PROGRAM

3rd Nordic Metabolomics Conference 2023

October 18-20, 2023
Trondheim, Norway

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Program

Wednesday 18th October

17:00	**Registration opens**
18:00-20:00	**Early-career researcher event** Chairs: Maria Karoline Andersen and Matteo Sangermani, Norwegian University of Science and Technology
18:00	Workshop: "Pressure creates diamonds: How to make stress your friend in academia". Speaker: **Henrik Herrebrøden, Kristiania University College.**
20:00	Walking towards pub through Bakklandet
20:30	Social pub event for early-career researchers at Kieglekroa, Trondheims oldest pub

Thursday 19th October

08:00	**Registration opens**
08:45-10:25	**Welcome and Session 1: Spatial metabolomics** Chair: Tone Frost Bathen, Norwegian University of Science and Technology
08:45 - 09:00	Welcome from local committee and Nordic Metabolomics Society
09:00 - 09:40	**Ron Heeren, Maastricht University** *Spatial Metabolomics: from single cells to translational diagnostics*
09:40 - 09:55	**Maria Karoline Andersen, Norwegian University of Science and Technology** *Optimal storage condition and time of fresh frozen tissue sections prior to spatial metabolite detection with MALDI MSI*
09:55 - 10:10	**Ingela Lanekoff, Uppsala University** *Spatial metabolomics - revealing molecular distributions correlating with disease*
10:10 - 10:25	**Ellen Marie Botne Quinsgaard, Norwegian University of Science and Technology** *Studying metabolic changes during EMT using MALDI MSI*
10:25 - 11:00	**Break with refreshments**
11:00 - 13:00	**Session 2: Metabolomics and lifestyle** Chair: Otto Savolainen, Chalmers University of Technology
11:00 - 11:40	**Kati Hanhineva, University of Turku** *Metabolite profiling in food and nutrition research*
11:40 - 11:55	**David Chamoso-Sanchez, Universidad San Pablo-CEU** *Metabotyping the obesity: new factor analysis-based strategies for classifying from multiplatform metabolomics data children with obesity*
11:55 - 12:10	**Samira Prado, Örebro University** *Mapping the effects of plant-based proteins on human metabolic profiling*

12:10 - 12:25	**Sergio Polakof, University of Clermont Auvergne** *Exploring the impact of plant protein vs. animal protein-rich diets in men at cardiometabolic risk: insights from plasma metabolome signatures*
12:25 - 12:40	**Julia Debik, Norwegian University of Science and Technology** *Exploring sources of variation in the female serum metabolome in light of breast cancer risk factors, in healthy participants of the HUNT2 study*
12:40 - 12:55	**Hany Ahmed, University of Turku** *Plasma metabolic profiling shows reversible changes in metabolites linked to psychological traits: A metabolomics study of the effects of alcohol withdrawal in patients with alcohol use disorder*
13:00 - 14:00	**Lunch**
14:00 - 15:00	**Session 3 Part I: Microbiome and host metabolism** Chair: Margrét Þorsteinsdóttir, University of Iceland
14:00 - 14:40	**Coral Barbas, Universidad CEU San Pablo** *Analytical Challenges in the Analysis of Microbiota related Metabolites*
14:40 - 15:00	**Santosh Lamichhane, University of Turku** *Gut Microbiome and Novel Bile Acids: New Insights into the Progression to Islet Autoimmunity*
15:00 - 16:00	**Poster session with refreshments**
16:00 - 16:45	**Session 3 Part II: Microbiome and host metabolism** Chair: Margrét Þorsteinsdóttir, University of Iceland
16:00 - 16:15	**Daniel Globisch, Uppsala University** *Chemoselective Metabolomics – New Chemical Biology Tools to Explore Microbiome and Diet Metabolism*
16:15 - 16:30	**Stefania Noerman, Chalmers University of Technology** *Oral microbiome associates with salivary metabolome and sugars profile*
16:30 - 16:45	**Youngsun Lee, University of Helsinki** *Effect of Fermentation on Sorghum Phenolic Compounds*
17:30	**Departure from hotel to Nidarosdomen**
18:00 - 19:00	**Consert in Nidarosdomen**
19:30	**Conference dinner**

Friday 20th October

07:30	**Running group/morning walk**
08:45 - 10:25	**Session 4: Computational metabolomics** Chair: Julia Debik, Norwegian University of Science and Technology
08:45 - 09:25	**Johan Westerhuis, University of Amsterdam** *Analysis of longitudinal intervention studies with multivariate metabolomics data*

09:25 - 09:40	**Yingxiao Yan, Chalmers University of Technology** *Adjusting for covariates and assessing modeling fitness in machine learning using MUVR 2.0.*
09:40 - 09:55	**Lu Li, Simula Metropolitan Center for Digital Engineering** *From static to dynamic, how to analyze postprandial metabolomics data?*
09:55 - 10:10	**Maximilian Wess, Norwegian University of Science and Technology** *Registration-based Integration of Spatial Multi-Omics Data for Prostate Cancer Classification*
10:10 - 10:25	**Yannek Nowatzky, Bundesanstalt für Materialforschung und -prüfung (BAM):** *Fragmentation site prediction for non-targeted metabolomics using graph neural networks*
10:25 - 11:00	**Break with refreshments**
11:00 - 11:45	**Gold sponsor session** Chair: Daniel Globisch, Uppsala University
11:00 - 11:20	**Bruker Nordic, Cristian De Gobba, Application Specialist** *Bruker 4D-Lipidomics: Exploring the lipidome at the speed of PASEF*
11:20 - 11:40	**Metabolon**
11:45 - 12:40	**Speed-presentations** Chair: Daniel Globisch, Uppsala University
	Alya Ghina Ahram, Norwegian University of Science and Technology *Plasma NMR metabolites of psoriasis and common immune-mediated inflammatory diseases in HUNT and UK Biobank*
	Sisi Deng, University Hospital Tübingen *Quantitative NMR serum spectroscopy deciphers metabolomic and lipidomic heterogeneity in endometriosis and pelvic inflammatory disease*
	Gaute H. Bø, UiT The Arctic University of Norway *Absolute quantification of short-chain fatty acids, organic acids and amino acids in feces using liquid chromatography-mass spectrometry*
	Paula Cuevas-Delgado, Universidad San Pablo-CEU *Untargeted metabolomics sample treatment strategies for renal tissue: a comparative study of solid phase microextraction (SPME) and homogenization-solid liquid extraction (Homo-SLE)*
	Sydney Mwasambu, Uppsala University *Metabolomics Investigation of Colonic Intraluminal Environment*
	Ida Marie Marquart Løber, Aarhus University *Metabolomics-based drug screening – a pilot study*
	Abhibhav Sharma, Norwegian University of Science and Technology *Comprehensive multi-omics analysis of breast cancer reveals distinct prognostic subtypes.*
	Viktor Skantze, Fraunhofer-Chalmers Research Centre for Industrial Mathematics *Analysis and prediction of postprandial metabolic response to multiple dietary challenges using dynamic mode decomposition*

	Sander J.T. Guttorm, University of Oslo *Global LC-MS multi-omics for investigating the effects of High Intensity Training (HIT)*
	Sara Rocha, University of Turku *Metabolic impact of whole grain diets on brain regions in a pig feeding trial*
12:40 - 13:15	**Poster session**
13:15 - 14:00	**Lunch**
14:15 - 15:40	**Session 5: Clinical metabolomics** Chair: Santosh Lamichane, University of Turku
14:15 - 14:55	**Guro F. Giskeødegård, Norwegian University of Science and Technology** *The metabolic lifespan of breast cancer*
14:55 - 15:10	**Gyuntae Bae, University Hospital Tübingen** *Stratification of ovarian cancer borderline from high-grade serous carcinoma patients by quantitative serum NMR spectroscopy of metabolites, lipoproteins, and inflammatory markers*
15:10 - 15:25	**Zoe S. Grenville, University of Oxford** *Perturbations in the blood metabolome up to a decade before prostate cancer diagnosis in 4,387 matched case-control sets from the European Prospective Investigation into Cancer and Nutrition*
15:25 - 15:40	**Aidan McGlinchey, Örebro University** *In-utero exposures to per- and polyfluoroalkyl substances and the human fetal liver metabolome: a cross-sectional study*
15:40 - 16:00	**Awards and goodbye**
16:00 - 16:30	**Light departure snack available**

Poster overview

P01	Annotating Unknown PFAS Compounds in Biological Matrices Using Real-Time Library Search and MSn	Brandon Bills, Sunandini Yedla, Ed George, Juan Sanchez, Tim Stratton, Ralf Tautenhahn, Vlad Zabrouskov
P02	*Spatial Characterization of Steroid Hormones in Breast Cancer Tissue by MALDI Mass Spectrometry Imaging*	Feng Wang, Sebastian Krossa, Marco Giampà, Siver Andreas Moestue, Guro F. Giskeødegård, Tone F. Bathen
P03	*Absolute quantification of short-chain fatty acids, organic acids and amino acids in feces using liquid chromatography-mass spectrometry*	Gaute H. Bø, Sietske S. Grijseels, Marie Mardal, Terje Vasskog, Veronika K. Pettersen
P04	*Multi-metabolic signature of controlled modification of dietary carbohydrate quality*	Cecilia Martinez Escobedo, Rikard Landberg, Clemens Wittenbecher
P05	*Urinary phenotyping of acute SARS-CoV-2 infection connects clinical diagnostics with metabolomics and links immune activation to antiviral nucleosides and SIRT1*	Caterina Lonati, Georgy Berezhnoy, Nathan Lawler, Reika Masuda, Aditi Kulkarni, Samuele Sala, Philipp Nitschke, Laimdota Zizmare, Daniele Bucci, Claire Cannet, Hartmut Schäfer, Yogesh Singh, Nicola Gray, Samantha Lodge, Jeremy Nicholson, Uta Merle, Julien Wist, Christoph Trautwein
P06	*The Implications of Mitochondrial DNA in Prostate Cancer Development*	Elen Telumyan, Elise Midtbust, Wei Wang Sebastian Krossa, Maria K. Andersen, Morten Rye Beck, Magnar Bjørås, May-Britt Tessem
P07	*Targeted metabolomic assay for therapeutic drug monitoring in patients with adenine phosphoribosyltransferase deficiency*	Margret Thorsteinsdottir, Unnur A. Thorsteinsdottir, Hrafnhildur L. Runolfsdottir, Finnur F. Eiriksson, Vidar O, Edvardsson, Runolfur Palsson
P08	*Correlating human gut microbiota metabolites and composition in a longitudinal study*	Matteo Sangermani, Solveig M. Jorgensen, Indri Desiati, Tone F. Bathen, Guro F. Giskeødegård
P09	*Untargeted metabolomics sample treatment strategies for renal tissue: a comparative study of solid phase microextraction (SPME) and homogenization-solid liquid extraction (Homo-SLE)*	Paula Cuevas-Delgado, Natalia Warmuzińska, Kamil Łuczykowski, Barbara Bojko, Coral Barbas
P10	*QComics: Recommendations and Guidelines for Robust, Easily Implementable and Reportable Quality Control of Metabolomics Data*	Núria Estanyol-Torres, Álvaro González-Domínguez, Carl Brunius, Rikard Landberg, Raúl González-Domínguez
P11	*Automated sample preparation and analysis of steroid hormones, bile acids, perfluoroalkyls, oxylipins and non-steroidal anti-inflammatory drugs in human plasma using UHPLC-MS/MS*	Samira Salihovic, Tove Slettvoll, Therese Koivula, Alicia Edin, Johan Normark, Matej Orešič, Sara Cajander, Tuulia Hyötyläinen

