. Firstly, a decline in lipid classes related to energy metabolism (PG, CL, BMP, CAR) suggested mitochondrial dysfunction, evident in both schizophrenia and depression across multiple brain regions. This observation was further supported by decreased levels of PUFA residues inschizophrenia and MDD, potentially due to increased oxidative stress associated with dysfunctional mitochondria. Secondly, elevated levels of signaling lipid classes (LPC, CE, Cer) indicated neuroinflammation, more prominent in major depressive disorder, especially in cortical regions.The research was supported by the Russian Science Foundation grant No. 22-15-00474.

Vitamins B7 and B12 deficiency in standard cell culture rewires adipocyte metabolism

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The in vitro microenvironment profoundly affects the capacity of cell culture to model physiological and pathophysiological states. Central to the modern optimisation of cell culture is the development of physiologic media, which highlight the major impact that specific nutrients can have on a variety of cellular processes. One of the most commonly used media today – DMEM, lacks vitamins B7 (biotin) and B12 (cobalamin), yet little is known about the impact of this on cultured cells. Importantly, these vitamins act as cofactors for several key enzymes involved in glucose, amino acid, and lipid metabolism. Presently, there are three major existing fatty acid profiling papers of 3T3-L1 adipogenesis, each of which show large quantities of odd-chain fatty acids. Addressing this abnormal fatty acid profile is important as 3T3-L1 adipocytes are widely used as a model of in vivo white adipose tissue, and ensuring metabolic fidelity is key to improving the translatability of findings from in vitro experiments. Here, we confirm that 3T3-L1 adipocytes accumulate large amounts of odd-chain fatty acids in vitro. Since vitamins B7 and B12-dependent enzymes regulate the activity of several pathways that converge at the point of lipid metabolism, we further show that the addition of both vitamins are sufficient to eliminate most odd-chain fatty acids, whilst boosting total fatty acid synthesis. Vitamins B7 and B12 supplementation also reduced methylmalonate production, a toxic metabolite, and altered nutrient fluxes towards the TCA cycle. Functionally, insulin sensitivity was not affected, however vitamin B7 supplementation increased leptin secretion in these adipocytes, representing a new avenue to study the link between leptin and cellular metabolism or lipid burden. These results highlight the importance of vitamins to adipocyte metabolism as well as how the nutrient microenvironment in standard cell culture can inadvertently compromise our understanding and discoveries of cell metabolism and function.

Ceramide scoring for cardiovascular disease risk prediction using Agilent RapidFire SPE-MS/MS

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Cardiovascular diseases (CVDs) have been steadily rising in recent years, culminating in their contribution to 32.0% of worldwide fatalities in 2021. Ceramides, a class of sphingolipids, have been used as biomarkers for CVD and diabetes. Specifically, four ceramide species (Cer d18:1/16:0, Cer d18:1/18:0, Cer d18:1/24:0, and Cer d18:1/24:1) are utilized for predicting Major Adverse Cardiovascular Events (MACE). We present an efficient screening method for these four ceramides in human plasma, employing the Agilent Rapidfire capabilities for online SPE-MS/MS high-throughput analysis (~15 secs/sample). The use of authentic deuterium labelled standards enables absolute quantitation of each ceramide within physiologic dynamic ranges. A validation of our methodology using biological samples was supported by highly correlated values when compared to established LC-MS/MS methods employed in research labs and clinics. Our results illustrate the potential of the Rapidfire for high-throughput screening in both research and clinical applications, addressing scalability issues present in existing technologies while ensuring robust and reproducible outcomes.

Lipid alterations in brain and blood of depression patients: potential lipid-based prediction model for the detection of depressive symptoms in the general population

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Depression considerably contributes to the total burden of mental disease, and is among leading causes of disability. However, its mechanisms and molecular signatures remain elusive. Recent studies have suggested that lipids and lipid metabolism is significantly impacted in depressive disorders. Here we conducted a study of brain and blood plasma lipid alterations associated with depressive phenotypes. First, we investigated whether brain lipid abundances in depression would be significantly different from healthy controls using lipidomics profiling across 36 brain regions in 14 individuals. Considering the small sample size, our results show promising trends in lipid changes in the diseases across the different brain structures. Together with reported findings on shared genetic etiology between depressive phenotypes and plasma lipid levels, these results suggest inherent and global lipid metabolism alterations inthis mental disorder. Hence, we next considered a lipidomic study of blood profiles associated with depressive symptoms and investigated the potential of lipids as depression biomarkers. For this purpose, using direct-infusion mass spectrometry, we assessed blood plasma lipid levels in 604 individuals from a general population and investigated their association with self-reported anxiety and depression symptoms. We observed a significant correlation between lipid levels and the severity of self-reported depression symptoms. Additionally, we found that the lipid profiles of volunteers with high depression scores were paralleled by those of 32 clinically depressed patients included in the study design. Our results demonstrate that lipid alterations are not only found in the blood and brain of clinically depressed patients, but can be detected in individuals exhibiting depressive symptoms from the general population, highlighting the significance and robustness of lipid metabolism alterations in depression. The research was supported by the Russian Science Foundation grant No. 22-15-00474.

