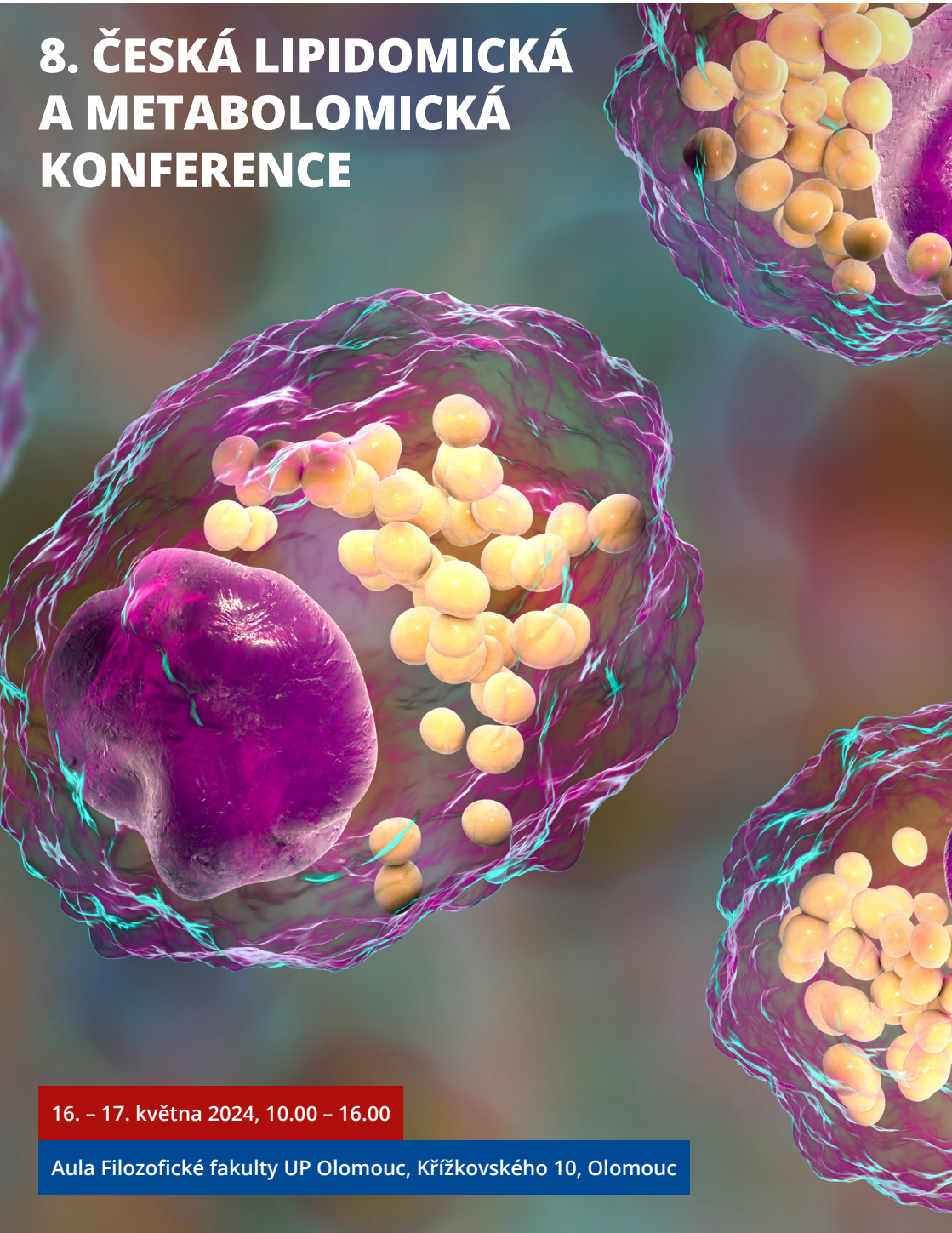
Lipidomická sekce
České společnosti pro biochemii a molekulární biologii

FAKULTNÍ NEMOCNICE OLOMOUC

Univerzita Palackého v Olomouci

8. ČESKÁ LIPIDOMICKÁ A METABOLOMICKÁ KONFERENCE



16. – 17. května 2024, 10.00 – 16.00

Aula Filozofické fakulty UP Olomouc, Křížkovského 10, Olomouc

VĚDECKÝ VÝBOR

RNDr. Ondřej Kuda, Ph.D., Fyziologický ústav AV ČR, v.v.i., Praha

doc. RNDr. Josef Cvačka, Ph.D., Ústav organické chemie a biochemie AVČR, v.v.i., Praha

prof. RNDr. David Friedecký, Ph.D., Fakultní nemocnice a Univerzita Palackého v Olomouci

prof. Ing. Michal Holčapek, Ph.D., Univerzita Pardubice

doc. Ing. Miroslav Lísa, Ph.D., Univerzita Hradec Králové

MÍSTNÍ ORGANIZAČNÍ VÝBOR

prof. RNDr. David Friedecký, Ph.D., organizátor, Fakultní nemocnice a Univerzita Palackého Olomouc

Mgr. Aleš Kvasnička, Mgr. Eliška Ivanovová, Mgr. Barbora Pisklákova, Univerzita Palackého Olomouc

Radka Klásková, Ing. Petr Hrabálek, Fakultní nemocnice Olomouc

Další informace najdete na: https://biochemie.fnol.cz/konference-a-seminare

PROGRAM

ČTVRTEK 16. 5. 2024

10:00 – 10:15
Opening of the Conference
O. Kuda, D. Friedecký

10:15 – 12:15
Lipidomics Advances: From Cellular Dynamics to Structural Analysis
Chair: M. Lísa

O. Kuda **Tracking Neutral Lipid Metabolism in Hepatocytes and Adipocytes** *(Institute of Physiology CAS, Prague) 25min*

O. Kozlov **Analysis of FAHFA isomers in biological samples using RP-LC fractionation and chiral SFC/MS** *(University of Hradec Králové) 20min*

T. Čajka **LC-HDX-MS for enhancing structure elucidation of unknowns** *(Institute of Physiology CAS, Prague) 25min*

J. Cvačka **Use of mass spectrometry with UV photodissociation for structural analysis of ester lipids** *(Institute of Organic Chemistry and Biochemistry CAS, Prague) 25min*

J. Ngere **Comprehensive data acquisition workflow on Orbitrap Astral MS for untargeted lipidomics to achieve deep lipidome coverage with high confidence annotations** *(Pragolab) 15min*

12:15 – 13:45 Lunch, posters

13:45 – 15:20
Exploring Metabolic Frontiers: Novel Insights and Analytical Approaches
Chair: M. Holčapek

D. Olešová **Potential Novel Regulators of Adipose Tissue Thermogenesis: Insights from Multi-omics Analysis.** *(Biomedical Research Center SAV, Bratislava) 20min*

M. Moos **The role of glucose and trehalose metabolism by cyclic pentose phosphate pathway in pathogen resistance and host protection in Drosophila** *(University of South Bohemia, České Budějovice) 20min*

M. Vondráčková **LORA: Lipid Over-Representation Analysis Based on Structural Information** *(Institute of Physiology CAS, Prague) 20min*

J. Procházková **Lipidomic analyses and single-cell profiling of glycosphingolipids in non-tumor and tumor breast in vitro models and clinical samples** *(Institute of Biophysics CAS, Brno) 15min*

J. Zrostlíková Targeted lipidomics and metabolomics using LC/MS triple quadrupole Agilent 6495D *(Altium) 10min*

15:20 – 16:00 Coffee break, posters

16:00 – 17:45
Innovations in Clinical Lipidomics: From Screening to Therapeutic Insights
Chair: J. Cvačka

M. Holčapek Clinical Validation of Pancreatic Cancer Screening Test and Investigation of Mechanism of Observed Dysregulations
(University of Pardubice) 25min

E. Cífková Brain lipidomic and metabolomic changes in psychotic relapse relevant to schizophrenia *(University of Hradec Králové) 15min*

A. Kvasnička Clinical lipidomics in cardiology and rheumatology
(Palacky University Olomouc) 20min

L. Řimnáčová Fully validated lipid fatty acid analysis after transmethylation by isotope-coded derivatization and GC-PICI-MS
(Biology Centre, CAS, České Budějovice) 20min

M. Maldini Quantitation and structural characterization of lipid mediators by high-resolution mass spectrometry *(SCIEX) 10min*

19:00 – 22:30 Dinner, Konvikt Restaurant

PÁTEK 17. 5. 2024

9:00 – 10:40
Integrative Omics: Unraveling Complex Metabolic Pathways
Chair: T. Čajka

G. Koellenperger Workflows enabling simultaneous targeted and non-targeted metabolomics *(University of Vienna, Austria) 30min*

T. Pluskal Lessons learned from repository-scale machine learning on tandem mass spectra
(Institute of Organic Chemistry and Biochemistry CAS, Prague) 25min

D. Friedecký Clinical applications of targeted metabolomics
(Palacký University Olomouc) 25min

D. Maxa Mass spectrometer radicalisation - OAD-TOF *(Shimadzu) 10min*

10:40 – 11:20 Coffee break, posters

11:20 – 12:50
Next-Generation Analytical Techniques: Advancing Metabolomics Research
Chair: T. Pluskal

M. Lísa LC/MS analysis of polar metabolites and endocannabinoids in spontaneous preterm birth human placenta samples *(University of Hradec Králové) 25min*

A. Behner Metabolomic changes in malted barley after pulsed electric field (PEF) treatment *(University of Chemistry and Technology, Prague) 20min*

L. Najdekr Can lipid profiles reflect the true biological age and healthy ageing? *(IMTM, Palacký University Olomouc) 20min*

D. Vláčil Bruker 4D-Lipidomics™: Exploring the lipidome at the speed of PASEF® *(Bruker) 10min*

12:50 – 13:00 Closing of the Conference

12:30 – 14:00 Light lunch

SBORNÍK ABSTRAKT

Tracking Neutral Lipid Metabolism in Hepatocytes and Adipocytes

Ondrej Kuda, Dovilė Milonaitytė, Martina Rombaldova, Tomas Cajka

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Triglyceride (TG) cycling is the continuous process of partial triglyceride degradation and re-synthesis (re-esterification) within cellular stores. This project focuses on understanding TG turnover and the mechanisms of futile substrate cycling in adipocytes and hepatocytes. Using a combination of molecular biochemistry, mass spectrometry, and bioinformatics, we model the interconnected pathways at the stereospecific level of acylglycerol substrates and products. This tracer-based approach to metabolic flux analysis allows us to study biochemical reactions that would otherwise be experimentally inaccessible. Our data indicate a rapid and continuous redistribution of fatty acyls within acylglycerols, with specific spatial distributions of reactions and preferred stereo- and regio-specific combinations of acyls in acylglycerols. We observe both quantitative and qualitative differences in acylglycerol metabolism between hepatocytes and adipocytes, suggesting that not all TG combinations can be synthesized everywhere and that cells have fatty acyl-specific preferences. Our results reveal cell type-specific biochemical mechanisms beyond TG cycling.

Supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) – Funded by the European Union – Next Generation EU.

Analysis of FAHFA isomers in biological samples using RP-LC fractionation and chiral SFC/MS

Oleksandr Kozlov[1], Ondřej Kuda[2], Martin Riečan[2], Miroslav Lísa[1]

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Branched fatty acid esters of hydroxy fatty acids (FAHFAs) represent a recently discovered class of endogenous lipids with promising therapeutic potential for diabetes and inflammation. Their common structure includes a fatty acid (e.g. oleic acid, OA) esterified to the hydroxyl group of a hydroxy fatty acid (e.g. hydroxystearic acid, HSA) by its carboxyl head (abbreviated as OAHSA). Depending on the position of the hydroxyl group in the acyl chain, FAHFAs can exist as different positional isomers, each providing two enantiomers with distinct roles in physiological processes. Moreover, a variety of combinations of fatty acids and hydroxy fatty acids occurred in biological samples. This complexity creates significant challenges for FAHFA analysis, demanding powerful analytical techniques.

This study focused on developing a novel SFC/MS method for the enantioselective analysis of FAHFAs. The 21 commercially available representatives from the FAHFA family, primarily with HSA backbone, were selected for the method development with using of polysaccharide-based chiral stationary phases. A detailed evaluation of key chromatographic parameters on separation efficiency was conducted. The optimized chiral SFC/MS method utilizes a methanol-acetonitrile mobile phase modifier, enabling the separation of the targeted compounds in 5 min. While the method achieved good enantioselectivity, successfully resolving most FAHFA racemic pairs into single enantiomers, positional isomers within the FAHFAs remained poorly distinguished by SFC, which makes the developed method hardly applicable to the analysis of complex biological samples. To address this limitation, we propose a two-step approach: RP-LC fractionation followed by chiral SFC/MS analysis. Lipid extracts from white adipose tissue and rice were fractionated after careful identification based on the retention time of standards and characteristic fragments in tandem mass spectra. Subsequently, the developed chiral SFC/MS method was used to determine the enantiomeric composition of the FAHFAs in collected fractions. The developed approach provides a valuable tool for future research on the biological roles of FAHFAs in health and disease.

This work was supported by the Czech Science Foundation (project no. 24-12234S). O.K. wishes to acknowledge project 2200/04/2024-2026 as part of the „Competition for 2024-2026 Postdoctoral Job Positions at the UHK" for the support.

LC-HDX-MS for enhancing structure elucidation of unknowns

Tomas Cajka, Jiri Hricko, Stanislava Rakusanova, Kristyna Brejchova, Michaela Novakova, Lucie Rudl Kulhava, Veronika Hola, Michaela Paucova, Ondrej Kuda

Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, 14200 Prague, Czech Republic

Liquid chromatography with mass spectrometry (LC-MS)-based metabolomics detects thousands of molecular features (retention time–m/z pairs) in biological samples per analysis. However, the metabolite annotation rate remains low, with 90% of signals classified as unknowns [(1)]. Researchers employ tandem mass spectral libraries and challenging in-silico fragmentation software to enhance the metabolite annotation rates [(2)]. Here, we investigated the potential of hydrogen/deuterium exchange mass spectrometry (HDX-MS) with reversed-phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) as an additional layer of structural information in LC-MS-based untargeted metabolomics. Specifically, we evaluated the effectiveness of two approaches using hypothetical targets: the post-column addition of deuterium oxide and the on-column RPLC-HDX-MS and HILIC-HDX-MS methods. The post-column addition of D2O in LC-MS exhibited limitations, particularly in achieving complete H/D exchange for the polar metabolites. However, the LC-HDX-MS setups, where mobile phases included D2O, demonstrated improved performance with complete H/D exchange for the evaluated metabolites. To illustrate the practical application of LC-HDX-MS, we applied this methodology and the in-silico fragmentation software MS-FINDER to an unknown compound detected in various biological samples, including plasma, serum, tissues, and feces during metabolomic profiling, subsequently identified as N1-acetylspermidine [(3)].

The work was supported by the Czech Science Foundation (21-00477S) and the National Institute for Research for Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) funded by the European Union—NextGenerationEU.

1. Rakusanova, S. et al. Toward building mass spectrometry-based metabolomics and lipidomics atlases for biological and clinical research. TrAC-Trend Anal. Chem. 158, 116825 (2023).
2. Kind, T. et al. Identification of small molecules using accurate mass MS/MS search. Mass Spectrom. Rev. 37, 513–532 (2018).
3. Cajka, T. et al. Hydrophilic interaction liquid chromatography–hydrogen/deuterium exchange–mass spectrometry (HILIC-HDX-MS) for untargeted metabolomics. Int. J. Mol. Sci. 25, 2899 (2024).

Využití hmotnostní spektrometrie s UV fotodisociací pro strukturní analýzu esterových lipidů

Josef Cvačka[1,2], Lukáš Cudlman[1,2], Barbora Kloudová[1,2], Vladimír Vrkoslav[1], Aleš Machara[1], Miroslav Polášek[2]

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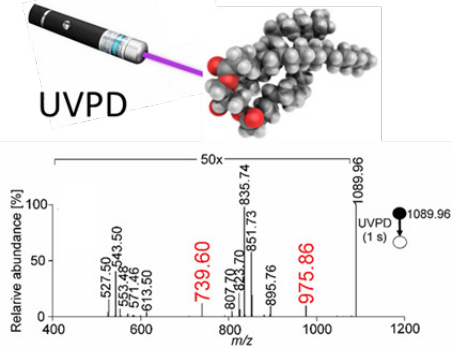

Podrobná znalost struktury lipidů je důležitá pro hlubší porozumění komplexních rolí lipidů v patologii mnoha onemocnění včetně kardiovaskulárních. V hmotnostní spektrometrii se pro strukturní analýzu nejčastěji využívá kolizně indukovaná disociace (CID). Tato technika poskytuje cenné informace o lipidech, avšak některé strukturní detaily, například polohy dvojných vazeb, popsat nedokáže. Nedávné pokroky v instrumentaci umožnily využívat nové fragmentační techniky, např. fotodisociaci UV zářením (UVPD). Interakce iontů s UV fotony vedou k fotochemickým reakcím a fotoproduktům, které lze využít pro strukturní analýzu lipidů.

V této práci jsme se zabývali strukturní analýzou esterových lipidů pomocí UVPD. Aktivace lithných aduktů voskových esterů (WE) a estolidů triacylglycerolů (TG-EST) pomocí UV laseru (213 nm) poskytovala informativní hmotnostní spektra s fragmenty, které nebyly dosud pozorovány a popsány. Domníváme se, že na esterových skupinách probíhaly Norrishovy a Norrish-Yangovy fotochemické reakce.

Absorpce UV fotonů vedla k excitaci karbonylu, který abstrahoval γ-vodík z alifatického řetězce. Vzniklý biradikál se buď fragmentoval přímo (Norrishova reakce typu II), nebo se nejprve interně rekombinoval na substituovaný oxetan-2-ol nebo cyklobutan (Norrish-Yangova reakce). Ve spektrech WE se tvořily aldehydové fragmenty odpovídající kyselinovým a alkoholovým řetězcům a fragmentové ionty mastných kyselin. TG-EST obsahují několik esterových skupin, a proto byla jejich fragmentace složitější. UVPD spektra umožnila odlišit izomerní estolidy lišící se polohou esterové vazby v jednotce FAHFA. Rozdíly byly výrazné ve spektrech MS3 CID/UVPD s lithným aduktem FAHFA jakožto prekurzorem pro UVPD. Spektra MS3 CID/UVPD dále umožnila odlišit regioizomery lišící se substituenty v sn-1/3 a sn-2 polohách na glycerolu. Interakce fotonů s dvojnými vazbmi vedla k fragmentům, které jednoznačně určovaly jejich polohy. Tyto fragmenty bylo snadné poznat, protože se vzájemně lišily o 24,0000 Da. V případě TG-EST bylo možné získat ionty související s dvojnou vazbou pomocí MS3 HCD/UVPD nebo MS4 CID/CID/UVPD. Využití nové metody strukturní analýzy lipidů jsme demonstrovali na biologických vzorcích.

Tato práce vznikla za podpory projektu Národní institut pro výzkum metabolických a kardiovaskulárních onemocnění (Program EXCELES, ID: LX22NPO5104), financovaného Evropskou unií – Next Generation EU.

Potential Novel Regulators of Adipose Tissue Thermogenesis: Insights from Multi-omics Analysis.

Dominika Olešová[1], Adhideb Ghosh[3], Aleš Kvasnička[2], Lívia Petrisková[1], Patrik Štefanička[4], Dana Dobešová[2], Lucia Balážová[1], Christian Wolfrum[3], David Friedecký[2], Miroslav Baláž[1]

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[4] Bory Hospital, Ivana Kadlečíka 2, 841 03 Bratislava, Slovakia.

In the quest to combat obesity and its associated metabolic complications, the activation of thermogenic adipocytes has emerged as a promising therapeutic strategy. Brown adipose tissue (BAT) shows unique metabolic properties, characterized by its ability to release energy as heat through thermogenesis. Studies have shown that increasing the activity or amount of BAT could help improve systemic metabolic control, alleviate metabolic complications such as dyslipidaemia, impaired insulin secretion, and insulin resistance.

We established a biobank comprising paired human deep neck brown and subcutaneous white adipose tissue (WAT) samples obtained from patients undergoing neck surgery (N=48) with varying levels of thermogenic activation. Through quantitative real-time PCR analysis of all adipose tissue biopsies, we identified 15 patients exhibiting enrichment of UCP1 mRNA in BAT. In this study, we employed a multi-omics approach to identify key regulators of adipose tissue thermogenic activity.