Poster overview

P12	*Unraveling Lipidomics Complexity: Overcoming False-Positives after Software Assisted Annotation for Building a comprehensive in-house human plasma MS library for Accurate Lipid Annotation*	Sara Martínez, Ana Gradillas, Coral Barbas
P13	*Metabolomics Investigation of Colonic Intraluminal Environment*	Sydney Mwasambu, Weifeng Lin, Daniel Globisch
P14	*CE-MS-based strategy to assess the metabolic signature of testicular cancer in human seminal plasma*	Maricruz Mamami-Huanca, Constanza Fernández-Hernández, Ángeles López-Gonzálvez, Nina Mørup, Francisco J. Rupérez, Antonia García, Serge Rudaz, Serge Nef, Kristian Alsmtrup, Coral Barbas, Víctor González-Ruiz
P15	*Global metabolomics reveals severe 3-nitropropionic acid intoxication in a Norwegian patient*	Hanne Bendiksen Skogvold, Mazyar Yazdani, Elise Mørk Sandås, Anja Østeby Vassli, Erle Kristensen, Dagfinn Haarr, Helge Rootwelt, Katja Benedikte Prestø Elgstøen
P16	*Decontamination of Aflatoxin B1 by Lactic Acid Bacteria*	Jenna Lemmetty, Youngsun Lee, Swantje Bredehorst, Tiina Laitila, Henry N. Maina
P17	*Age-dependent differences in serum metabolites linked to breast cancer risk: A high-resolution mass spectrometry study of pre-diagnostic serum samples from the Norwegian Trøndelag Health Study (HUNT2 study)*	Katarzyna Mrowiec, Agata Kurczyk, Karol Jelonek, Lucyna Ponge, Julia Debik, Guro F. Giskeødegård, Tone F. Bathen, Piotr Widłak
P18	*Spatial multi-omics to uncover prostate cancer heterogeneity*	Maria K. Andersen, Elise Midtbust, Sebastian Krossa, Maximillian Wess, Therese S. Høiem, Christine Aaserød Pedersen, Elen Telumyan, Guro F. Giskeødegård, Morten B. Rye, May-Britt Tessem
P19	*Metabolomic study of metabolites in winter damaged soils that can impact plant growth*	Marit Almvik, Monica Fongen, Pia Heltoft Thomsen, Karin Juul Hesselsøe, Trygve Aamlid
P20	*In silico expansion of the phospholipidome compositional profile and polar metabolome characterization in Haemophilus influenzae Rd KW20 using multiplatform metabolomics and probabilistic modelling*	Miguel Fernández-García, Manuel Ares-Arroyo, Emilia Wedel, Natalia Montero, Coral Barbas, Mª Fernanda Rey-Stolle, Bruno González-Zorn, Antonia Garcí
P21	*Metabolomics-based drug screening – a pilot study*	Ida Marie Marquart Løber, Jørgen Bo Hasselstrøm, Kirstine Lykke Nielsen
P22	*Comprehensive multi-omics analysis of breast cancer reveals distinct prognostic subtypes*	Abhibhav Sharma, Julia Debik, Bjørn Naume, Hege Oma Ohnstad, Oslo Breast Cancer Consortium (OSBREAC), Tone F. Bathen, Guro F. Giskeødegård

Poster overview

P23	*Lipid removal during sample pretreatment - effective ways to reduce matrix effects during HILIC LC-MS analysis of nucleotides and their derivatives*	Adela Pravdova, Ondrej Hodek, Thomas Moritz
P24	*Mass spectral fingerprinting metabolomics – flexible, high throughput metabolomics for sample screening*	Alastair Ross
P25	*Metabolic profiles reflect weight loss maintenance and the composition of diet after very-low-energy diet*	Mari Näätänen, Anna Kårlund, Santtu Mikkonen, Anton Klåvus, Otto Savolainen, Marko Lehtonen, Leila Karhunen, Kati Hanhineva, Marjukka Kolehmainen
P26	*Rapid and efficient LC-MS/MS diagnosis of inherited metabolic disorders: a semi-automated workflow for analysis of organic acids, acylglycines, and acylcarnitines in urine*	Barbora Piskláková, Jaroslava Friedecká, Eliška Ivanovová, Eva Hlídková, Vojtěch Bekárek, Matúš Prídavok, Aleš Kvasnička, Tomáš Adam, David Friedecký
P27	*Quantification of steroids in stool samples using LC-MS*	Ilia Evstafev, Matilda Kråkström, Matej Orešič, Alex M. Dickens
P28	*Metabolomics assessment of colistin induced toxicity*	Ioanna Barla, Ioanna Dagla, Aikaterini Daskalopoulou, Maria Panagiotopoulou, Maria Kritikaki, Panagiotis Dalezis, Nikolaos Thomaidis, Antonis Tsarbopoulos, Dimitris Trafalis, Evagelos Gikas
P29	*LC-MS method development for analysis of vitamins, hormones, and neutransmitter*	Nikola Gabriela Matusevica, Maros Mastrak, Kristaps Klavins
P30	*Serum metabolome profiling in early detection of lung cancer*	Piotr Widłak, Karol Jelonek, Mateusz Smolarz, Agata Kurczyk, Witold Rzyman
P31	*Diet (habitual Western vs Mediterranean) and food type (organic vs conventional) significantly affects different groups of plasma metabolites, a randomized, controlled intervention trial*	*Carlo Leifert, Per Ole Iversen*
P32	*Establishing appropriate levels of internal standards in quantitative targeted metabolomics research: profiling lipid mediators*	Pedro Araujo, Sarah Iqbal, Marit Espe, Elisabeth Holen
P33	*Plasma NMR metabolites of psoriasis and common immune-mediated inflammatory diseases in HUNT and UK Biobank*	Alya Ghina Aqila Arham Abhibhav Sharma, Lavinia Paternoster, George Davey Smith, Bjørn Olav Åsvold, Kristian Hveem, Guro Giskeødegård, Ben Brumpton, Mari Løset

Poster overview

P34	*Quantitative NMR serum spectroscopy deciphers metabolomic and lipidomic heterogeneity in endometriosis and pelvic inflammatory disease*	Sisi Deng, Laimdota Zizmare, André Koch, Lukas Schimunek, Daniele Cefaro, Madhuri Salker, Claire Cannet, Hartmut Schaefer, Yogesh Singh, Jürgen Andress4, Bernhard Krämer, Christoph Trautwein
P35	*Altered plasma metabolite levels can be detected years before a glioma diagnosis*	Sebastian Löding, Ulrika Andersson, Henrik Antti, Benny Björkblom, Beatrice Melin
P36	*Simultaneous Quantitation and Discovery (SQUAD) metabolomics: an intelligent combination of targeted and untargeted workflows in a single injection*	Bashar Amer, Siegrun Mohring, Eugen Damoc, Tabiwang N. Arrey, Jingjing Huang, David Bergen, Rahul Ravi Deshpande, Daniel Hermanson, Vlad Zabrouskov, Susan S. Bird
P37	*High-throughput metabolite exchange across organs provides unique insights to understand underlying metabolic perturbations in progressive obesity and insulin-resistance in minipigs*	Imene Bousahba, Jérémie David, Florence Castelli, Céline Chollet, François Fenaille, Didier Rémond, Nathalie Poupin, Sergio Polakof
P38	*Using labeling probes and isotope tagging for detection and quantification of short chain fatty acids by LCMS in biological samples*	Rikard Fristedt, Rikard Landberg
P39	*Comprehensive plasma steroidomics in patients with different stages of prostate cancer disease*	Sergey Girel, Pavel A. Markin, Elena Tobolkina, Julien Boccard, Natalia E. Moskaleva , Serge Rudaz, Svetlana A. Appolonova
P40	*Analysis and prediction of postprandial metabolic response to multiple dietary challenges using dynamic mode decomposition*	Viktor Skantze, Mats Jirstrand, Carl Brunius, Ann-Sofie Sandberg, Rikard Landberg, Mikael Wallman
P41	*Unraveling the chemical ecology of successful monoculture farming in termites using LC-MS metabolomics*	Nanna Hjort Vidkjær, Suzanne Schmidt, Erin Cole, Christine Beemelmanns, Michael Poulsen
P42	*Metabolic biomarkers on the surface of cutaneous melanoma*	Skaidre Jankovskaja, Peter Spégel, Johan Engblom, Kari Nielsen, Gustav Christensen, Chris Anderson, Tautgirdas Ruzgas
P43	*Biochemical composition of soy-based meat alternatives examined using non-targeted metabolomics approaches*	Jasmin Raita, Hany Ahmed, Kang Chen, Ville Koistinen, Kati Hanhineva
P44	*Understanding the role of matrix polysaccharides of cell wall in altering aspen cuticle chemistry integrating mass spectrometry with multivariate tools*	Madhusree Mitra, Hans Stenlund, Annika I. Johansson, Marta Derba-Maceluch, Ewa J. Mellerowicz