Concordant inter-laboratory derived concentrations of ceramides in human plasma reference materials via authentic standards

Federico TORTA and the participants to the ceramides harmonization ring trial

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In this community effort, we compared measurements between 34 laboratories from 19 countries, utilizing mixtures of labelled authentic synthetic standards, to quantify by mass spectrometry four clinically used ceramide species in the NIST (National Institute of Standards and Technology) human blood plasma Standard Reference Material (SRM) 1950, as well as new suite of candidate plasma reference materials. Participants either utilized a provided validated method and/or their method of choice. Our results are the most precise (intra-laboratory CV ≤ 4.2%) and concordant (inter-laboratory CV < 14%) community-derived absolute concentration values reported to date for four clinically used ceramides in the commonly analyzed SRM 1950. Collectively, the results from this study provide a base for translation of lipidomic technologies to clinical applications.

Introducing clinical mass spectrometry-based sex steroid hormone analysis in Singapore

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Background and Objectives: Sex steroid hormones, such as testosterone, estradiol, and progesterone, play pivotal roles in human health. Knowing their levels is necessary for various purposes including assessing fertility and menopausal status, serving as biomarkers for hormone-related disorders, such as prostate and cervical cancers, and monitoring hormone replacement therapy. Accurate measurement of these hormones is challenging, given their broad concentration ranges and structural similarities. Commonly used immunoassays, while convenient and cost-effective, exhibit limitations in accuracy and specificity. Liquid chromatography-mass spectrometry (LC-MS) is the globally recognized gold standard for hormone analysis, offering high sensitivity, specificity, and the capability to simultaneously measure multiple hormones. SLING aims to deliver an accurate and reliable sex steroid hormone testing service under a Healthcare Services Act (HCSA) license, utilizing a commercial LC-MS kit.

Methods: Employing Tecan's Steroid Panel LC-MS kit, target analytes are extracted from human serum via solid-phase extraction in a 96-well format and subjected to LC-MS analysis through multiple reaction monitoring for quantification.

Results: The method, optimized to separate and detect all 18 steroids, overcame challenges posed by multiple pairs of isobaric compounds. The lower limit of detection (LLOQ) was established at 0.01 ng/mL. The use of authentic internal standards allows for providing absolute concentrations for each hormone. A robust quality management system (QMS), compliant with ISO 15189, ensures comprehensive quality control and assurance. The ongoing method validation, aligned with Clinical & Laboratory Standard Institute C62A guidelines, focuses on clinical MS analysis with a specific emphasis on evaluating commutability. Beyond traditional validation, a comprehensive quality control and assurance protocol will be implemented in routine clinical sample analysis for long-term method validation.

Conclusions: SLING aims to position itself as clinical MS laboratory in Singapore, offering mass spectrometry-based measurements of sex steroid hormones. This method is also well-suited for cohort studies involving a large number of samples. Our future objective is to establish an Asian-specific hormone reference interval (RI). The implemented QMS ensures the service's quality, enhancing its reliability and adherence to international standards, ultimately contributing to improved patient care and safety.

Nano-LC-MS based lipidomics for single cell applications

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Introduction. Lipidomics is the comprehensive analysis of molecular lipid species. Separation of lipids using liquid chromatography followed by analysis by mass spectrometry has become the method of choice for lipidomic studies. Many biological specimens including single cell extracts are limited in sample amounts and hence require highly sensitive analysis methods. Conventional LC-MS based lipidomics does not offer the sensitivity required for the comprehensive lipidomic analysis of these samples. Nano-LC-MS offers high sensitivity but is technically challenging to implement in terms of robustness and reproducibility. This work describes the development and optimization of nano-LC-MS using micropillar array-based columns for robust and reproducible lipidomic analysis.