Through lipidomic, metabolomic, and transcriptomic analyses, we systematically characterized both BAT and WAT collected from patients with active brown fat. Using this approach, we identified 240 lipids species, 59 metabolites and 5722 transcripts differentially regulated between BAT and WAT. Robust evidence shows enrichment of membrane and mitochondrial lipids in BAT, while neutral glycerolipids and fatty acids predominate in WAT. To identify novel targets with potential to activate BAT thermogenesis, we conducted pathway analysis on the lipidomic data and identified several lipid-processing genes, including DGAT2, PISD, PTDSS1, CHPT1 and PEMT as interesting candidates for further functional validation. Moreover, multi-omics factor analysis showed that purine metabolites emerge as the most prominent features driving BAT activation.

These findings provide a groundwork for understanding the metabolic differences between brown and white adipose tissue. In this work we elucidated key regulators of adipose tissue thermogenic activity, with purine metabolites, and several lipid-associated genes emerging as new promising drivers of BAT activation.

The role of glucose and trehalose metabolism by cyclic pentose phosphate pathway in pathogen resistance and host protection in Drosophila

Michalina Kazek[1], Lenka Chodáková[1], Katharina Lehr[1], Lukáš Strych[1], Pavla Nedbalová[1], Ellen McMullen[1], Adam Bajgar[1], Stanislav Opekar[2], Petr Šimek[2], Martin Moos[2], Tomáš Doležal[1]

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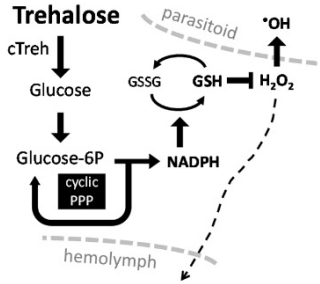
In different situations, the cell uses the glycolysis - pentose phosphate pathway (PPP) linkage in different ways, depending on how much ATP, NADPH and de novo nucleotide production (e.g. for proliferation) it requires. If it mainly needs ATP, it uses glycolysis (possibly in conjunction with oxphos in the mitochondria). If the cell mainly needs nucleotides, it uses the non-oxidative pentose pathway. If it requires NADPH and nucleotides, it uses the oxidative PPP to produce NADPH and divert ribose 5-phosphate for de novo nucleotide synthesis; if it needs NADPH and ATP, it uses the oxidative PPP and returns to downstream glycolysis via the non-oxidative PPP and continues ATP production. If the cell primarily needs to produce NADP (e.g. in the case of oxidative stress), it uses the cyclic PPP.



The activation of immune cells requires a remodeling of cell metabolism to support immune function. We investigate these metabolic changes by studying the infection of Drosophila larvae by parasitic wasps. Neutralization of the parasitoid egg involves the differentiation of lamellocytes that encapsulate the egg. A melanization cascade is initiated that produces toxic molecules to destroy the egg; at the same time, the capsule formed protects the host from the toxic response. We combine transcriptomics and metabolomics, including HRMS tracing of 13C-labeled glucose and trehalose, and genetic manipulation of sugar metabolism to study changes in metabolism, specifically in Drosophila hemocytes. We found that hemocytes increase the expression of several carbohydrate transporters, and thus the uptake of sugar during infection. These carbohydrates are metabolized by increased glycolysis, which is accompanied by lactate production, and the PPP, in which glucosa-6-phosphate is re-oxidized to maximize NADPH production. The oxidative PPP is required for lamellocyte differentiation and resistance, as is systemic trehalose metabolism. In addition, fully differentiated lamellocytes utilize a cytoplasmic form of trehalase to cleave trehalose into glucose within the cell, to drive cyclic PPP. Intracellular trehalose metabolism is not required for lamellocyte differentiation, but its downregulation increases reactive oxygen species levels, which is associated with increased resistance and decreased fitness. Thus, our results suggest that sugar metabolism in immune cells, and especially in the cyclic PPP, may be important not only for fighting infection, but also for protecting the host from its own immune response and ensuring sufficient fitness of the survivor.

The work was supported by GACR 20-09103S and Marie Skłodowska-Curie grant No 867430 to M.K.

1. Kazek M. et al. The role of glucose and trehalose metabolism by cyclic pentose phosphate pathway in pathogen resistance and host protection in Drosophila. PLOS Biology (2024)

LORA: Lipid Over-Representation Analysis Based on Structural Information

Michaela Vondráčková[1], Dominik Kopczynski[2], Nils Hoffmann[3], Ondřej Kuda[1]

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With the increasing number of lipidomic studies, there is a need for efficient and automated analysis of lipidomic data. One of the challenges faced by most existing approaches to lipidomic data analysis is lipid nomenclature. The systematic nomenclature of lipids contains all available information about the molecule, including its hierarchical representation, which can be used for statistical evaluation. The Lipid Over-Representation Analysis (LORA) web application (https://lora.metabolomics.fgu.cas.cz)

analyzes this information using the Java-based Goslin framework, which translates lipid names into a standardized nomenclature. Goslin provides the level of lipid hierarchy, including information on headgroups, acyl chains, and their modifications, up to the 'complete structure' level. LORA allows the user to upload the experimental query and universe datasets, select a grammar for lipid name normalization, and then process the data. The user can then interactively explore the results and perform lipid overrepresentation analysis based on selected criteria. The results are graphically visualized according to the lipidome hierarchy. The lipids present in the most over-represented terms (lipids with the highest number of enriched shared structural features) are defined as Very Important Lipids (VILs). For example, the main result of a demo dataset is the information that the query is significantly enriched with ‚glycerophospholipids' containing ‚acyl 20:4' at ‚sn-2 position'. These terms define a set of VILs (e.g., PC 18:2/20:4;O and PE 16:0/20:4(5,8,10,14);OH). All results, graphs, and visualizations are summarized in a report. LORA is a tool focused on the smart mining of epilipidomics datasets to facilitate their interpretation at the molecular level.

Supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) – Funded by the European Union – Next Generation EU.

Lipidomic analyses and single-cell profiling of glycosphingolipids in non-tumor and tumor breast in vitro models and clinical samples

Jiřina Procházková[1], Barbora Hradilová[1,3], Radek Fedr[1,2], Barbora Kvokačková[1,2,3], Josef Slavík[4], Ondrej Kováč[4], Miroslav Machala[4], Jiří Navrátil[5], Pavel Fabian[6], Karel Souček[1,2,3]

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Epithelial-mesenchymal plasticity is the shape-shifting ability of tumor cells, which helps them to leave the site of the primary tumor, enter the circulation system, and form metastases in distant organs. During this process of tumor dissemination, cancer cells actively change their epithelial phenotype and adopt a more migratory mesenchymal-like phenotype. In this study, we asked a question if the composition of glycosphingolipids (GSLs) is changed during epithelial-to-mesenchymal transition (EMT), and if so, can single-cell profiling of GSL-related surface epitopes present in primary tumors serve as a tool for evaluation of tumor progression or at least prediction of EMT status in patients with breast cancer (BCa)?

Total levels of selected GSLs were analyzed by HPLC-MS/MS in in vitro EMT models derived from breast tissue to reveal the association of GSLs with the phenotypic status of cells (epithelial vs. mesenchymal). These associations were then investigated in clinical samples of breast non-tumor and tumor tissue using a set of commercial antibodies recognizing GSL-related epitopes together with lineage and EMT surface markers. Our data show changes in the surface presence of Gb3, SSEA3, and SSEA4 between epithelial cells and/or stromal mesenchymal-like cells based on BCa disease status. Breast epithelial cells appeared more positive for surface SSEA1 than their paired stromal mesenchymal-like counterparts. We plan further lipidomic and single cell-based phenotypic analyses as they are essential to validate effectively the unique associations we observed between the surface presence of specific GSL-related epitopes (e.g. Gb3, SSEA3, SSEA1) and distinct breast cancer phenotypes and disease status.

In conclusion, the prognostic value of GSLs as markers of EMT is very limited. However, single-cell profiling of surface GSL-related epitopes may provide a useful description of heterogeneity present in subpopulations of epithelial and stromal mesenchymal-like cells residing in the breast tissue microenvironment of BCa patients with different disease statuses.

The study was supported by the Internal Support of Research Program of the Institute of Biophysics of the CAS (J.P.), by the Czech Science Foundation project GA CR 21-11585S (K.S.), and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU (K.S.).

Clinical Validation of Pancreatic Cancer Screening Test and Investigation of Mechanism of Observed Dysregulations

M. Holčapek[1], O. Peterka[1,2], Z. Dolečková[2], M. Kanásová[2], Z. Vaňková[1], R. Jirásko[1], B. Mohelníková Duchoňová[3], M. Loveček[3], O. Urban[3], B. Melichar[3], J. Trna[4], K. Kašparová[2], Z. Jirsa[2]

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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. 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 14 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. 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. 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 in multiple types of cancer [2]. The next goal 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. D. Wolrab et al., Sci. Rep. 11 (2021) 20322.

Brain lipidomic and metabolomic changes in psychotic relapse relevant to schizophrenia

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Schizophrenia is a mental disorder characterized by reoccurring psychotic relapses that may have deleterious effects on brain structure, function, and treatment outcome. The characterization of metabolomic and lipidomic changes in the individual brain parts including the hippocampus, prefrontal cortex, striatum, and cortex induced by single or reoccurring psychotic episodes using the MK-801 application in the preclinical models provides valuable information on the response of the immune system to recurrent episodes. Male rats were assigned to groups with a single MK-801 injection and a regimen of five daily MK-801 injections and control groups. Metabolomic analysis of individual brain extracts was performed using the reversed-phase LC/MS, while lipidomic analysis using SFC/MS experiments. Obtained results provide significant changes for repeated MK-801 injections group in metabolic pathways associated with inflammation (quinolinic acid, glutamic acid, ceramide, and cholesterol ester).