Poster overview

P45	*Chloroplastic ascorbate level may regulate arginine metabolism through ascorbate – protein interactions*	Roland Tengölics, Dávid Tóth, Fayezeh Aarabi, Anna Karlsson, André Vidal-Meireles, László Kovács, Soujanya Kuntam, Tímea Körmöczi, Alisdair R. Fernie, Elton P. Hudson, Balázs Papp, Szilvia Z. Tóth
P46	*Global LC-MS multi-omics for investigating the effects of High Intensity Training (HIT)*	Sander J.T. Guttorm, Maria T.K.T Nguyen, Nurtene Dernjani, Elise M. Sandås, Hanne B. Skogvold, Mazyar Yazdani, Helge Rootwelt, Per Ola Rønning, Steven R.H. Wilson, Katja B.P. Elgstøe
P47	*Clinical metabolomics and lipidomics: what we have done and where we are going*	David Friedecký, Aleš Kvasnička, Dana Doběšová, Barbora Piskláková, Eliška Ivanovová
P48	*Metagenomic study of the human gut microbiome*	Indri Desiati, Tone F. Bathen, Guro F. Giskeødegård, Matteo Sangermani
P49	*Network analysis reveals systematic alterations in lipidome profiles in early-onset hyperuricemia, gout, and the effect of urate-lowering treatment*	Aleš Kvasnička, David Friedecký, Radana Brumarová, Markéta Pavlíková, Kateřina Pavelcová, Jana Mašínová, Lenka Hasíková, Jakub Závada, Karel Pavelka, Pavel Ješina, Blanka Stibůrková
P50	*Metabolic impact of whole grain diets on brain regions in a pig feeding trial*	Sara Rocha, Topi Meuronen, Retu Haikonen, Anna Kårlund, Joseph F Urban Jr., Gloria Solano-Aguilar, Olli Kärkkäinen4, Kati Hanhineva
P51	*An integrated understanding of the metabolic benefits of a novel double-targeted intervention using genetically engineered probiotic expressing aldafermin with dietary changes on NAFLD*	Ambrin Farizah Babu, Valeria Iannone, Johnson Lok, Carlos Gomez-Gallego, Giuseppe D''Auria, Ruben Vazquez-Uribe, Troels Holger Vaaben, Mareike Bongers, Santtu Mikkonen, Maija Vaittinen, Ida Tikkanen, Mikko Kettunen, Anton Klåvus, Ratika Sehgal, Dorota Kaminska, Jussi Pihlajamaki, Hani El-Nezami, Morten Otto Alexander Sommer, Marjukka Kolehmainen, Kati Hanhineva
P52	*Spatial multiomics show lipid metabolism alterations in prostate cancer*	Maria K. Andersen, Elise Midtbust, Sebastian Krossa, Trond Viset, Øystein Størkersen, Michiel Vandenbosch, Britt S.R Claes, Marco Giampà, Therese S. Høiem, Ron M.A Heeren, Guro F. Giskeødegård, Morten B. Rye, May-Britt Tessem
P53	*The optimization of the endocannabinoids' measurement method for LC-MS*	Katarzyna Miniewska, Matilda Kråkström, Ilia Evstafev, Tukka Rönko, Tuomas Lindeman, Tuulia Hyötyläinen, Matej Oresic, Alex Dickens

Poster overview

P54	*Reproducibility and Data Pooling for large scale studirs – A Interlaboratory comparison of metabolomics analyses of plasma using biocrates kit technology*	Jerzy Adamski, Gözde Ertürk Zararsiz, Gabi Kastenmüller, Jiamin Zheng, Rupasri Mandal, Lisa St. John-Williams, Kendra Adams, J. Will Thompson, Michael P. Synder, Kevin Conterpois, Songije Chen, adia Ashrafi, Sumeyya Akyol, Alexander Cecil, Ali Yilmaz, Stewart Graham, Thomas M. O`Connell, Teodoro Bottiglieri, Karel Kalecky, Tuan Hai Pham, Jerzy Adamski, Therese Koal, Jutta Lintelmann, Dernot Poschet, Jennifer Kirwan, Sven Schuchardt, Xue Li Guan, Daisuke Saigusa, David Wishart
P55	*Circulating lipoprotein subfractions and microRNAs as potential biomarkers for improved risk prediction of myocardial infarction: the HUNT study*	Julie Caroline Sæther, Marie Klevjer, Guro Fanneløb Giskeødegård, Tone Frost Bathen, Bruna Gigante, Turid Follestad, Helge Rørvik Røsjø, Torbjørn Omland, Erik Madssen, Anja Bye
P56	*Immobilized Enzymes on Magnetic Beads for Separate Mass Spectrometric Investigation of Human Phase II Metabolite Classes*	Ioanna Tsiara, Amelie Riemer, Mario S.P. Correia, Ana Rodriguez Mateos, Daniel Globisch
P57	*Comparison of serum metabolome profiles of four types of solid cancers by MS and NMR approaches*	Katarzyna Mrowiec, Julia Debik, Karol Jelonek, Agata Kurczyk, Lucyna Ponge, Guro F. Giskeødegård, Tone F. Bathen, Piotr Widłak
P58	*Biochemical profiling of porcine burn wound healing following treatment with acellular skin grafts*	Óttar Rolfsson, Aristotelis Kotronoulas, Christian Christiansen, Adrian Lopez Garcia de Lomana Giorgios Stamatakis, Marieke Heijink, Martin Giera, Martina Samiotaki, Himar Kjartansson, Randolph Stone II
P59	*Integration of proteomics and metabolomics data in a case-control study of Graves disease*	Arve Ulvik, Klaus Meyer, Johnny Laupsa Borge, Hans Olav Ueland
P60	*Longitudinal associations of sleep duration, vitamin D, and grain intake with serum fatty amides measured by LC-MS from childhood to adolescence*	Iman Zarei, Darren R. Healy, Sonja Soininen, Aino-Maija Eloranta, Marko Lehtonen, Marjukka Kolehmainen, Timo A. Lakka, Kati Hanhineva
P61	*Isotope-guided metabolomics dissects kidney arginine metabolism*	Maria Chrysopoulou, Johannes Jägers, Markus Bleich, Nina Himmerkus, Markus Rinschen
P62	*Analysis of biological samples by fast HILIC separations of nucleotides, and RPLC separation of 3-NPH derivatized carboxylic acids with LC-MS/MS*	Ondrej Hodek, Adela Pravdova, Thomas Moritz

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P63	*Dynamics of gut metabolome and microbiome maturation during early life*	Anna-Katariina Aatsinki, Santosh Lamichhane, Heidi Isokääntä, Partho Sen, Matilda Kråkström, Marina Amaral Alves, Anniina Keskitalo, Eveliina Munukka, Hasse Karlsson, Laura Perasto, Minna Lukkarinen, Matej Oresic, Henna-Maria Kailanto, Linnea Karlsson, Leo Lahti, Alex M Dickens
P64	*Multi-omics fingerprint of in vitro bioengineered heart-on-chip platform*	Ilaria Gisone, Maria Guirro, Lorena García, Nerea Abasolo, Federico Vocci, Núria Canela, Salvador Fernández-Arroyo
P65	Metabolite changes during curative treatment of Prostate Cancer	Kristina Lundquist, Camilla Thellenberg-Karlsson, Henrik Antti
P66	*An integrated molecular networking based non-targeted PFAS analysis workflow enables the identification of novel targets in NIST plasma*	Juan Moises Sanchez, Marynka Ulaszewska, Ralf Tautenhahn
P67	*Effects of FODMAPs and gluten on irritable bowel syndrome, from self-reported symptoms to molecular profiling*	Elise Nordina, Per M. Hellströmb, Rikard Landberga, Carl Bruniusa
P68	*Global metabolomics and lipidomics in a university hospital setting*	Katja B Prestø Elgstøen, Hanne B. Skogvold, Sander J.T. Guttorm, Mazyar Yazdani, Elise Sandås Sand, Helge Rootwelt
P69	*Metabolomic and lipidomic approaches in mindfulness-based intervention for health-care students*	Isabel Meister, Sergey Girel, Izadora Furlani, Claire Holman, Françoise Jermann, Julien Boccard, Camille Piguet, Serge Rudaz
P70	*A quantitative method for analysis of the oat specific compounds avenanthramides and avenacosides in human plasma samples*	Marina Armeni, Rikard Fridstedt, Otto Savolainen, Rikard Landberg
P71	*Combining real time and post-acquisition quality control (QC) for metabolomics workflows*	Aiko Barsch, Patrick Groos, Nikolas Kessler, Matthias Szesny, Sven W. Meyer, Ilmari Krebs, Heiko Neuweger, Matthew R. Lewis, Cristian De Gobba
P72	*Non-targeted metabolomics of urine to characterize benign and malignant canine mammary cancer subtypes*	Robin Moore, Alessandra Estrela-Lima, Olli Kärkkäinen, Soile Turunen, Anna Hielm-Björkman

ORAL PRESENTATIONS

Session 1: Spatial Metabolomics

Title

Optimal storage condition and time of fresh frozen tissue sections prior to spatial metabolite detection with MALDI MSI

Authors
Maria K. Andersen[1], Elise Midtbust[1], Britt S.R. Claes[2], Marco Giampà[3], Benjamin Balluff[2], Juan Carlos Cabellos Guillem[1], Ron M.A. Heeren[2], Therese S. Høiem[1], Sebastian Krossa[1], May-Britt Tessem[1]

Affiliations
[1]Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway. [2]Maastricht MultiModal Molecular Imaging institute (M4I), Maastricht University, Maastricht, The Netherlands. [3]Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

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Abstract

By preserving spatial localization, MALDI-MSI is a powerful method to analyse metabolites in the search for novel clinical markers in heterogenous cancer tissue. However, large patient cohorts are required to find robust biomarkers, making analysis logistically challenging and often resulting in long-term storage. We aimed to identify the optimal long-term storage conditions of fresh frozen tissue sections to be analyzed for spatial metabolite distribution with MALDI-MSI.

Fresh frozen serial sections (10µm) from non-cancerous human prostate tissue were stored under three different conditions: 1) Frozen at -80°C, 2) vacuum at room temperature (RT) in the dark, and 3) vacuum at -80°C. Sections were stored with these conditions for different time frames: 1.5 months, 7 months, and 3 years. Serial sections were obtained from four samples for which three technical replicates were used per condition. All sections were analyzed with MALDI-TOF MSI in negative ion mode (*m/z* 40-1000) and subsequently stained with HES and annotated for epithelial glands and stroma. So far, one of the four samples has been computationally analyzed. Analyte localization and metabolite degradation were evaluated to assess analytical quality.

Citrate (*m/z* 191) and taurine (*m/z* 124) were used to assess analyte localization as they have previously been determined to have high intensity specifically in epithelium and stroma, respectively. The mean citrate levels in epithelium were divided by the mean levels in stroma, while the reverse calculation was done for taurine. A high ratio between epithelium and stroma indicated good mass localization. Although there were some variations between the technical replicates, sections stored at vacuum, both at -80°C and RT, had the best mass localization of citrate and taurine. Storage time did not negatively affect the mass localization for any of the conditions. Metabolite degradation was determined by the ratio of ATP to ADP and AMP within each section with a high ratio indicating high metabolic integrity. Sections stored at -80°C, both with and without vacuum packing, were far superior to vacuum storage at RT with the least metabolic degradation. The ATP/(ADP+AMP) ratio was stable between 1.5 and 7 months of frozen storage but showed a clear decline for all storage conditions after over 3 years.

In conclusion, our preliminary results suggest that vacuum packing improves analyte localization and frozen storage is crucial to limit metabolic degradation. How the different storage conditions affect the lipids and other metabolites also detected in this dataset will be investigated in future data analysis.

Title
Spatial metabolomics - revealing molecular distributions correlating with disease

Authors (presenting author underlined)
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Abstract text

Spatial metabolomics has the potential of sheading new light on disease states by directly mapping the chemistry in healthy and diseased tissue regions. The PA nano-DESI MSI technique utilizes localized liquid extraction followed by electrospray ionization to spatially map detected ions in tissue. Specifically, the chemical material is desorbed into a flowing liquid bridge between two fused silica capillaries for subsequent electrospray ionization at the inlet of the mass spectrometer. Here, we present using mass spectrometry imaging (MSI), particularly pneumatically assisted nanospray desorption electrospray ionization (PA nano-DESI), of a rodent model for ischemic stroke and human brain tissue affected by multiple sclerosis.