Methods. Lipid standards and bovine liver lipid extracts were purchased from Avanti Lipids. For conventional LC-MS, lipids were separated on a Thermo Scientific™ Accucore™ C18 column connected to a Thermo Scientific Vanquish™ Horizon HPLC system. For nano LC-MS lipids were separated on a Thermo Scientific µPAC™ - column connected to a Thermo Scientific VanquishNeo UHPLC system. Data were acquired on a Thermo Scientific Orbitrap IQ-X Tribrid Mass Spectrometer equipped with UVPD (213 nm laser). Thermo Scientific LipidSearch™ 5.0 software was used for lipid annotation anddata processing.

Preliminary Data. Dilution series of lipid standards and bovine liver lipid extracts were analyzed using both the conventional LC-MS as well as nano-LC-MS. µPAC columns are built by precise micro machining chromatographic separation beds into silicon. This leads to lower backpressure, high resolution, robustness and high reproducibility. The flow rates and run time for the nLC were optimized for greater separation and resolution of the eluted lipid species. Repeated injections of the same sample- showed a maximum retention time drift of 0.1 minutes. We further benchmarked the developed nLC system to the conventional high flow HPLC/ESI MS system in terms of lipidome coverage and sensitivity. We also found an increase in sensitivity of all lipid classes going up to almost 10 times for certain lipid species such as acyl-carnitines. There was also an increase in dynamic range of almost 2 orders of magnitude. For similar amounts of injected lipids, we were able to annotate almost twice the lipid species in nLC compared to conventional LC separation using LipidSearch software.

Novel Aspect. nLC-MS based method for lipidomics is developed using micropillar array-based columns to approach sensitivities.

Untargeted metabolomic profiling reveals distinct metabolomic profiles in patients with metabolic dysfunction-associated steatotic liver disease

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Introduction. Liquid chromatography-high resolution mass spectrometry (LC-HRMS)-based untargeted approaches offer opportunities for global profiling of metabolome in clinical applications. Leveraging on a combination of data-dependent acquisition (DDA) and data-independent acquisition (DIA or SWATH-MS) on SCIEX TripleTOF 6600, we analyzed liver tissues and blood plasma samples from 109 obese patients undergoing bariatric surgery spanning early stages of metabolic dysfunction-associated steatotic liver diseases (MASLD).

Methods. Metabolites and lipids were extracted using modified chloroform-methanol biphasic extraction methods for both biological matrices. Organic and aqueous fractions were separated using RRHD Eclipse Plus C18 and SeQuant ZIC-cHILIC columns prior to DDA and DIA acquisitions, respectively. For broad metabolome coverage, we designed a hybrid workflow for metabolite identification via fragment ion spectral matches (MS/MS-level) and matches by precursor ion matches (MS1-level). For metabolite quantification, we built a project-specific spectral library directly from the DDA data and extracted quantitative measurements from peak areas of precursor and product ions from the SWATH-MS data. Data were subjected to further quality control screening measures based on the coefficient of variation, detection frequency, signal-to-noise ratio, and others.

Preliminary Results. The final semi-quantitative data included 1104 metabolites in the liver and 962 in the plasma. Over 60% of compounds were identified at the MS/MS level via matching to public reference libraries such as NIST, HMDB and LipidBlast using MetaboKit software suite. Global projection by principal component analysis indicated that both glycerolipids (GL including DAGs and TAGs) and non-GL metabolome in the liver progressively changed from no pathology to high grades of MASLD. By contrast, the plasma metabolome did not exhibit patterns consistent with pathological and histological assessment of the diseases. Pronounced changes in the hepatic lipidome were observed as expected, including increased net storage of glycerolipids and mildly decreased levels of other lipid classes such as PC, PE, SM and ceramides. Interestingly, in the hepatic lipidome, TGs with 0 – 5 double bonds in the acyl chains were positively associated with the histological scores, whereas those containing more polyunsaturated fatty acids chains were negatively associated with the scores. Beyond the lipids, we observed significant increases in anti-oxidant and anti-inflammatory mediators such as CoQ10 intermediates, alpha-tocopherol, taurine, and 6 amino acids associated with steatosis. Unlike the lipidome-wide remodeling in the liver, the plasma metabolome showed limited changes attributable to the histological assessments in this cohort of early-stage MASLD patients. However, certain plasma TG species showed significant differences correlated with the degree of steatosis than others, highlighting the potential as more specific biomarkers of disease progression in early MASLD.