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Clinical lipidomics in cardiology and rheumatology

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Lipidomics has reached the point where it is possible to perform routine high-throughput analyses in a clinical setting. The commercial availability of various labelled internal standards and standard mixtures has enabled the transition to the precise quantification of lipids of interest. One of the most promising areas of medicine where clinical lipidomics is showing robust results is in cardiovascular risk assessment. Many independent studies in large international cohorts (n > 30 000) have identified and validated selected sphingolipids (especially ceramides) as promising prognostic markers for cardiovascular events and death. Elevated levels of ceramides, a class of sphingolipids circulating in plasma, have been associated with an increased risk of CVD events and death in primary and secondary prevention. The Coronary Event Risk Test (CERT) has been developed and is routinely used by specialised clinical laboratories. This presentation will show how plasma ceramides can be used to calculate the CERT score and how sample collection can be streamlined in the hospital setting. In addition, a novel application of the CERT score in the cohort of patients with hyperuricemia and gout will be presented and discussed together with a comprehensive lipidomic analysis of this unique rheumatology cohort.

Fully validated lipid fatty acid analysis after transmethylation by isotope-coded derivatization and GC-PICI-MS

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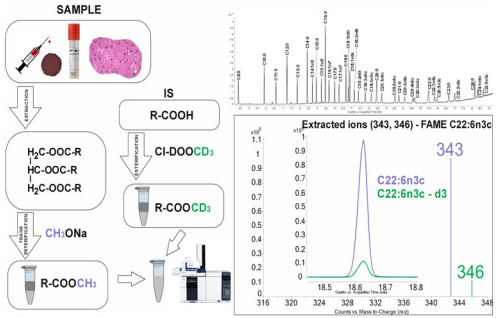
Fatty acids (FAs) are essential components of lipids and an important source of energy for organisms. Polyunsaturated fatty acids (PUFA, mainly omega 3, 6 and 9) play a key role in the structure of cell membranes, promote immunity and support the development of the nervous system. The determination of the omega-3 index (DHA and EPA levels in the blood) is an important medical indicator of health status and is usually measured by GC-MS quantification of transmethylated fatty acyl methyl esters (FAME) using one or more appropriate internal standards. However, the availability of individual standards is very difficult when comprehensively analyzing lipid FA in a complex biological matrix.



In this study, we present a comparison between a new method for the determination of lipid-bound fatty acids using isotope-coded derivatization (ICD) and a conventional technique using only one internal standard. The paired internal FAME standards with a D3 mass shift are first prepared by esterification of an FA standard mixture with D3-labeled methyl chloroformate. The D3 FAME standards are then mixed with a FAME sample obtained by transmethylation of a lipid extract with sodium methoxide. The approach was investigated with FAME detection using EI and isobutane PICI-MS ionization. The latter ionization mode proved to be clearly advantageous, especially for PUFA, where the EI mass spectra of the FAME/FAME-D3 pair are complex and very similar due to double bond migration during the EI ionization process. Instead, the PICI spectra of FAME contain intense molecular [M+H]+ ions and distinct mass shifts that allow direct quantification of FAME versus the FAME-D3 standard in biological samples. The method has been fully validated according to generally accepted analytical guidelines. The efficiency of the transesterification method for the most important lipid classes was also investigated and showed values between 72% and 100%.

The described method was successfully applied to the validated GC-PICI-MS analysis of 37 FA in different biological samples, i.e. dried blood spots, human serum, or bird sperm. Excellent validation criteria were achieved. The coefficient of determination R2 in linear regression was ≥ 0.99 for all FAME, the LLOQ was 50 ng/mL, and the precision and accuracy were in the range of 0.2 – 15 % and 82 – 122 %, respectively.

Workflows enabling simultaneous targeted and non-targeted metabolomics

Harald Schoeny, Lisa Panzenboeck, Veronika Fitz, Helena Hyo Gyoung Kim, Christina Brenner, Felina Hildebrand, Gunda Koellensperger (Invited speaker)

Department of Analytical Chemistry, Faculty of Chemistry and Vienna Metabolomics Center (VIME), University of Vienna, Austria.

The different facets of mass spectrometry-based metabolomics navigate the conflicting goals of analytical throughput and metabolome coverage. Customized workflows involve clever combinations of orthogonal chromatographic separations, wide-panel internal standardization and high resolution mass spectrometry. We will discuss different strategies merging metabolomics and lipidomics into one run. Moreover, we will present new approaches based on latest generation tandem high-resolution mass spectrometry offering MS/MS acquisition rates > 100 Hz, this way unlocking the potential for high throughput /high metabolome coverage strategies. Multiple MS-experiments can be merged within a single analytical run, increasing the sampling depth for both essential scopes of metabolomics, i.e. the identification and quantification of small molecules. We will showcase the power of the simultaneous targeted and non-targeted metabolomics, in the frame of on-going clinical studies.

BIO: Gunda Koellensperger is a respected scientist with a distinguished career in analytical chemistry. She earned her academic credentials at the Technical University of Vienna, where she received her Diploma (Dipl.-Ing.) in 1995 and her Doctorate (Dr. techn.) in 1998, both in the field of analytical chemistry. Her doctoral thesis explored the use of scanning force microscopy to investigate small particles. Her professional journey is marked by significant contributions to environmental and life sciences, particularly through her expertise in inductively coupled plasma mass spectrometry. After completing her habilitation at the University of Natural Resources and Life Sciences, Vienna (BOKU), where she focused on elemental trace analysis and speciation, she progressed through various academic positions. These include roles as Assistant and Associate Professor at BOKU, and later as a guest professor at Humboldt University in Berlin. Currently, Gunda Koellensperger holds the position of Full Professor for Environmental Chemistry at the University of Vienna. She has also been involved with the Austrian Centre for Industrial Biotechnology (ACIB), where she has significantly influenced the fields of metabolomics and analytical chemistry through both leadership in core facility management and innovative research. Her research has resulted in numerous important publications in the field of analytical chemistry, with significant work in the development of methodologies for analyzing complex biological systems and environmental samples. (generated by ChatGPT 4.0)

Lessons learned from repository-scale machine learning on tandem mass spectra

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Mass spectrometry is the primary method for characterizing biological and environmental samples at a molecular level. Despite this, the interpretation of mass spectra remains a challenge to overcome. Existing methods heavily rely on limited spectral libraries and human expertise, so we have taken an orthogonal approach. Here, we introduce a foundation transformer-based model pre-trained in a self-supervised way on millions of unlabeled mass spectra from our new GeMS (GNPS Experimental Mass Spectra) dataset. We show that by learning to predict masked spectral peaks and chromatographic retention orders, our model discovers rich molecular representations, which we name DreaMS (Deep Representations Empowering the Annotation of Mass Spectra). Fine-tuning the neural network for predicting spectral similarity, molecular fingerprints, chemical properties, and the presence of fluorine from mass spectra yields state-of-the-art performance across all tasks. This underscores the practical utility of DreaMS across diverse spectrum interpretation tasks and establishes it as a foundation for future advances in the field.

Klinické aplikace cílené metabolomiky

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V posledních letech si omické techniky na úrovni nízkomolekulárních analytů našly své místo při hledání biomarkerů známých onemocnění i v rutinní diagnostice. S tím, jak se metody analýzy metabolitů a lipidů stále zdokonalují a využívají se při studiu různých onemocnění, je stále jasnější, že metabolické poruchy hrají klíčovou roli v mnoha patofyziologických a patobiochemických procesech. Cílené i necílené přístupy pak poskytují nový komplexní pohled na metabolické profily odpovídající změnám u široké škály onemocnění. V oblasti dědičných metabolických poruch zachraňuje rychlé zavedení metabolomiky v podobě vícesložkové analýzy malých molekul velký počet dětí. Metabolomické a lipidomické studie v poslední době ukázaly nové slibné biomarkery pro zánětlivé stavy nebo pro predikci rizika ischemické choroby srdeční a akutního koronárního syndromu, obezity a hyperlipidemie, rakoviny, Alzheimerovy choroby a dalších. Nezbytnou součástí je hodnocení pomocí vícerozměrných statistických metod a následné použití bioinformatických nástrojů, které umožňují získat komplexní pohled. Tato prezentace představí nové poznatky ve výše uvedených oblastech a shrne výsledky našich studií, které nabízejí potenciál v oblasti laboratorní diagnostiky. Z části bude věnována pozornost také nové direktivě IVD-R, která je nezbytnou podmínkou pro zavedení omics metod do rutinního diagnostického procesu.

LC/MS analysis of polar metabolites and endocannabinoids in spontaneous preterm birth human placenta samples

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Spontaneous preterm delivery presents one of the most complex challenges in obstetrics and is a leading cause of perinatal morbidity and mortality. Although it is a common endpoint for multiple pathological processes, the mechanisms governing the etiological complexity of spontaneous preterm birth and the placental responses are poorly understood. This work aimed to evaluate metabolomic changes in placental tissues from a well-defined cohort of women who experienced spontaneous preterm birth (n = 72) and healthy full-term deliveries (n = 30). Metabolomic profiling of polar metabolites and endocannabinoids was performed using an ultra-high performance liquid chromatography-mass spectrometry platform based on a C18 column for 100% aqueous phases and water – acetonitrile – propan-2-ol mobile phase using two different gradients for polar and middle-polar metabolites. The resulting data were assessed using multi- and univariate statistical methods followed by unsupervised clustering. A comprehensive metabolomic evaluation of the placenta revealed that spontaneous preterm birth was associated with significant changes in the levels of N-acylethanolamines, monoacylglycerols, and polar metabolites involved in intracellular energy metabolism and biochemical activity, including amino acids, purine metabolites, and small organic acids (1).

This work was supported by the Czech Science Foundation project no. 22-13967S

1. Cífková, E. et al. Metabolomic Analysis of the Human Placenta Reveals Perturbations in Amino Acids, Purine Metabolites, And Small Organic Acids in Spontaneous Preterm Birth. EXCLI J. 23, 264 – 282 (2024).