Ischemic stroke was induced in mice by occlusion of the middle cerebral artery (MCAO), followed by reperfusions before the animals were sacrificed and the brain tissue snap frozen. Cryo-sectioned tissue sections of 12 µm were subsequently imaged with quantitative PA nano-DESI coupled to an orbitrap mass spectrometer. Region-of-interest analysis comparing the healthy hemisphere to the one subjected to MCAO revealed distinct chemical alterations. Specifically, lysophosphatidylcholine and long-chain carnitienes species were accumulated in the ischemic regions whereas phophatidylcholine, glucose, carnitine and short-chain carnitines species were depleted. With these results, we estimated the activities of the carnitine transporting enzymes and report a disruption in the beta-oxidation that is specific to the damaged caused by ischemia.

Human post-mortem donated and flash-frozen brain tissue of multiple sclerosis and healthy tissue were obtained from a biobank and cryo-sectioned into thin tissue sections. Subsequent sections were either stained to identify individual sub-regions of diseased states or imaged with quantitative PA nano-DESI coupled to an orbitrap mass spectrometer. Following, the data of the respective subregions were compared with a focus on oxidized lipids, including leukotrienes, prostaglandins, and hydroxyeicosatetraenoic acids. The results show significant alterations of all types of the oxidized with hydroxyeicosatetraenoic acids and leukotriene species being highly increased in remyelinating lesions and slightly increased in chronic lesions compared to normal-appearing white matter. Contrarily, PGE2 was decreased in both chronic and remyelinating lesions. This suggest an increased inflammatory activity through both LOX and COX in the multiple sclerosis lesions and the border regions.

Overall, we show the additional dimension of metabolomics that is achievable with techniques providing spatially resolved data, the high applicability of PA nano-DESI for imaging of tissue sections, and present specific results for increased mechanistic understanding of stroke and multiple sclerosis.

Title
Studying metabolic changes during EMT using MALDI MSI

Authors (presenting author underlined)
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Abstract text

The Epithelial-Mesenchymal Transition (EMT) is a process that occurs during early metastasis. Here, stationary, quickly dividing epithelial cells gain mesenchymal features that allow them to quickly migrate but at the cost of slower cell division. This process requires metabolic rearrangements.
In our project we are using MALDI MSI to study the metabolic changes that occur in the breast cancer cell line MDA MB 468 during EMT. The cells are cultured directly on ITO-coated slides and EMT is induced using 25ng/mL EGF. Using this method, we have found that the levels of more than 30 unique lipid species are altered during the phenotypic transition. Identification of these species with SolariX suggest that EMT regulates both phosphatidylethanolamine and phosphatidylinositol metabolism.
Traditional lipidomics methods determine the average lipid levels of cell populations. However, EMT is highly heterogeneous, and single-cell gene expression analyses have revealed a spectrum of intermediate EMT-states with different properties. Taking advantage of the spatial resolution of MALDI MSI we are exploring the heterogeneity of the metabolic responses to EGF, and how these changes are distributed within cell populations. We aim to identify metabolic responses associated with strong responses to stimuli and acquisition of a mobile cellular phenotype.

Session 2: Metabolomics and lifestyle

Title
Metabotyping the obesity: New factor-analysis-based strategies for classifying from multiplatform metabolomics data children with obesity

Authors
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Abstract text
The dramatic increase in childhood obesity prevalence has resulted in specific needs in defining obesity-associated entities. Due to the inherent complexity of obesity, metabolomics approaches allow a broader understanding of its pathophysiology. Many metabolomics studies have been performed in adults; however, there is a need to deepen the understanding of this disease in children and how it affects their development.

Integrating data from different analytical platforms provides improved coverage of complex sample metabolomes, allowing better understanding of the underlying biological mechanisms associated with a given phenotypic pattern. Factor Analysis makes it possible to integrate data, reduce dimensionality, and identify underlying factors that explain variance in metabolomic data. The combination of Factor Analysis and hierarchical clustering algorithms allows grouping the samples according to the information contained in a multi-platform analysis. Comprehensive metabolic phenotyping ("metabotype") of children with childhood obesity will also increase understanding of the disease's development.

110 children with obesity were analyzed by multiplatform untargeted metabolomics approach based on reversed-phase U(H)PLC-QTOF-MS/MS, GC-q-MS, and CE-TOF-MS analysis. It clearly shows that data integration based on dimensionality reduction techniques is a powerful tool to combine all metabolomic information obtained from different platforms. Three distinct metabotypes of our patients have been identified by Factor Analysis of the integrated multiplatform data in conjunction with a hierarchical cluster technique that was applied to the factors acquired. No metabotype is characterized by a higher number of individuals with genetic alterations (heterozygous variants in the leptin-melanocortin satiety pathway). This information is highly relevant in the clinical management of patients, allowing to establish an adequate treatment for everyone. The integration of data from different analytical platforms provides the opportunity to obtain a more complete understanding of biological systems and to identify different metabolic phenotypes for personalized therapy.

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Title
Mapping the effects of plant-based proteins on human metabolic profiling

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Abstract text

Human dietary shifts are partly due to food availability and, more recently, to maintain a sustainable ecosystem by eating less animal and more plant-based proteins. Distinct nutritional responses to these alterations are expected because of each person's heterogeneous genetic background and gut microbiota profile. Identifying metabolic signatures and biomarkers of dietary change adaptations may shed light on the mechanisms underlying common diseases and the different individual responses. In a comprehensive approach, we aim to map triggered metabolic pathways by plant-based protein supplementation. To this end, we performed two dietary interventions and participants included were healthy, between 18 and 45 years old, males and females, in a normoprotein diet (up to 1.2 g of protein/kilogram (kg) of body weight (BW)). The first intervention was a single-arm study (n=29), consisting of 4 weeks of baseline followed by 4 weeks of isolated pea protein (IPP) supplementation (week 1: 0.25, week 2: 0.50, week 3: 0.75, week 4: 1.00 g of protein from the IPP per kg of BW). The aim was to evaluate the amount of protein consumed from the IPP that affected gut-related metabolites. The second intervention was a double-blind, randomised, 3-arm parallel trial (n=59) with 2 weeks of baseline measurements followed by 4 weeks of isolated pea protein, concentrated pea protein or whey protein supplementation (all 4 weeks: 0.50 g of protein from the supplement per kg/BW). Hence, the second intervention aimed to investigate how the different proteins affected the diet- and gut-metabolites profile. For both studies, the diet was followed by 3 food diaries per week, compliance was measured by 24 h urea nitrogen, questionnaires were used to assess physical activity, gastrointestinal symptoms, stool consistency and frequency, and the body composition was measured consistently during the study. Targeted metabolomics in faeces and plasma for short chain fatty acids (SCFA) and tricarboxylic acid cycle (TCA) cycle, ammonia, as well about 200 targeted polar metabolites related with protein fermentation and known diseases markers (incl., indoles and phenols) were measured. For hypothesis generation, untargeted metabolomics were applied including lipidomics in plasma. Acetate (SCFA) and oxaloacetate (TCA cycle) changed significantly compared to the baseline samples in the faecal samples after the intervention. Tryptophan, pyrimidine and phenylalanine metabolisms were altered due to the intervention, as well as carnitine derivatives. Having a panel of diet- and gut- related metabolites can aid in investigating plant-based healthier diet adaptations in an individualised manner.

Title: Exploring the impact of plant protein vs. animal protein-rich diets in men at cardiometabolic risk: insights from plasma metabolome signatures

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Abstract text:

A dietary shift from plant protein (PP) sources to animal protein (AP) sources is encouraged both for environmental and health reasons, especially for limiting cardiometabolic risk (CMR). We aimed at characterizing the metabolic reorientations induced by a reasonable dietary shift from AP to PP sources in middle-age men at CMR.

We conducted a 1-month cross-over randomized controlled trial (NCT04236518) enrolling 19 healthy men with overweight and CMR. They randomly consumed 2 controlled iso-caloric diets (lunch and dinner provided) containing predominantly AP (66% AP:34% PP) or PP sources (37% AP:63% PP). Plasma metabolome (untargeted LC-MS) was assessed at the fasted state every 2 weeks and postprandially (6h follow up) at the end of each intervention period after the intake of a high-fat challenge meal (900kcal, lipids=80%E). Multivariate (LiMM-PCA) and univariate mixed model analyses were performed.

Plasma metabolome significantly differed between PP and AP diets at both fasted and fed states (*P* diet<0.01). At the fasted state, gut microbiota-related metabolites (e.g. indoleacrylic acid) and plant-derived metabolites (polyphenols degradation products, trigonelline, N-acetyl-ornithine) were higher after PP *vs* AP diets. Concomitantly, metabolites related to amino acid metabolism (lysine and its byproduct α-aminoadipic acid, branched chain amino acid (BCAA) catabolism products), gut microbiota-related metabolites (indoxyl sulfate) and meat or fish related food intake biomarkers (FIB) (methyhistidine, hydroxyprolines, EPA) were lower with PP *vs* AP-diets. The analysis of the postprandial metabolome revealed additional associations not visible at the fasting state, especially regarding lipid metabolism, with following PP vs AP-diets: lower levels of C3-acylcarnitine (related to BCAA catabolism) and higher postprandial increase of lipid species synthesized through the ω-oxidation (dicarboxylic acids C10, C12, C14, C16), a minor microsomal metabolic pathway.
While some of these metabolites are validated FIB (e.g. methylhistidine for meat intake) others might be promising new ones, such as N-acetyl-ornithine, recently associated to PP intake. Some of the metabolic pathways or metabolites reported here have also been associated to increased CMR (BCAA degradation and α-aminoadipic acid) or decreased CMR (indoles produced by the gut microbiota tryptophan catabolism) and their levels are compatible with improved metabolic health after PP *vs* AP-diets. Additionally, the postprandial response to a high-fat challenge meal revealed unexpected changes in lipid metabolism, suggesting the activation of specific compensatory mechanisms in case of lipid overload and β-oxidation saturation following PP *vs* AP-diets. Overall,

these results bring new mechanistic insights to unravel the subtle changes induced by plant-rich diets on CMR.

Title
Exploring sources of variation in the female serum metabolome in light of breast cancer risk factors, in healthy participants of the HUNT2 study.

Authors (presenting author underlined)
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Abstract text

Circulating metabolic profiles have repeatedly been found to associate with various disease outcomes in large-scale population studies. It is well-known that the circulating metabolic profile is dependent on both internal and external factors, however, the key determinants of most metabolites, and how to manipulate their levels, are yet unknown.

This study aimed to explore sources of variation in the female serum metabolome related to known breast cancer risk factors.