Metabolomic changes in malted barley after pulsed electric field (PEF) treatment

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Fusarium micromycetes are widespread pathogens causing fungal diseases of crops, including barley. Such contamination is usually associated with formation of mycotoxins posing a serious health risk to consumers. The malting of barley is susceptible for spreading of Fusarium pathogens and mycotoxins, as favorable conditions (temperature and humidity) during germination support the fungal development. Recently developing food processing technology significantly contributing to Fusarium fungi minimization, is the pulsed electric field (PEF), which also influences the metabolism of germinating barley. Here, we investigate the metabolomic changes during the individual technological steps of malting after the PEF treatment. Methanolic extracts of technological intermediates of malting were analyzed by metabolomic fingerprinting performed by ultra-high performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS). For data processing and interpretation, the freely available MS-DIAL – MS-CleanR – MS-Finder software platform was used. Additional software utility MS-CleanR able to effectively filter the 'blank' ions, improbable ions and various artefacts, preventing overfitting of multivariate statistical models and improving biomarkers' annotation rate. After creation of OPLS-DA binary models, separately for each technological intermediates, statistically significant features were selected using variable importance in the projection (VIP, VIP score>1) and the receiver operating characteristic (ROC, AUC=1) methods. Both filters were applied simultaneously to ensure proper statistical filtration and to keep only the statistically significant biomarkers relevant for further data interpretation. The evaluation of metabolomes of the treated and untreated samples enabled the tentative identification of up- and down-regulated biomarkers of the PEF treatment. The validity of biochemical interpretation of these biomarkers was strengthened by statistically significant Fusarium / barley genes selected by transcriptomics approach, and the multi–omics data–driven approach (OmicsAnalyst software). Final interpretation of data generated at both "omics" levels significantly contributes to the basic knowledge on the "germinating barley grain – pathogen" cross-talk.

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Can lipid profiles reflect the true biological age and healthy ageing?

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Lipidomics, the comprehensive analysis of lipid molecules within biological systems, has emerged as a powerful tool for understanding the complex interplay between lipids and human health. This work explored lipidomic profiles as potential indicators of true biological age and healthy ageing.

We collected human plasma samples (n = 1104) of various ages (19 – 73 y.o.) from a Transfusion department of University Hospital Olomouc (CZ). All samples can be considered healthy without significant pathologies, creating the perfect sample collection for studying ageing effects. The human plasma was analysed using ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). The data matrix was processed using Compound Discoverer 3.3 SP1 (Thermo Fisher Scientific, USA).

By approach of untargeted lipidomics, we have putatively annotated 182 lipids in positive ionisation mode and 130 lipids in negative ionisation mode (MSI ID Level 2). Furthermore, on MSI ID Level 3, we annotated 602 and 175 lipids in positive and negative ionisation modes, respectively. The first glimpse at the data set did not reveal any „one-molecule" markers. Still, we have observed trends associated with age in groups of phosphocholines (PC), ceramides (Cer) and hexosylceramides (HexCer). This finding is in concordance with the latest trends in small molecule research, where it is more effective to focus on a combined panel of molecules rather than a single entity. As expected, we have also observed differences in lipid profiles based on gender.

By unravelling the intricate relationship between lipids and ageing, lipidomics offers a unique opportunity to identify novel therapeutic targets and develop personalised interventions to mitigate age-related pathologies. Further investigation is warranted to validate and refine these findings, enabling the translation of lipidomic-based approaches into clinical practice for assessing and monitoring the ageing process.

POSTERY

1. **A. Langová** **Comparison of Bioinert and Conventional UHPLC Systems for Metabolomic Analysis**
   (University of Pardubice)
2. **E. Ivanovová** **Detailní LC-MS/MS analýza cukrů a jejich alditolů pro diagnostiku dědičných metabolických poruch**
   (Univerzita Palackého v Olomouci)
3. **B. Piskláková** **Hodnocení rizika kardiovaskulárních příhod pomocí skóre CERT**
   (Univerzita Palackého v Olomouci)
4. **V. Šubrtová** **Komplexní analýza nepolárních lipidů ve vzorcích plazmy a séra pomocí RP-UHPSFC/MS**
   (University of Pardubice)
5. **V. Vrkoslav** **Lipidom protizánětlivých a prozánětlivých makrofágů tukové tkáně**
   (Vysoká škola chemicko-technologická v Praze)
6. **Z. Dolečková** **Lipidomic test for early detection of pancreatic cancer – transfer to clinical practice**
   (Lipidica a.s.)
7. **J. Rozhon** **Lipidomická analýza pacientů se střádavými lysozomálními poruchami (LSD)**
   (Univerzita Palackého v Olomouci)
8. **P. Žáček** **Mass spectrometry Core laboratory in BIOCEV offers metabolomic, proteomic and lipidomic services for public**
   (Charles University, Prague)
9. **K. Brejchová** **Metabolic pathways of acylcarnitine synthesis** *(Institute of Physiology CAS)*
10. **S. Rakušanová** **Metabolomic changes across various matrices in mice in response to chow and high-fat diet**
    (Institute of Physiology CAS)
11. **M. Morozovová** **Permeability Assessment of Antiepileptic Drugs Using in vitro BBB Model**
    (Institute of Organic Chemistry and Biochemistry CAS)
12. **V. Kosek** **Stratification of patients with and without significant carotid plaque by LC-HRMS/MS plasma lipidomics**
    (University of Chemistryand Technology, Prague)
13. **P. Majerova** **Study on lipid changes in animal model for Type 2 diabetes**
    (Institute of Neuroimmunology SAV)
14. **V. Berka** **Untargeted lipidomics distinguish Streptococcus zooepidemicus mucoid and non-mucoid phenotype**
    (Contipro a.s.)
15. **Z. Vaňková** **Utilization of Comprehensive Online RP-UHPLC×UHPSFC/MS/MS configuration to Lipidomic Analysis of Human Plasma**
    (University of Pardubice)
16. **M. Riečan** **Androgen levels determination in ADTRP male and female knock-out mice**
    (Institute of Physiology CAS)

1) Comparison of Bioinert and Conventional UHPLC Systems for Metabolomic Analysis

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In recent years, metabolomics has been ranked as one of the leading omics approaches, offering direct information about the current state of the organism. This approach is utilized to identify and quantify metabolites in biological samples. Metabolomics might improve the understanding of physiological conditions and facilitate the characterization of metabolic alterations linked to serious diseases or the exploration of novel biomarkers. Metabolites are small molecules mostly containing polar function groups, e.g., phosphate and sulfate. Those ionic groups are typically challenging for chromatography because of the interaction with the metal surface of the instrument, resulting in poor peak shapes, reduced chromatographic resolution, and decreased sensitivity. A fully bioinert system compared to a conventional system, can solve these problems, especially for nucleotides containing one to three phosphate groups (e.g., AMP, ADP, ATP). Traditionally, bioinert columns are made from polyether ether ketone (PEEK), but materials like titanium and MP35N alloys are frequently favored over PEEK due to their superior capacity to resist high pressures.

In this study, a new metabolomics method was developed that focuses on ionic metabolites present in human plasma. This method can separate all proteinogenic amino acids, including isomers, such as leucine/isoleucine/norleucine, carnitines, nucleotides, and nucleosides. Optimized method was applied to NIST SRM 1950 human plasma, leading to the identification of more than 130 metabolites (in addition to lipids). The identification was based on retention times, mass shifts within a 3 mDa tolerance in full-scan mode, tandem mass spectrometry, and retention dependencies. At the same time, we cross-compared two different systems: bioinert and conventional, which included both bioinert and conventional columns. The results indicate that a fully bioinert system significantly improves peak shapes and resolution. In comparison to the effect of the column and the system, it is evident that the column has a more pronounced effect on enhancing the overall behavior of metabolites. However, even the bioinert system without bioinert column contributes also better results than the fully conventional system.

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2) Detailní LC-MS/MS analýza cukrů a jejich alditolů pro diagnostiku dědičných metabolických poruch

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Dědičné poruchy metabolismu (DMP) sacharidů se vyznačují abnormální hladinou cukrů a jejich alditolů v tělních tekutinách, které utváří strukturní isomery, tedy látky o stejné molekulové hmotnosti lišící se svou strukturou, které není snadné rozlišit. V rámci rutinního vyšetření vybraných metabolitů v moči se v Laboratoři dědičných metabolických poruch ve Fakultní nemocnici Olomouc provádí analýza založená na hydrofilní interakční chromatografii ve spojení s tandemovou hmotnostní spektrometrií, která slouží k detekci >60 metabolitů ze spektra purinů, pyrimidinů, N-acetylovaných aminokyselin, acylglycinů, cukrů, jejich alditolů a dalších diagnosticky významných markerů, avšak jednotlivé isomery cukrů a alditolů touto metodou nelze rozlišit. V případě potřeby jejich rozlišení se přistupuje ke druhostupňovým metodám. V klinické praxi se často jedná o koeluci hexitolů, tedy galaktitolu, mannitolu a sorbitolu. Galaktitol je klíčový marker galaktosémie, která bez správné léčby může přejít v život ohrožující stav, zatímco mannitol/sorbitol se v moči hromadí v důsledku jejich přítomnosti v řadě potravin, potravinových doplňků a léků. Rozlišení galaktitolu od mannitolu/sorbitolu je tedy naprosto stěžejní v rámci diagnostiky galaktosémie. V laboratoři k tomu využíváme GC-MS metodu, která je však pro urgentní případy příliš zdlouhavá vzhledem k časově náročné extrakci a derivatizaci vzorku, dlouhé analýze a vyhodnocení. S cílem urychlit a zjednodušit diagnostiku DMP sacharidů jsme vyvinuli LC-MS/MS metodu, která dokáže galaktitol od ostatních alditolů spolehlivě rozlišit pomocí jeho značeného standardu (galaktitol-13C6) na základě retenčního času. Nová LC-MS/MS metoda umožňuje simultánní analýzu 19 cukrů a 10 alditolů za 17 min. Jedná se tak o diagnostickou platformu pro více než 20 DMP sacharidů (poruchy pentosafosfátové dráhy, transportu glukosy, metabolismu galaktosy, fruktosy a glykogenu), která má potenciál v budoucnu nahradit GC-MS metodu.