Detailed serum metabolic profiles of 2283 healthy female participants within the Trøndelag Health Study (HUNT study) were obtained, through nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Machine learning was applied to predict metabolic levels in held-out individuals based on clinical parameters, lifestyle, socio-economic factors, and anthropometric measurements. Thereafter, the study cohort was clustered into three groups of individuals based on the abovementioned factors. Cluster 1 included obese and mostly inactive participants (n = 507); cluster 2 included the oldest participants, characterized by high blood pressure (n = 639); cluster 3 included the youngest and healthiest individuals (n = 1137). Metabolite levels were compared across the clusters.

We found that up to 32% of the variance in individual serum metabolites could be explained by known breast cancer risk factors. Clear metabolic differences were found between the clusters of participants: cluster 1 was characterized by significantly elevated serum levels of triglycerides, phospholipids, and apolipoprotein B; cluster 2 by elevated levels of cholesterol and apolipoproteins A1 and A2; and cluster 3 by decreased levels of lipoproteins, except for levels of high-density lipoprotein subfractions. Most of the small-molecular metabolites had highest levels in cluster 1, and lowest levels in cluster 3.

We report distinct metabolic signatures based on clinical parameters, lifestyle, socio-economic factors, and anthropometric measurements in a large population of healthy females.

Title

Plasma metabolic profiling shows reversible changes in metabolites linked to psychological traits: A metabolomics study of the effects of alcohol withdrawal in patients with alcohol use disorder

Authors (presenting author underlined)

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Abstract text

Background: Alcohol use disorder (AUD) is a multifaceted mental health disease with current treatments showing limited efficacy. Patients with AUD exhibit mood and behavioral symptoms and display alterations in plasma and brain metabolomes. Such alterations could potentially be used for novel treatment targets, patient stratification or recognition of high-risk individuals. However, less is known about the relationship between metabolites and psychological symptoms and the persistence of the metabolic changes associated with alcohol use following withdrawal.
Aim: To characterize the effects of alcohol abuse and withdrawal on plasma metabolome, associate findings with psychological traits and expand findings to cerebral metabolites.
Methods: Plasma samples from AUD subjects at the start (n=96) and at the end (n=64) of the withdrawal as well as healthy controls (n=32) were subjected to metabolomics analysis by LC-HRMS in four analytical modes. Following peak-picking and preprocessing, the features were subjected to groupwise comparisons to shortlist metabolites for annotation. Additionally, a targeted search for the altered plasma metabolites was performed in a separate metabolomics subset of corticospinal fluid and frontal cortex samples from deceased AUD patients and controls.
Results: AUD patients displayed marked changes in plasma metabolome from where 141 significantly ($q<0.05$) altered metabolites were annotated. Majority were related to energy and steroid metabolism, such as 16- and 18- carbon fatty acids and glycerophospholipids, 3-hydroxyvaleric acid and bile acids that correlated positively ($r > 0.2$, $p<0.05$) while xanthines, phenolsulfates and hippuric acid negatively ($r < -0.2$, $p<0.05$,) with psychological symptoms or alcohol intake. Frontal cortex and corticospinal fluid levels of 3-hydroxyvaleric acid and xanthines were also significantly ($q<0.05$) different between controls and AUD patients. Alcohol withdrawal reversed majority of observed plasma alterations.
Conclusions: Excessive alcohol use has a profound effect on plasma metabolites with observable overlapping traits in the cerebral metabolites. Our results demonstrate that alcohol is the driving force behind plasma metabolic alterations as three weeks of alcohol withdrawal was sufficient to reverse majority of observed alterations. Findings of metabolites correlated with psychological symptoms warrants for further mechanistic studies focusing on the role of host and gut microbiota-related metabolites in psychological wellbeing in AUD and other high-risk populations.

Session 3: Microbiome and host metabolism

Title
Gut Microbiome and Novel Bile Acids: New Insights into the Progression to Islet Autoimmunity

Authors
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Gut microbiota plays a critical role in regulating bile acid (BA) metabolism. Recent research suggests that gut microbes can produce many more BA conjugates than previously thought (Qinn et al.), including BA conjugates of 22 different amino acids, now referred to as bile acid amidates (BAAs). However, there is still limited knowledge about the regulation of these microbially-conjugated BAAs and their potential role in health and disease.

In our previous work, we found that microbial dysregulation of secondary BA pathways associates with progression to islet autoimmunity and type 1 diabetes (Lamichhane et al.). Here, we aimed to define the trajectories of BAAs in early life and their potential association with the progression to islet autoimmunity and type 1 diabetes. We developed and optimized a method to investigate the dynamics of 110 BAAs in longitudinal stool samples (n=303) collected at 3, 6, 12, 18, 24, and 36 months of age from children (n=74) who developed a single islet autoantibody (AAb) (P1Ab), multiple islet AAbs (P2Ab), and controls (CTRs) who remained AAb negative during the follow-up. Additionally, we analyzed the stool microbiome in a subgroup of these children.

Through factor analysis, we found that age had the most significant impact on both BAAs and microbiome profiles. We observed that in infancy, systemic BAAs and microbial secondary BA pathways were altered in the P2Ab group compared to the P1Ab and CTR groups. These findings suggest that dysregulated microbial BA metabolism in early life may contribute to the risk and pathogenesis of type 1 diabetes.

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Title

Chemoselective Metabolomics – New Chemical Biology Tools to Explore Microbiome and Diet Metabolism

Authors

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Abstract text

Metabolites produced by the gut microbiome play a crucial and diverse role on host physiology, which are detectable in a wide range of biological samples including feces, plasma, urine, and brain. Microbiota dysbiosis has been associated with the development of diseases, however, the metabolic link has yet to be detected. The detailed and targeted analysis of these metabolites is important for the discovery of biomarkers and unknown bioactive molecules. Mass spectrometric metabolomics is the method of choice for identification and quantification of these metabolites. Advanced methods at the interface of chemistry and biology coupled with metabolomics analysis are required but still limited. We have therefore developed a unique and multifunctional chemoselective probe with synthetic $^{13}C/^{12}C$ isotopically labelled analogues that allows for comparative and quantitative analysis of metabolites in human samples at low concentrations (1). We have termed this method quantitative **Quant**itative **S**ensitive **CHE**moselective **M**et**A**bolomics (*quant*-SCHEMA). Coupled to magnetic beads, this method allows the straightforward chemoselective extraction of metabolites from human samples (1-3). This isolation procedure of specific metabolite classes from sample matrices led to a substantial increase in mass spectrometric sensitivity by up to sixth orders of magnitude and facilitates the detection of metabolites at femtomolar quantities.

We have recently applied these chemoselective probes for analysis of dietary carbonyl-containing metabolites in human urine samples and SCFAs microbiome co-cultures with pathogens. For the first time, we have applied this methodology for large-scale analysis in a dietary intervention study with 156 samples for nutritional biomarker discovery (2). We have successfully found four potential dietary carbonyl biomarkers, which have not been reported before. Additionally, we have developed a chemoselective probe methodology for the absolute quantification of short-chain fatty acids (3). The targeted SCFA analysis was combined with global metabolomics on gut microbiome co-cultures with *Salmonella* and treated with antibiotics. The successes of these methodologies encourage the general use of this method in metabolomics studies.

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Title
Oral microbiome associates with salivary metabolome and sugars profile

Authors
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Abstract text

The health status of the oral cavity is pivotal. The associations between oral health and dental as well as non-communicable diseases such as cardiovascular disease have been firmly established. Oral cavity also harbors a plethora of sensory signaling and oral microbiome with its metabolic capacity which may influence host health. Unfortunately, the complex interplay between oral microbiota, the host, and exogenous exposures, such as diet, has not yet been well understood. We therefore performed a comprehensive profiling of saliva, as a biofluid from the oral cavity, to obtain understanding on the associations between salivary microbiome, metabolome, and sugars, as well as the impact of age, sex, and body mass index (BMI) on such associations. We also examined how the metabolome and sugars profiles were associated with microbial diversity (Shannon diversity index) and oral microbiome phylotypes ('orotypes') previously linked with participants' lifestyles and sugar intake (1). Saliva samples were collected from 427 participants aged 16-79 years old living in Umeå, Sweden. Untargeted metabolomics and sugar profiling data were performed for subsets of 416 and 200 participants, respectively. The most important microbial species that characterized the orotypes were selected using elastic net models adjusted for age and sex. The top 20 microbial species with the highest ranks were associated with sugars and metabolome profiles using partial Spearman correlation adjusted for the same covariates. Microbial species, sugars, and metabolite features differed by age, sex, and BMI were investigated using random forests. Preliminary data suggest that age appeared to be an important determinant of the metabolome profiles and, to some extent, of the sugars, and the least with oral microbiota. Moreover, sex, but not BMI, appeared to have effects on the molecular signatures of saliva. Microbial diversity, to a greater extent than orotypes, was associated with the metabolome and sugar profiles. Our data suggest that saliva is an important, yet neglected, easily accessible matrix to indicate participants' individual molecular phenotypes which may link oral health to dental and noncommunicable diseases.

1. Esberg A, Eriksson L, Hasslöf P, Haworth S, Holgerson PL, Johansson I. Using Oral Microbiota Data to Design a Short Sucrose Intake Index. Nutrients. 2021.

Title
Effect of Fermentation on Sorghum Phenolic Compounds

Authors (presenting author underlined)
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Abstract text

Sorghum (*Sorghum bicolor* L. Moench) has high contents of phenolic compounds, which carry both health benefits and antinutritional effects. These phenolic compounds can bind with proteins to form insoluble complexes, leading to decreased protein digestibility. Lactic acid bacteria (LAB) fermentation has been found to reduce and modify the phenolic compound and condensed tannin profiles in sorghum. The aim of this study was to assess the impact of LAB fermentation on the phenolic compounds present in white and red sorghum using a metabolomics approach. Whole grain flours of white and red sorghum from Uganda were fermented by two different lactic acid bacteria: *Weissella confusa* A16 and *Weissella confusa* VIII40. Both the native and fermented sorghums were extracted with 80% ethanol and analysed using ultra-performance liquid chromatography coupled with a photodiode-array detector and quadrupole time-of-flight mass spectrometry (UPLC-PDA-Q-TOF). This study identified 40 compounds, of which 37 were phenolic compounds. The changes of 23 compounds were distinct between native and fermented groups. Red sorghum had a higher flavonoid content, including catechin, (epi) catechin hexosides, 7,3',4'-trihydroxyflavone and several condensed tannins such as procyanidin B1 and procyanidin polymers compared to white sorghum. The native samples were statistically different from the fermented ones. The primary changes observed during fermentation were the liberation of phenolic acids from their conjugated forms and an elevation in phenolamines. Notably, LAB fermentation proved efficient in breaking down larger molecules, such as condensed tannins, into their smaller counterparts. This promising result suggests that sorghum fermented with LAB may lead to enhanced protein digestibility. Further studies, such as *in vitro* protein digestibility and enzymatic activity studies, can elucidate the mechanisms of the strain and their effects on protein digestibility.