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3) Hodnocení rizika kardiovaskulárních příhod pomocí skóre CERT

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Lipidy jsou obecně spojovány se vznikem a patologií aterosklerotických kardiovaskulárních onemocnění. Na základě doporučení Evropské kardiologické společnosti a Evropské společnosti pro aterosklerózu z roku 2019 se jako základní hodnocení těchto onemocnění doporučuje metrika SCORE (Systematic Coronary Risk Evaluation). SCORE vychází z kombinace faktorů, jako je věk, pohlaví, kouření, krevní tlak a celkový cholesterol. Velkou roli ovšem v těchto zánětlivých onemocněních hrají nejen cholesterol a triacylglyceroly, ale i ceramidy a fosfatidylcholiny, které bývají spojovány s infarkty myokardu, ischemickou chorobou srdeční, cévní mozkovou příhodou a obecně se zvýšenou mortalitou. Pro vytvoření jednoduchého skóre pro predikci těchto kardiovaskulárních příhod byly kombinovány naměřené koncentrace ceramidů a fosfatidylcholinů s pokročilými statistickými modely. Z těchto vytvořených skóre má velký potenciál především CERAM (Mayo Clinic, USA), CERT1 a CERT2 (Zora Biosciences, Finsko). CERAM a CERT1 zahrnují výpočet tohoto skóre pouze v rámci koncentrace ceramidů, kdežto CERT2 zohledňuje i koncentraci fosfatidylcholinů. V rámci této práce byla u kohorty pacientů (n=600) z 1. IK FNOL změřena koncentrace vybraných ceramidů a fosfatidylcholinů v krevním séru. Pro tento účel byla optimalizována LC-MS/MS metoda (1,2) pro analýzu ceramidů: Cer 18:1/16:0, Cer 18:1/18:0, Cer 18:1/24:0, Cer 18:1/24:1, a fosfatidylcholinů: PC 16:0/16:0, PC 14:0/22:6, PC 16:0/22:5. Analýza byla provedena pomocí kolony Acquity BEH C18 2.1 × 75 mm x 1.7 µm (Waters), HPLC přístroje Exion LC (SCIEX) a hmotnostního spektrometru QTRAP 6500+ (SCIEX). Mobilní fáze A obsahovala 10mM octan amonný + 0.1% kyselinu mravenčí a mobilní fáze B 10mM octan amonný v acetonitrilu:2-propanolu (4:3, v/v) + 0,1% kyselinu mravenčí. Vyhřívání kolony bylo nastaveno na 60 °C, průtok na 0.5 ml/min, nástřik vzorku 0.5 µl a doba analýzy 5 min. Koncentrace analytů byly vypočítány na základě jejich příslušných stabilních izotopově značených interních standardů. Následně bylo vypočítáno CERT skóre, jeho kvartily a dle těchto výsledků byly stanoveny rizikové skupiny. Tyto výsledky byly navíc podrobeny korelační analýze s rutinními biochemickými parametry. Výsledky ukazují, že tato rychlá kvantitativní analýza a následný výpočet CERT skóre mají velký potenciál pro predikci kardiovaskulárních příhod a jejich prevenci.

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4) Komplexní analýza nepolárních lipidů ve vzorcích plazmy a séra pomocí RP-UHPSFC/MS

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Nepolární lipidy tvoří skupinu důležitých biomolekul, které jsou klíčové pro výrobu a skladování energie ve všech buňkách. Do této skupiny lze zařadit acylglyceroly, steroly (ST) a sterol estery (SE). Každý z těchto lipidů také hraje významnou roli v buněčné homeostáze, nicméně význam jednotlivých lipidů v těchto procesech není dosud přesně znám. Tyto látky jsou sice hojně zastoupeny v lidské plazmě/séru, ale i přesto je jejich analýza komplikovaná, a to z mnoha důvodů, zejména pak kvůli velkému množství izomerů. Proto jsou zapotřebí vysokoúčinné separační metody, které umožní jednotlivé izomery rozlišit. Vzhledem ke komplexnosti biologických vzorků je analýza lipidů založená zejména na hmotnostní spektrometrii (MS) ve spojení s předřazenou separační technikou. Velmi výhodnou kombinací pro analýzu nepolárních lipidů je ultra-vysokoúčinná superkritická fluidní chromatografie v systému s obrácenými fázemi (RPUHPSFC), kterou lze snadno kombinovat s ionizací elektrosprejem. UHPSFC technika umožňuje dva separační přístupy v lipidomické analýze. Prvním přístupem je separace dle lipidových tříd (separace podobná HILIC módu), která primárně dělí lipidy na základě polární skupiny, což vede ke koeluci lipidů z jedné třídy do jednoho chromatografického píků. Tento přístup je výhodný zejména pro kvantitativní analýzu. Druhým přístupem je separace jednotlivých druhů lipidů (separace podobná chromatografii v systémech s obrácenými fázemi (RP) u kapalinové chromatografie), která separuje jednotlivé lipidy na základě délky mastného acylového řetězce a počtu dvojných vazeb, což umožňuje i separaci izomerů s odlišnými polohami dvojné vazby.

Hlavním cílem této práce bylo vyvinout a zoptimalizovat RP-UHPSFC/MS metodu pro analýzu nepolárních lipidů, jmenovitě mastných kyselin (FA), ST, SE a mono- (MG), di- (DG) a tri-acylglycerolů (TG) ve vzorcích plazmy a séra. Kromě separačních a ionizačních podmínek byly optimalizovány i extrakční protokoly, kde byla jako nejvhodnější zvolena selektivní extrakce nepolárních lipidů do hexanu v kombinaci s chemickou derivatizací pomocí benzoylchloridu, díky které se významně zvyšuje odezva nepolárních lipidů a to zejména FA, ST, MG a DG. Benzoylace navíc poskytuje stabilnější deriváty sterolů a umožňuje detekci FA v režimu záznamu pozitivních iontů, bez nutnosti přepínání polarity ve srovnání s analýzou nederivatizovaných forem. Při optimalizaci přípravy vzorku byl také vyřešen problém enzymatické degradace TG za vzniku DG nahrazením původní proteinové precipitace za Folchovu extrakci.

Zoptimalizovaná metoda byla použita pro analýzu nepolárních lipidů v lidské plazmě a séru, přičemž s využitím kombinace přesně určené hodnoty m/z, specifických fragmentů v MS/MS a retenčních závislostí byly identifikovány stovky nepolárních lipidů, včetně rozlišení polohy a pozice dvojných vazeb. Kromě zvýšení počtu identifikovaných lipidů metoda také poskytuje podrobnější informace o struktuře ve srovnání se současným stavem.

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5) Lipidom protizánětlivých a prozánětlivých makrofágů tukové tkáně

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S inzulinovou rezistencí, aterosklerózou a diabetem 2. typu je relativně nově spojován také mírný chronický zánět v tukové tkáni. V tukové tkáni jsou kromě adipocitů přítomny i buňky imunitního systému. Jednou z důležitých skupin těchto buněk jsou makrofágy. Makrofágy lze rozdělit na základě jejich znaků na prozánětlivé a protizánětlivé(1). Pro lepší pochopení probíhajících dějů je důležité zabývat se analýzou lipidomu těchto dvou zmíněných skupin buněk. V rámci projektu jsme optimalizovali LC-MS metodu pro lipidomickou analýzu makrofágů a zjišťovali jsme rozdíly v lipidomu protizánětlivých a prozánětlivých makrofágů tukové tkáně.

Tuková tkáň byla odebrána v průběhu transplantace z povrchu ledviny dárců. Makrofágy ze vzorku každého jedince byly izolovány a průtokovou cytometrií rozděleny na prozánětlivé a protizánětlivé. Hlavním problémem analýzy bylo liminované množství makrofágů, kreré lze z jednoho vzorku zístkat Lipidy byly extrahovány metodou MTBE adaptovanou na malé množství vzorku(2). Pro separaci byla použita kolona Acquity UPLC BEH C18 (1,7 µm; 2,1 mm) při teplotě 55°C. Binární gradient mobilní fáze byl míchán s těchto směsí: A) acetonitril/voda (60:40 (v/v)) a B) acetonitril/2-propanol (90:10 (v/v)) s přídavkem kyselina mravenčí (0,1 %) a mravenčanu amonného (10 mm)) do obou směsí.

Získané křivky retenčních závislostí pro jednotlivé třídy lipidů byly následně použity k potvrzení anotace lipidů ve vzorcích lipidomu makrofágů. Optimalizovanou LC-MS metodu jsem aplikovali na analýzu lipidomů makrofágů. Bylo zjištěno, že větší celkové množství lipidů obsahovaly prozánětlivé makrofágy. Z analýza hlavních komponent vyplynulo, že vzorky prozánětlivých makrofágů vykazují vyšší rozptyl v rámci skupiny. Vulkánový graf ukázal významný nárůst koncentrace některých konkrétních lipidů (např. fosfatidylcholinů, fosfatidylethanolaminů, sfingomyelinů) v případě prozánětlivých makrofágů. Tyto lipidy obsahovaly převážně nasycené a mononenasycené mastné kyseliny.

Práce byla podpořena projektem Národní institut pro výzkum metabolických a kardiovaskulárních onemocnění (Program EXCELES, ID LX22NPO5104) – Financováno Evropskou unií - Next Generation EU.

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6) Lipidomic test for early detection of pancreatic cancer – transfer to clinical practice

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Pancreatic cancer (PaC) has one of the worst prognoses among solid malignancies. The main reason is the late diagnosis due to the lack of symptoms at an early stage. Moreover, no screening program for PaC is available. A new method for the early diagnosis of PaC was developed at the University of Pardubice [1]. The diagnostic test is based on Folch extraction of lipids from plasma to organic solvent, accurate and high-throughput quantification of lipid species from 7 lipid classes using UHPSFC/MS in a total run time of 8 min [2]. The lipidomic profiles of cancer patients and healthy controls are measured together with samples of unknown classification and quality control samples. Then, multivariate data analysis (MDA) is used to create orthogonal partial least squares (OPLS) models for differentiating patients and controls, which can be applied to samples with unknown classification. The whole process is patent protected. The spin-off company Lipidica is the user of the patent and works on its transfer to clinical practice as the LDPC test (Lipidomic Diagnostics of Pancreatic Cancer). The methodology (analysis, data processing and multivariate statistical analysis) was transferred successfully from the University of Pardubice to Lipidica. Furthermore, the precision of the test based on biological material was investigated for more than 200 cancer patients and 240 healthy control samples of plasma and serum. The next step is clinical performance study that focuses on high-risk groups of people for the outbreak of PaC (i.e. the lifetime risk is higher than 5%). The result of LDPC test will be correlated with current diagnostic approaches (endoscopic ultrasound and magnetic resonance imaging). After successful clinical validation of LDPC test, the method could be implemented in clinical practice and later in the national screening program for PaC.