Session 4: Computational metabolomics

Title
Adjusting for covariates and assessing modeling fitness in machine learning using MUVR 2.0.

Authors (presenting author underlined)
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Abstract text
Machine learning algorithms are frequently used for modelling and feature selection in metabolomics. The MUVR algorithm was shown to generate predictions and highly relevant variable selections with minimal bias at state-of-the-art performance (1). However, a general framework for assessing prediction and overfitting across cross-validation strategies is still lacking. In addition, adjusting for covariates is a highly desired, but largely lacking trait in machine learning. We aimed to address these issues in the MUVR framework.

The original MUVR algorithm incorporated partial least squares and random forest modelling and performed variable selection through recursive elimination within a repeated double cross-validation framework. In brief, the MUVR algorithm uses the data of metabolic features and a target variable and selects features that give optimal prediction performance.

The updated MUVR algorithm v 2.0 was extended to include elastic net, allowing covariate adjustment. Using simulated and real-world data, we observed that upon adjusting for covariates in MUVR-elastic net metabolite features that are associated with covariates were less frequently selected as important. In addition, similar attempts at adjusting for covariates in PLS and RF were unsuccessful. The new MUVR functionality may benefit epidemiological and other studies where covariate adjustment directly during machine learning analysis is desired while maintaining the highest levels of safeguards against overfitting.

In addition, we further introduced a framework for the systematic assessment of prediction performance and modelling overfitting based on resampling tests, which compares the prediction performance using the actual target variable vs a resampled target variable, as well as a reference state as a null hypothesis condition to assessing model overfitting. We recommend this framework as part of general procedures in machine learning since it provides not only useful information about modelling prediction performance and overfitting, but also insights into data-driven choices of machine learning strategy. Comparing MUVR to less complex cross-validation (CV) strategies in both regression and classification, we showed that although no strategy can completely eliminate overfitting, the MUVR procedure has considerable informatics benefits from achieving an automated selection of variables of interest with state-of-the-art performance without inducing bias.

While applications focused on the analysis of metabolomics data, MUVR can also be applied to other types of high-dimensional data where variable selection is of interest. The R package *MUVR* is freely available with data, tutorial, and scripts at https://github.com/MetaboComp/MUVR.

Shi L, Westerhuis JA, Rosén J, Landberg R, Brunius C. Variable selection and validation in multivariate modelling. *Bioinformatics*. 2019;35(6):972-980.

Title
From static to dynamic, how to analyze postprandial metabolomics data?

Authors (presenting author underlined)
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Abstract text
The transition of metabolomics data analysis from a static to a dynamic perspective following a meal intake can improve the understanding of metabolic mechanisms, reveal biomarkers for early diagnosis of metabolic diseases and advance precision nutrition and medicine. However, analyzing such longitudinal metabolomics data is challenging, hampered by the complexities of temporal dynamics, high dimensionality and high biological variability. Conventional analysis methods mainly focus on clustering methods that rely on summaries of data across individuals or on univariate methods that analyze one metabolite at a time, failing to provide a compact summary that can reveal the underlying patterns, i.e., groups of subjects, clusters of metabolites, and their temporal profiles.

In this talk, we discuss the analysis of postprandial metabolomics data using multiway data analysis (also referred to as tensor factorizations), demonstrate how to extract the underlying patterns in the data as well as how to reveal both the static and dynamic markers. We arrange the postprandial metabolomics data as a three-way array: *subjects* by *metabolites* by *time points*, and assess the performance of different analysis methods on both simulated and real data sets. We generate simulated postprandial metabolomics data with known ground truth using mathematical metabolic models. The real data corresponds to NMR (Nuclear Magnetic Resonance) spectroscopy metabolomics measurements of plasma samples collected during a meal challenge test from the $COPSAC_{2000}$ cohort. We consider the analysis of the postprandial metabolomics data using principal component analysis (PCA) of the fasting (static) state, the CANDECOMP/PARAFAC (CP) model of the dynamic part, and joint analysis of fasting and dynamic parts (coupled through the *subjects* mode) using Coupled Matrix and Tensor Factorizations (CMTF).

Our results show that the proposed separate analyses of the static and dynamic signals from the postprandial metabolomics data are able to reveal the metabolic differences at the fasting and dynamic states. Furthermore, we demonstrate that jointly analyzing static and dynamic data through CMTF models yields a complete picture of static and dynamic markers for the same subjects revealing more interpretable patterns.

(1) Li L, Yan S, Bakker BM, Hoefsloot H, Chawes B, Horner D, Rasmussen MA, Smilde AK, Acar E. Analyzing postprandial metabolomics data using multiway models: A simulation study. bioRxiv: https://www.biorxiv.org/content/10.1101/2022.12.19.521154v2

Title
Registration-based Integration of Spatial Multi-Omics Data for Prostate Cancer Classification

Authors
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Abstract

Aim: To truly understand the cancer biology of heterogenous tumors, it is crucial to use analytical methodology capable of capturing the complexities of multiple omics levels, as well as the spatial heterogeneity of cancer tissue. Different molecular imaging techniques, such MALDI mass spectrometry imaging (MALDI TOF-MSI) and spatial transcriptomics (ST) achieve this goal by spatially detecting metabolites and mRNA, respectively. To take full computational advantage of such multi-omics data, the individual measurements need to be integrated into one dataset. The main aim of this work is therefore to provide a computational pipeline that generates a spatially integrated multi-omics dataset for biomarker detection in prostate cancer. For this purpose, we have developed GreedyFHist, a powerful tool to co-register heterogeneous serial sections.

Methods: Tissue cores (n=32) were taken from 2 mm whole prostate slices extracted from the middle of the prostate after prostatectomy (N=8 patients). Each core was cryo-sectioned (10µm, n=3), stained with Hematoxylin, Erythrosine & Saffron (after MALDI-TOF MSI) or Hematoxylin & Eosin (before ST) and analyzed for various omics technologies with two sections for MALDI TOF-MSI (positive and negative ion mode) and one section for ST. Then serial histology sections were co-registered using GreedyFHist. To measure the effect of tissue heterogeneity between serial sections we computed the median relative landmark registration error (median-rTRE) using manually placed matching landmarks on each section (40 landmarks on average). We compared the performance of GreedyFHist to 7 other registration algorithms.

Results and discussion: Our experiments show that GreedyFHist accurately aligns serial histology sections with a median-rTRE of 0.0088 (~ 38.27 µm; after removal of 9 outliers). This is the best score achieved in a selection of 8 registration algorithms. Other algorithms reached a median-rTRE between 0.244 (~982.334 µm) and 0.284 (~1141.741 µm).

Conclusions: Our study revealed that we can spatially integrate several molecular imaging techniques into one dataset within a median registration error of 0.0088. For the future we will analyze our integrated multi-omics dataset to uncover the relationship between spatial metabolomics and spatial transcriptomics and use this knowledge for prostate cancer classification.

Title
Fragmentation site prediction for non-targeted metabolomics using graph neural networks

Authors (presenting author underlined)
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Abstract text

Motivation
The potential of non-targeted metabolomics to uncover new biological insights, identify biomarkers or monitor clinical disease progression cannot be emphasized enough. However, spectral reference data is incomplete, and most compound mass spectra in non-targeted metabolomics experiments cannot be annotated with spectral search alone. At the same time, the identification and classification of unknown compounds are far from trivial. One reason is the current lack of understanding about how new molecules will fragment when subjected to tandem mass spectrometry (MS/MS). Existing *in silico* fragmentation methods, such as MetFrag [1] and CFM-ID [2], imitate the fragmentation process but their accuracy is limited due to the way they integrate and engineer molecular features. We investigate the ability of graph neural networks (GNNs) to learn and recognize relevant structural groups associated with bond cleavage during MS/MS.

Methods
Graph neural networks (GNNs) have been used in the context of drug property prediction with great success. Their ability to learn and aggregate relevant features from neighboring atoms and substructures through graph convolution or message passing makes them a powerful tool for predicting molecular properties. This approach opposes traditional hand-crafted molecular descriptors. However, in non-targeted metabolomics, GNNs have only been used for direct property and peak predictions rather than as a foundation of *in silico* fragmentation frameworks. We deploy and test various types of to predict the break tendencies of bonds during MS/MS fragmentation and predict the resulting fragment ions.

Results
Trained on the NIST17 spectral library, preliminary results show significant improvement over CFM-ID tested with CASMI 2016 challenge. Predicted spectra for the challenge compounds have a much higher cosine similarity of 0.7 on average (median) to their experimental spectra compared CFM-ID (version 4.4.7), which achieves an average cosine score of 0.62. Simultaneously, our model predicts retention time and collision cross section, which is valuable for compound identification and elimination of false matches. Despite the early prototype, our results show that GNN have a great potential to improve the accuracy of *in silico* fragmentation algorithms.

[1] Ruttkies, Christoph, et al. "MetFrag relaunched: incorporating strategies beyond in silico fragmentation." *Journal of cheminformatics* 8.1 (2016): 1-16.
[2] Wang, Fei, et al. "CFM-ID 4.0: more accurate ESI-MS/MS spectral prediction and compound identification." *Analytical chemistry* 93.34 (2021): 11692-11700.

Session 5: Clinical metabolomics

Title: Stratification of ovarian cancer borderline from high-grade serous carcinoma patients by quantitative serum NMR spectroscopy of metabolites, lipoproteins, and inflammatory markers

Authors (presenting author underlined)
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Abstract text (396 words)

Background: Traditional diagnosis is based on histology or clinical-stage classification which provides no information on tumor metabolism and inflammation, which, however, are both hallmarks of cancer and are directly associated with prognosis and severity. This project was an exploratory approach to profile metabolites, lipoproteins, and inflammation parameters (glycoprotein A and glycoprotein B) of borderline ovarian tumor (BOT) and high-grade serous ovarian cancer (HGSOC).

Methods: This project included 201 serum samples of which 50 were received from BOT and 151 from high-grade serous ovarian cancer (HGSOC), respectively. All the serum samples were validated and phenotyped by 1H-NMR-based metabolomics with in vitro diagnostics research (IVDr) standard operating procedures generating quantitative data on 38 metabolites, 112 lipoprotein parameters, and 5 inflammation markers. Uni- and multivariate statistics were applied to identify NMR-based alterations. Moreover, biomarker analysis was carried out with all NMR parameters and CA-125.

Results: Ketone bodies, glutamate, 2-hydroxybutyrate, glucose, glycerol, and phenylalanine levels were significantly higher in HGSOC, while the same tumors showed significantly lower levels of alanine and histidine. Furthermore, alanine and histidine and formic acid decreased and increased, respectively, over the clinical stages. Inflammatory markers glycoproteins A and B (GlycA and GlycB) increased significantly over the clinical stages and were higher in HGSOC, alongside significant changes in lipoproteins. Lipoprotein subfractions of VLDLs, IDLs, and LDLs increased significantly in HGSOC and over the clinical stages, while total plasma apolipoprotein A1 and A2 and a subfraction of HDLs decreased significantly over the clinical stages. In biomarker analysis, glycoprotein inflammation biomarkers behaved in the same way as the established clinical biomarker CA-125. Moreover, CA-125/GlycA, CA-125/GlycB, and CA-125/Glycs are potential biomarkers for diagnosis, prognosis, and treatment response of epithelial ovarian cancer (EOC). Last, the quantitative inflammatory parameters clearly displayed unique patterns of metabolites, lipoproteins, and CA-125 in BOT and HGSOC with clinical stages I–IV.

Conclusion: 1H-NMR-based metabolomics with commercial IVDr assays identified altered metabolites and lipoproteins relevant to EOC development and progression and showed that inflammation (based on glycoproteins) increased along with malignancy. As inflammation is a hallmark of cancer, glycoproteins, thereof, are promising future serum biomarkers for the diagnosis, prognosis, and treatment response of EOC. This was supported by the definition and stratification of three different

inflammatory serum classes which characterize specific alternations in metabolites, lipoproteins, and CA-125, implicating that future diagnosis could be refined not only by diagnosed histology and/or clinical stages but also by glycoprotein classes.

Bae, G., Berezhnoy, G., Koch, A., Cannet, C., Schäfer, H., Kommoss, S., Brucker, S., Beziere, N., & Trautwein, C. (2023). **Stratification of ovarian cancer borderline from high-grade serous carcinoma patients by quantitative serum NMR spectroscopy of metabolites, lipoproteins, and inflammatory markers.** Frontiers in molecular biosciences, 10, 1158330.

Title

Perturbations in the blood metabolome up to a decade before prostate cancer diagnosis in 4,387 matched case-control sets from the European Prospective Investigation into Cancer and Nutrition

Authors

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Abstract text

Measuring pre-diagnostic blood metabolites may help identify novel risk factors for prostate cancer. Using data from 4,387 matched case-control pairs from the European Prospective Investigation into Cancer and Nutrition (EPIC), we investigated the associations of individual metabolites and three previously defined metabolite patterns with prostate cancer risk. 148 metabolites were measured by mass spectrometry (AbsoluteIDQ p180 Kit, Biocrates Life Sciences AG). Multivariable-adjusted conditional logistic regression was used to estimate the odds ratio (OR) per standard deviation increase in metabolite concentration and pattern score (OR_{1SD}) for prostate cancer overall, and for aggressive (high grade, advanced stage, and death combined), advanced (stage greater than T_2 or with nodes or metastases), and high grade disease (Gleason 8+ or equivalent), and prostate cancer death. We corrected for multiple-testing using the Benjamini-Hochberg method. Six phosphatidylcholines (PCs) were inversely associated with advanced prostate cancer diagnosed within 10 years of recruitment. There were no significant associations between metabolites or metabolite patterns and overall, aggressive, or high grade prostate cancer. Metabolite patterns 1 (64 PCs and three hydroxysphingomyelins) and 2 (two acylcarnitines, glutamate, ornithine, and taurine) were significantly inversely associated with advanced prostate cancer; when stratified by follow-up time, these associations were observed for diagnoses within 10 years of recruitment (OR_{1SD} 0.80, 95% Confidence Interval (CI) 0.66-0.96 and OR_{1SD} 0.76, 95% CI 0.59-0.97, respectively) but not after longer follow-up (OR_{1SD} 0.95, 95% CI 0.82-1.10 and OR_{1SD} 0.85, 95% CI 0.67-1.06). Pattern 3 (8 lyso PCs) was significantly associated with prostate cancer death (OR_{1SD} 0.82, 95% CI 0.68-0.98). We identify several associations between metabolites and risk of advanced prostate cancer, which appeared most evident among men diagnosed within a shorter time since recruitment. Our results suggest that the plasma metabolite profile changes in response to prostate cancer up to a decade before detection of advanced stage disease.

Title
In-utero exposures to per- and polyfluoroalkyl substances and the human fetal liver metabolome: a cross-sectional study

Authors (presenting author underlined)

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Abstract text (word count: 315)

Background
Per- and polyfluoroalkyl substances (PFAS) are classed as Endocrine Disrupting Compounds (EDCs) but continue to be used in many products. This includes firefighting foams, flame retardants, utensil coatings and waterproofing of food packaging. PFAS exposure aberrantly modulates lipid, metabolite and bile acid (BA) levels, increasing susceptibility to onset and severity of metabolic diseases, such as diabetes and non-alcoholic fatty liver disease (NAFLD). To date, most studies in humans have focused on PFAS-exposure effects in adults. In this study we now demonstrate that PFAS are present in the human fetal liver and that they have metabolic consequences for the human fetus.

Methods
Human fetal livers from elective termination of pregnancies between 11-19 weeks of gestation (n = 78) were analysed by both targeted and untargeted metabolomic analyses of lipids, polar metabolites, BAs and PFAS, as well as with RNA sequencing. Stringent bioinformatic and statistical methods were applied to this data to investigate the association of PFAS exposure with hepatic metabolic pathways.

Findings
Metabolites associated with PFAS were identified in the fetal liver and these varied with gestational age. Conjugated BAs were markedly positively associated with fetal age. Several amino acids, fatty acids and sugar derivatives in fetal livers were inversely associated with PFAS exposure, while the BA glycolithocholic acid (GLCA) was markedly positively associated with all quantified PFAS. Furthermore, 7α-hydroxy-4-cholesten-3-one (C4), a marker of BA synthesis rate, was strongly positively associated with PFAS levels and was detectable as early as gestational week 12.

Interpretation

The data show direct evidence for in-utero effects of PFAS exposure on specific key hepatic products. Our results provide evidence that PFAS exposure, with potential future consequences, manifests in the human fetus as early as the first trimester of gestation. Furthermore, the profiles of metabolic changes resemble those observed in perinatal PFAS exposures. Such exposures are already linked with susceptibility, initiation, progression and/or exacerbation of a wide range of metabolic diseases.

POSTER PRESENTATIONS

P01

Title

Annotating Unknown PFAS Compounds in Biological Matrices Using Real-Time Library Search and MSn

Authors (presenting author underlined)

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Abstract text

PFAS are synthetic chemicals characterized by a carbon backbone with near or complete saturation with fluorine. Valued for their hydrophobic and oleophobic traits, PFAS have been used for decades in numerous industrial and commercial applications. These compounds resist degradation, are known to bioaccumulate, and have emerged as a prolific environmental contaminant. Characterizing these chemicals can be difficult due to the thousands of variations of the synthesized chemicals, isomeric structures, and the limited availability of reference standards. In our work we use the Real-Time Library Search on the Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer with the new PFAS method in Compound Discoverer to simplify detection and characterization of unknown PFAS.

Extracted plasma spiked with 30 PFAS compounds was analyzed with data dependent MS^3 methods using Real-Time Library Search. Cosine scores were generated in real time based on matching experimental MS^2 fragments to library spectra. Compounds with sufficient scores triggered additional scanning behavior during the run to aid in structure elucidation. In addition, an inclusion list was used to further focus $ddMS^2$ data collection on compounds with a negative mass defect, a common trait of PFAS compounds. Due to spectral similarities between PFAS, it was found that PFAS not in the spectral library were not only flagged as likely targets, but specific relevant scanning behavior could be selected in real time based on the class of PFAS.

Data was processed using the PFAS processing method in Compound Discoverer. Compounds were assigned a class score based on known PFAS fragments and graphed by their Kendrick mass defect as a function of m/z to help identify homologous series. The Real-Time Library Search filter allowed the instrument to focus analysis on likely PFAS compounds to produce a more concise data set in a single injection. This increased the number of compounds found in plasma extract from 18 to 25 of the 30 standards when compared to a similar untargeted data dependent analysis without Real-Time Library Search.

P02

Title

Spatial Characterization of Steroid Hormones in Breast Cancer Tissue by MALDI Mass Spectrometry Imaging

Authors

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Abstract text

Background

Estrogen and its signaling pathway are targets for pharmacological inhibition of hormone receptor-positive breast cancer. Molecular tumor heterogeneity of breast cancer is recognized as a major feature associated with resistance to treatments and as a determinant of prognosis. However, there are no established methods for high-resolution quantification of steroid hormones with spatial information in breast cancer tissue. Matrix-assisted laser desorption ionization (MALDI)-mass spectrometry imaging (MSI) enables mapping of the spatial distribution of several molecules in tissues allowing localization, detection, identification and quantitation of compounds in complex biological matrices. Here, we aimed to optimize a MALDI-MSI-protocol for analyzing steroid hormones and their sulfates in human breast cancer tissue.

Methods

Snap-frozen tumor tissue were obtained from hormone-receptor positive patient-derived breast cancer xenografts (MAS98.06), wherein animals received drinking water supplemented with 17β-estradiol (4 µg/mL) in order to promote tumor growth.[1] The sections of 10 µm thickness were mounted onto conductive slides, vacuum-packed and store at -80°C until the analysis.[2] For measuring steroid hormones, the sections were pre-treated with the derivatization agent, Girard's Reagent T (5 mg/mL in 80% methanol and 0.1% trifluoroacetic acid) containing the internal standard (d_4-cortisol, 10 µg/mL).[3] The sections were sprayed with α-cyano-4-hydroxycinnamic acid (10 mg/mL in 80% acetonitrile and 0.2% trifluoroacetic acid, 2.7 µg/mm^2 density) as the MALDI matrix for analyzing steroid hormones, while the 9-aminoacridine hydrochloride monohydrate matrix (10 mg/ml in 90% methanol, 2.5 µg/mm^2 density) was used for determining steroid sulfates.[3,4] All sections were imaged by use of the rapifleX

MALDI Tissuetyper™ mass spectrometer (Bruker Daltonics, Bremen, Germany). To further identify sulfates, on-tissue tandem mass spectrometry (MS/MS) analysis was conducted. Data were analyzed using the SCiLS lab software (Version 2021c Pro, GmbH, Bremen, Germany).

Results and Future Plans

By use of on-tissue derivatization, steroid hormones, including androstenedione, estrone, and progesterone, were localized in their derivatized form [mass to charge (m/z) 384.3, m/z 400.3, m/z 428.3, respectively] in patient-derived breast cancer xenografts. Cholesterol sulfate (m/z 465.3) was detected in these xenografts. We will further validate the protocol using breast cancer tissue obtained from patients with estrogen receptor-positive breast cancer, with the aim of investigating if heterogeneity in steroid hormones and sulfates is associated with patient outcome. The steroid hormones and their sulfates will be identified by comparing m/z values with public databases, and validated with MS/MS. The technical and spatial resolving ability will be evaluated by determination of coefficient of variation and fold changes.