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7) Lipidomická analýza pacientů se střádavými lysozomálními poruchami (LSD)

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Poruchy metabolismu sfingolipidů, řadící se do skupiny lysozomálních střádavých onemocnění (LSD), vznikají v důsledku genetické mutace, která vede k deficitu enzymů potřebných pro lysozomální degradaci sfingolipidových substrátů. Tato studie je zaměřená na vybraná LSD (Niemann-Pickova choroba typu C (NPC), Niemann-Pickova choroba typu A a B (NPA/B), Fabryho choroba (MF), Gaucherova choroba (MG), mukolipidóza (ML) a deficit lysozomální kyselé lipázy (LAL)) s cílem získat lipidové profily pacientů trpících vybranými LSD a odhalit změny v profilech pacientů v porovnání s kontrolní skupinou.

V rámci studie byla provedena cílená lipidomická analýza séra pacientů s NPC (n = 1), NPA/B (n = 2), MF (n = 4), MG (n = 8), ml (n = 1) a LAL (n = 4) a kontrol (n = 38). Pomocí vysokoúčinné kapalinové chromatografie (Exion LC, Sciex; kolona BEH C8 1,7 um, Waters) ve spojení s tandemovou hmotnostní spektrometrií (Qtrap 6500+, Sciex) bylo v krevním séru semikvantifikováno téměř 700 lipidů patřících do 19 lipidových tříd a podtříd a dále známých biomarkerů těchto onemocnění, jako jsou lysoGb3, lysoHexCer, lysoSM, lysoSM-509 pro onemocnění MF, MG, NPA/B a LAL.Multivariantní statistická analýza odhalila výrazné změny v lipidomu pacientů a kontrol.l. Kromě známých biomarkerů pro vybraná LSD byly u pacientů trpících LAL odhaleny systematické změny na úrovni ceramidů, hexosylceramidů, fosfatidylinositolů a mastných kyselin ve srovnání s kontrolní skupinou. U pacientů s MF došlo k systematickým změnám fosfatidylcholinových plasmalogenů a fosfatidylcholinů ve srovnání s kontrolami.

V této studii byla demonstrována využitelnost cílené lipidomické metody pro diagnostiku a studium pacientů s LSD. Hromadění lipidů v krviv důsledku těchto onemocnění koresponduje s provedeným experimentem, který poukazuje na zapojení vybraných podskupin sfingolipidů a glycerofosfolipidů do patobiochemie LSD.

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8) Mass spectrometry Core laboratory in BIOCEV offers metabolomic, proteomic and lipidomic services for public

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In our facility we offer services in metabolomics, lipidomics and proteomics. We can tailor our services to meet any specific requirements, including method development sample preparation, final chemical analyses and basic statistical evaluation. The interconnection among the Omics provides comprehensive information crucial for understanding of biological systems. In our laboratory we offer the advantage to analyze metabolites, lipids and proteins in the same sample(1). To accomplish this task our laboratory is equipped with hi-end instrumentation and software.

Metabolomics: In order to achieve better metabolite coverage in targeted or untargeted setup we can combine using liquid chromatograph connected with tribrid mass spectrometer (Fusion or Ascend, Thermo) and gas chromatograph with mass detector (GCxGC/MS, Pegasus4D). Furthermore, we offer specific services including dynamic headspace sampling for volatiles(2), or isotope tracing analysis both for volatiles and non-volatiles.

Lipidomics: We are focused on targeted analyses using shotgun approach of mainly phospholipids such as phosphatidyl cholines, ethanolamines, serines, inositols, glycerol and phosphatidic acid including their lyso-forms. We can also detect diacyl and triacyl-glycerols(3).

Proteomics: We provide a bottom-up proteomic analyses in combination with nano-flow liquid chromatography. We are able to perform proteomic analysis in wide variety of biological matrices. Our routine services include analysis of cell lysates, immunoprecipitations, phosphoproteome profiling or thermal proteome profiling.

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9) Metabolic pathways of acylcarnitine synthesis

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Acylcarnitines are important markers in metabolic studies of many diseases, including metabolic, cardiovascular, and neurological disorders. We reviewed analytical methods for analyzing acylcarnitines with respect to the available molecular structural information, the technical limitations of legacy methods, and the potential of new mass spectrometry-based techniques to provide new information on metabolite structure. We summarized the nomenclature of acylcarnitines based on historical common names and common abbreviations, and we propose the use of systematic abbreviations derived from the shorthand notation for lipid structures. The transition to systematic nomenclature will facilitate acylcarnitine annotation, reporting, and standardization in metabolomics. We have reviewed the metabolic origins of acylcarnitines important for the biological interpretation of human metabolomic profiles. We identified neglected isomers of acylcarnitines and summarized the metabolic pathways involved in the synthesis and degradation of acylcarnitines, including branched-chain lipids and amino acids. We reviewed the primary literature, mapped the metabolic transformations of acyl-CoAs to acylcarnitines, and created a freely available WikiPathway WP5423 to help researchers navigate the acylcarnitine field. The WikiPathway was curated, metabolites and metabolic reactions were annotated, and references were included. We also provide a table for conversion between common names and abbreviations and systematic abbreviations linked to the LIPID MAPS or Human Metabolome Database.

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10) Metabolomic changes across various matrices in mice in response to chow and high-fat diet

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The rapid expansion of metabolomics has generated significant interest in understanding the role of small molecule metabolites in various biological processes. Liquid chromatography-mass spectrometry (LC-MS) has emerged as the most widely utilized chromatography-MS tool for analyzing both polar and nonpolar metabolites. However, a single extraction method or instrumental platform cannot capture the true breadth and scope of polar metabolites (metabolome) and complex lipids (lipidome). Thus, the task is to achieve high metabolite coverage using as few platforms as possible while maintaining the requisite precision and accuracy. We have developed and validated an LC-MS workflow to extract complex lipids and polar metabolites from mouse plasma, liver, and feces. This "all-in-one" extraction method employs a mixture of methanol, methyl tert-butyl ether, and water to isolate sub-groups of compounds. Analysis of complex lipids is performed using reversed-phase LC (RPLC) in both positive and negative electrospray ionization (ESI) modes, while polar metabolites are separated using hydrophilic interaction chromatography (HILIC) in ESI(+) and RPLC in ESI(–). We employ simultaneous MS1 and MS/MS spectra acquisition in data-dependent mode for each platform to enhance data acquisition. We applied this workflow to samples from mice subjected to systemic energy balance (chow diet) and chronic nutrient stress (high-fat diet). Our method enabled the annotation of over 400 simple and complex lipids and more than 100 polar metabolites based on a matrix-type approach.

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11) Permeability Assessment of Antiepileptic Drugs Using in vitro BBB Model

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This study estimates the permeability of selected antiepileptic drugs (AEDs) through our developed in vitro blood-brain barrier (BBB) model. The BBB model we used consisted of a differentiated monolayer of three cell types: immortalized human brain microvascular endothelial cells, primary human brain vascular pericytes, and immortalized human astrocytes, cultured on transwell carriers. Firstly, transepithelial electrical resistance (TEER) was measured to verify the barrier integrity. Then, the AEDs were introduced into the apical medium, and the cells were incubated for 4h. The compounds were collected from both the apical and basolateral compartments, and quantitatively analyzed. The quantitative analysis of drug permeability was performed using HPLC-MS with a single quadrupole detector. In the methodological approach, we utilized a gradient elution program on a C18 column with a mobile phase A composed of water, methanol, formic acid (950:50:1), and acetonitrile (mobile phase B). Although the developed HPLC MS analysis seems to be appropriate, our results indicate that some of the measured compounds accumulated at the boundary, or did not penetrate properly the BBB model at all. Thus, further evaluation of the in vitro BBB model is necessary.

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12) Stratification of patients with and without significant carotid plaque by LC-HRMS/MS plasma lipidomics

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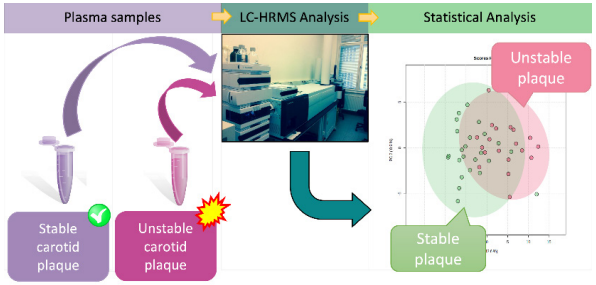

Atherosclerosis is a major cause of ischemic stroke, and early detection of advanced atherosclerosis in the carotid artery is important for reducing morbidity and mortality. What is even more important is not only detection of atherosclerosis but early determination whether the patients are at high risk of an event with adverse effects as the size of the plaque does not necessarily reflect its potential to trigger such events.We studied whether plasma lipidomics profile can be used as a diagnostic tool for stratification of stable or unstable plaques without the need of removing the carotid plaque. This study used liquid chromatography high-resolution tandem mass spectrometry lipidomics to characterize lipid profiles in patients' plasma and found that patients with significant and complicated (i. e. vulnerable) atherosclerotic plaque had distinct lipid profiles compared to those with insignificant plaques.

The lipid classes that were most predictive of vulnerable plaque were lysophosphoethanolamines, fatty acyl esters of hydroxy fatty acids, free fatty acids, plasmalogens, and triacylglycerols. Most of these compounds were found decreased in plasma of patients with unstable plaques which enabled sufficient performance of a statistical model used for patient stratification. Plasma lipidomes measured by liquid chromatography-mass spectrometry show differences in patients with stable and unstable carotid plaques, therefore these compounds could potentially be used as biomarkers for unstable plaque in future clinical diagnosis.

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13) Study on lipid changes in animal model for Type 2 diabetes

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Type 2 diabetes (T2D) is a complex metabolic disorder characterized by hyperglycemia and associated with retinopathy, nephropathy, neuropathy, and cardiovascular diseases. The impact of diabetes on the central nervous system (CNS), particularly concerning cognitive dysfunction, remains poorly understood. CNS complications may include stroke, alterations in the blood-brain barrier (BBB), or disruptions in the transport functions of the cerebral microvasculature. Substantial epidemiological evidence suggests a strong association between T2D and neuroinflammation and cognitive impairment, attributed to the failure of glucose absorption in neurons for energy production. Diabetes is also linked to lipid alterations. In obese individuals, the excess triglycerides and free fatty acids released into insulin-sensitive organs result in the release of other lipids, such as sphingolipids, ultimately leading to inflammation and insulin resistance. Several studies have demonstrated a direct connection between insulin receptor signaling and ganglioside GM3.

In this study, we utilized ob/ob mice, an animal model for T2D, that exhibit obesity, hyperinsulinemia, and hyperglycemia. We used this animal model to characterize changes in lipid production in the CNS. The semi-targeted screening revealed significant changes in the levels of GD1a d38:1 and GD1a d40:1 in the brain tissue of ob/ob mice. We found elevated levels of GD1 gangliosides, which have anti-inflammatory properties. This indicate that GD1 can modulate inflammatory processes in ob/ob mice.

The work was supported by APVV-22-0313, VEGA 2/0078/22, ICGEB CRP/SVK23-01

14) Untargeted lipidomics distinguish Streptococcus zooepidemicus mucoid and non-mucoid phenotype

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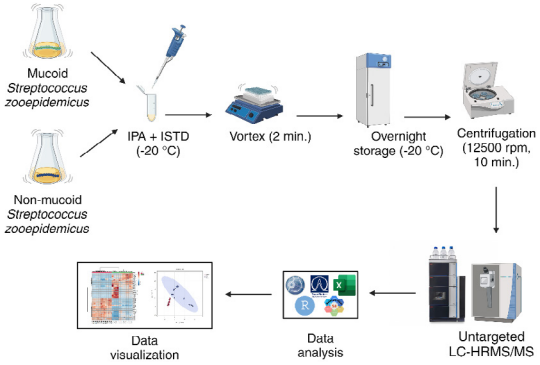

Hyaluronan is produced via enzymes HasA and HasB in S. zooepidemicus. HasA is anchored in the plasma membrane. The plasma membrane lipid composition is crucial for proper HasA function. However, comprehensive lipid composition of S. zooepidemicus plasma membrane is not available. Hence, we applied untargeted LC-HRMS/MS DDA lipidomics to unravel the lipids in plasma membrane and compare mucoid and non-mucoid phenotype. 120 unique lipid species from 13 lipid classes were identified. Digalactosyldiacylglycerols (DGDG), fatty acids (FA), monogalactosyldiacylglycerols (MGDG) and cardiolipins (CL) were the most abundant lipid classes. CL, DGDG, LNAPE, PI and SM were significantly up-regulated in non-mucoid phenotype, while FA and MGDG were down-regulated. In general, statistical analysis revealed non-mucoid phenotype tends to form shorter and less saturated lipid species than mucoid phenotype. Differences in lipidome could be linked with structural differences of plasma membrane where length and saturation of lipid species is crucial for fluidity, permeability, and proper membrane protein function maintenance. The difference could be an underlying factor for HasA dysfunction in non-mucoid phenotype and lays the ground for further research.

15) Utilization of Comprehensive Online RP-UHPLC×UHPSFC/MS/MS configuration to Lipidomic Analysis of Human Plasma

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In contemporary research, increased attention is paid to the distinguishing of individual lipid species and isomers. Detailed characterization of the human lipidome offers valuable information into the pathophysiology of various diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer. Chromatographic separation coupled by mass spectrometry detection represents the most widely used approach for lipidomic analysis. The conventional one-dimensional chromatographic techniques may have limited ability to separate and identify all these diverse components. However, multidimensional techniques bring the potential to combine various separation mechanisms enhancing the resolution and separation of lipids.

The main goal of this study was the development of a new comprehensive online RP-UHPLC×UHPSFC/MS/MS method applicable for the characterization of wide range of lipids in human plasma samples. Reversed-phase UHPLC with C18 column (150 x 0.5 mm, 1.9 µm) applied in first dimension (1D) allows the separation of lipids with low flow rate of mobile phase (8 µL/min). UHPSFC in second dimension (2D) is used due to the high speed of the analysis (35 s, 10 x 2.1 mm, 1.7 µm column). The development includes detailed optimization such as flow rate from 1D, matching the loop sizes, and a rather fast separation in 2D. The individual lipid species were identified according to the retention behaviors in both dimensions, mass accuracy of molecular adducts, and characteristic fragment ions measured by MSE and fast DDA mass spectrometry acquisition modes.

Continuous 4D lipidomic analysis represents a novel strategy for the comprehensive analysis of lipids in biological samples. Due to the orthogonality of both systems and their connection to MS, we were able to identify more than 300 lipid species from 16 lipid subclasses. So far, this is probably the first connection of UHPLC and UHPSFC in this configuration for lipidomic analysis, using very short columns in the 2D (10 mm), short sampling time (0.55 min), and using gradient elution in both dimensions.

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16) Androgen levels determination in ADTRP male and female knock-out mice

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Androgens are the collective term for steroid hormones, which stimulates or controls the development of masculine characteristics by binding to androgen receptors. The major androgens in the body are testosterone (17β-hydroxyandrost-4-en-3-one), androstenedione, and dihydrotestosterone secreted by cells of the testes, ovaries, and adrenal glands. Androgen dependent tissue factor pathway inhibitor regulating protein (ADTRP) was initially identified as a regulator of tissue factor pathway inhibitor (TFPI) which is an inhibitor of blood coagulation connected with bleeding and clotting disorders. Later studies suggest that androgen regulation of ADTRP expression is on the transcriptional level through direct binding of the androgen receptor to the androgen response element region at the regulatory region of the gene. The measurement of androgen levels in biological samples of plasma and tissues has proven to be a great challenge due to lack of precision and sensitivity of various commercially available testosterone assays, resulting in limited utility. Various analytical methods based on radioimmunoassay, enzyme immunoassay, high-performance liquid chromatography (HPLC) as well as gas chromatography, have been used in the quantification of testosterone. The current project was aimed at the development of a fast and simple HPLC–MS/MS method suitable for measurement of testosterone and other androgens in a limited amount of plasma and adipose tissue samples from ADTRP KO and WT male and female mice.

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PREZENTACE FIREM

Targeted lipidomics and metabolomics using LC/MS triple quadrupole Agilent 6495D

Jitka Zrostlíková

Altium International

Besides high resolution and accurate mass (HRAM) mass spectrometers used in the discovery phase of experiments, the “omics” analysis often utilize targeted analytical strategies. These approaches facilitate the analysis of large sets of samples with high throughput, easy data processing, better limits of quantification and data reproducibility. The overall data quality and the lower frequency of false positive and false negative results lead to more reliable statistical analysis and data interpretation. Considering the physico-chemical properties of biologically active compounds, LC/MS triple quadrupole is usually the technique of choice. The instrumentation of LC/MS triple quadrupoles has undergone a great progress in recent years, especially in terms of detectability and coverage of very wide sets of analytes per method. In this presentation the new Agilent LC/MS triple quadrupole 6495D will be presented as well as ready workflows for targeted metabolomics and lipidomics. The lipidomics method comprises 763 human lipids from 44 classes. The metabolomic method enables profiling of 500 metabolites including the problematic polar analytes, such as aminoacids, polycarboxylic acids and phosphorylated metabolites. Sample preparation is automated using Agilent Bravo robot.

Radikalizace hmotnostního spektrometru – OAD-TOF

David Maxa

Shimadzu, ČR

Systém OAD-TOF je Q-TOF LCMS s inovativní fragmentační technologií společnosti Shimadzu, využívající neutrální radikály pro štěpení dvojných vazeb. Díky této technologii je možné získat diagnostické fragmenty, které nelze analyzovat běžnou fragmentační technikou (CID), kdy se ionty fragmentují srážkou s inertním plynem, jako je argon nebo dusík. Neutrální radikály O/OH/H, generované pomocí mikrovlnného výboje, specificky oxidují/disociují dvojné vazby mezi uhlíky, což je užitečné pro identifikaci struktury organických sloučenin, jako jsou např. lipidy. Tuto techniku lze použít pro monovalentní ionty a záporné ionty, které je obtížné fragmentovat pomocí radikálových reakcí s elektrony a anionty, a poskytuje zcela nové strukturní informace. V kombinaci s LCMS-9050, který se může pochlubit prvotřídní přesností hmoty, vysokou skenovací rychlostí a rychlým přepínáním polarity, umožňuje OAD-TOF vytvářet inovativní aplikace.

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

Mariateresa Maldini

SCIEX, Italy

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 with 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 that of high-end triple quadrupole instruments. In addition to traditional fragmentation by collision-induced dissociation (CID), 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. They 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. The ZenoTOF 7600 instrument, however, has a tunable electron beam to produce EAD-based fragments that can distinguish lipid mediator isomers.

Herein, the use of the ZenoTOF 7600 system to quantitate and fully characterize lipid mediators and other small-molecule metabolites using both CID- and EAD-based fragmentation will be demonstrated. 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.

Unveiling the Lipidome with Unprecedented Speed and Depth: Bruker 4D-Lipidomics™ Empowered by PASEF® Technology

Daniel Vlačil

Bruker, ČR

Lipidomics, the comprehensive analysis of a cell’s lipid profile, is pivotal for understanding biological functions and disease mechanisms. Traditional lipidomic analysis methods face challenges such as limited throughput, sensitivity, and dynamic range. Bruker’s 4D-Lipidomics™, utilizing the revolutionary PASEF® (Parallel Accumulation Serial Fragmentation) technology, addresses these challenges, offering an enhanced solution for lipidomic research.

Bruker’s 4D-Lipidomics™ system integrates high-resolution mass spectrometry with the PASEF® technique to accelerate ion accumulation and fragmentation processes. This integration allows for an exponential increase in MS/MS data acquisition without compromising sensitivity or resolution. The technology allows for the detection of thousands of lipid species across a wide range of concentrations and molecular classes within a single experiment and brings a breakthrough not only for routine high-throughput screening and quantitation with timsTOF HT but also for single-cell research with timsTOF Ultra.

Bruker’s 4D-Lipidomics™, powered by PASEF®, represents a transformative advancement in lipidomics, enabling researchers to explore the lipidome with unparalleled speed, depth, and accuracy. This technology holds the potential to revolutionize our understanding of lipid metabolism and its role in health and disease, paving the way for new diagnostic and therapeutic approaches.

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

J. Ngere

Thermo Scientific

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 MS2

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 MS2 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 MS2 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 MS2 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.

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