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P03

Title
Absolute quantification of short-chain fatty acids, organic acids and amino acids in feces using liquid chromatography-mass spectrometry

Authors (presenting author underlined)
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Abstract text

The gut microbiome contributes to human physiology by producing beneficial metabolites. Among well-described microbial metabolites are short-chain fatty acids (SCFA) that have key roles in colonization resistance against pathogens, epithelial cell homeostasis, and immune system development. Low levels of SCFA are implicated in gut microbial dysbiosis linked to a risk of chronic infections and allergic diseases. SCFA thus provide insights into the health status of the host. However, limited knowledge of SCFA physiological concentrations prevents their utilization as diagnostic biomarkers.

We have adapted a targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for absolute quantification of 13 SCFA, together with associated Krebs cycle intermediates and 14 amino acids, using 3-nitrophenylhydrazine as a derivatization reagent. Quantifying absolute levels of metabolites in feces is challenging due to the complexity of the fecal matrix and inter-individual variation. To overcome these challenges, we have evaluated the matrix effect using germ-free animals, carefully monitored recovery and reproducibility for every step of the sample processing workflow, and normalized for variations in water content by dry weight measurement.

Finally, we used the method on meconium samples from 40 healthy vaginally born infants. We detected SCFA in the nanomolar range for the low abundant SCFA (including valerate and hexanoate) and concentrations between 0.7-41.6 μmol/g dry weight for acetate, the most abundant SCFA. This highly sensitive targeted approach will allow the description of SCFA and associated metabolites at absolute levels and their concentration range in various clinical settings.

P04

Title
Multi-metabolic signature of controlled modification of dietary carbohydrate quality.

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Abstract text

Unhealthy components of the habitual diet pose a constant challenge on central pathways of the human metabolism, which, in turn, increases the risk for cardiometabolic diseases (CMD). The acute metabolic impact of high glycemic index (GI) carbohydrates (CHO) is well documented in clinical trials and has plausible links to cardiometabolic health. In addition, prospective observational studies have shown that diets with high GI are associated with an increased risk of type 2 diabetes and coronary artery disease. Deriving multi-metabolite signatures from a controlled modification of dietary GI holds great potential in elucidating the metabolic adaptations to CHO quality in humans and understanding the long-term effects on CMD risk.

The aim of this study was to develop a multi-metabolite signature (MMS) that captures the long-term metabolic adaptation to CHO quality. The primary data source was the MedGICarb study, a 12-week randomized controlled dietary intervention trial with 135 participants in two parallel arms: low GI and high GI (1). Apart from the GI of CHO, all participants received a consistent Mediterranean background diet. We generated LC-MS based (RP ESI+/- and HILIC ESI+/-) untargeted metabolomics data from pre- and post-intervention plasma samples. We estimated the effect of the dietary intervention in linear regression models to preselect features that responded to the dietary intervention. Then, we used cross validated elastic net regression to train a multi metabolite model that predict the dietary intervention group (70% of participants) and evaluated the predictive performance in an internal validating sample (30% of participants).

After pre-processing, we assessed 9116 raw metabolomic features. A total of 481 FDR-significant metabolite features (FDR<0.1) were preselected. The elastic net regression procedure selected 15 metabolite features that were significantly associated with high vs low GI of dietary carbohydrates in the validation set (p-value=0.01). A weighted MMS based on these metabolite features showed a good performance in predicting the dietary intervention group (accuracy=0.725).

Our results indicate that a multi-metabolite signature captures the metabolic adaptation to dietary carbohydrate quality. Identification of these features and relating them to cardiometabolic endpoints in prospective cohort studies may elucidate the link between dietary carbohydrate quality and disease risk.

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P05

Title: Urinary phenotyping of acute SARS-CoV-2 infection connects clinical diagnostics with metabolomics and links immune activation to antiviral nucleosides and SIRT1

Authors (presenting author underlined)

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Abstract text (400 words)

Background. The stratification of patients suffering from SARS-CoV-2 infection or Long COVID is still a critical challenge. Besides the broad dysregulation of immune homeostasis, it is now clear that multiple biochemical pathways are profoundly modulated by the virus, a phenomenon referred to as phenoconversion. Consequently, quantitative metabolomics has been increasingly advocated as a useful tool to describe not only the consequences of virus-host interactions but also the influences exerted by demographic factors and comorbidities which affect patient recovery and treatment effectiveness. The aim of the present study was to characterize the urinary metabolic signature of acute infection and determine metabotypes associated with COVID-19 pathological hallmarks. In addition, we assessed the concentration of recently identified antiviral nucleosides and their association with other metabolites and clinical data. Finally, we investigated the potential connection between kynurenine dysregulation and the anti-inflammatory NAD+/sirtuins pathway.

Methods and Findings. Urinary metabolomic profiles of 243 infected individuals (AcuteCOV) were analyzed using a combination of quantitative NMR spectroscopy and LC-MS-based technologies. Results were compared to urinary data extracted from a historical cohort of noninfected subjects (CTR). Correlations between metabolite concentration and clinical variables or inflammatory mediators were likewise explored. SIRT1 serum concentrations were determined using ELISA. Urinary metabolomics captured clinical exacerbation and stratified patients into classes of disease severity, with a discrimination ability comparable to that of clinical biomarkers. Unique metabolite clusters were also generated based on age, sex, and BMI. Kynurenines showed the strongest fold change in clinically deteriorated patients and higher-risk subjects. Changes in the concentration of deoxydidehydronucelosides (ddhNs) were significantly associated with either other metabolites (neopterin, kynurenines, quinolinic acid, taurine) or medical variables (CPR, EASIX, LDH, leukocyte, iron). Increased kynurenines together with reduced trigonelline excretion pointed towards disrupted NAD^+ de novo/salvage pathways. Our pilot analysis supported a reduced activation of antiviral SIRT1.

Conclusions. Using a large cohort of acutely infected individuals, we linked metabolite alterations to clinical data in order to more precisely recognize disease severity and potentially predict clinical complications. Our results confirm the huge potential of urinary metabolomic phenotyping for noninvasive diagnostic/prognostic screening. The observed association between ddhNs and the main COVID-19-related metabolite and inflammatory perturbations makes such nucleosides promising biomarkers linking viral load, host immune response, and metabolism. Finally, by establishing, for the

first time, a casual link between kynurenine accumulation and deranged NAD^+/SIRT1, we offer a novel mechanism through which SARS-CoV-2 manipulates host physiology, which can be targeted by specific intervention strategies.

P06

Title:
The Implications of Mitochondrial DNA in Prostate Cancer Development

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Abstract text
Several studies have proposed that prostate cancer (PCa) tissue has an increased utilization of succinate to drive energy production as a compensatory mechanism. Our project aimed to combine succinate measurements with mitochondrial DNA (mtDNA) mutation data to study patient heterogeneity. These investigations will improve the characterizing of metabolic changes driving PCa formation and progression.

Whole organ prostate slices that were used for tissue sample collection were obtained from 42 patients with (N=31) and without (N=11) relapse (10-15 years follow-up). Several samples were collected from each patient and were histopathologically identified as cancer (CA, N=123), field effect (FE (close to cancer), N=40) and normal (NP -noncancerous, N=37) and used for metabolomics and DNA analysis. Metabolomics data was acquired by 1H HR-MAS MRS on fresh tissue samples and the extent of mtDNA mutation load were measured by RT-qPCR assay on the same samples. Nonparametric Man-Whitney U and Kruskal-Wallis tests were used to analyze the differences between the samples groups and Pearson correlation analysis was used to find the association between succinate levels and mtDNA mutation loads.

The CA samples had significantly more mtDNA mutations (P=0.001) and increased succinate levels (P=0.03) compared to NP and FE. No significant difference was detected when comparing CA to FE samples and between FE and NP. To investigate age-differences, we categorized patients: 60-73 years as old (n=27) and 50-59 years as young (n=15). For the old patients, CA samples differed significantly from NP tissue for both mtDNA (P=0.004) and succinate (P=0.00172), which was not for young patients (P=0.215 and P=0.37). Comparing CA tissue between old and young patients we discovered a significant difference for mtDNA mutations (P=0.03) and succinate (P=0.01) for old patients. We detected a moderate and borderline significant, correlation between succinate levels and mtDNA mutations (r=0.3,

P=0.066) for old patients, while there was no correlation for young patients (r=0.165, P=0.27). In conclusion, mtDNA mutation rate and succinate levels vary in heterogeneous prostate tissue. We detected a visible but not significant decrease from CA to FE, which suggests a potential trend for mutational change in FE areas. Older patients had increased mutation and succinate levels implying that tumors of older patients are more reliant on succinate dependent energy mechanism. It will be interesting to further explore the prostate specific metabolites citrate and polyamines to investigate the metabolic shift towards higher malignancy stages.

P07

Title: Targeted metabolomic assay for therapeutic drug monitoring in patients with adenine phosphoribosyltransferase deficiency

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Adenine phosphoribosyltransferase (APRT) deficiency is a rare purine metabolism disorder characterized by excessive urinary excretion of the poorly soluble 2,8-dihydroxyadenine (DHA), causing kidney stone formation and chronic kidney disease (CKD). Treatment with a xanthine oxidoreductase (XOR) inhibitor, allopurinol or febuxostat, reduces DHA excretion and slows CKD progression (1,2). However, some patients continue to form kidney stones and progress to end-stage kidney disease despite treatment. Currently, therapeutic drug monitoring (TDM) is performed by urine microscopy, which lacks specificity and is operator dependent. Therefore, a more sensitive and reliable method for both TDM and clinical diagnosis in patients with APRT deficiency is needed.
The objectives of this study were to optimize and validate a targeted metabolomic UPLC-MS/MS based assay for TDM in patients with APRT deficiency.
The chemometric approach design of experiments (DoE) was utilized for optimization of the UPLC-MS/MS based assay for simultaneous quantification of DHA, adenine, allopurinol, oxypurinol and febuxostat in human plasma. Fractional factorial design was used to reveal significant experimental factors influencing peak area, retention time and resolution of all analytes and significant factors were optimized using central composite face design. The assay was validated and used for analysis of plasma samples from healthy controls and patients with APRT deficiency before and after treatment with the XOR inhibitors.
The targeted metabolomic UPLC-MS/MS based assay was successfully optimized by DoE. A strong interaction effect between several factors was observed, indicating that these variables cannot be independently controlled to obtain optimal conditions. Intra- and interday accuracy and precision were within the ±15% acceptance criteria for all analytes. The median (range) plasma concentration was 248 (224-395) ng/mL for DHA and 194 (159-284) ng/mL for adenine in untreated patients, and < 100 ng/mL for DHA and 533 (339-1034) ng/mL for adenine in those on treatment. DHA was not detected in plasma from healthy controls. The median plasma concentration for allopurinol, oxypurinol and febuxostat in patients receiving therapy was 687 (103-2901), 7945 (2199-10943) and 1628 ng/mL, respectively.
The UPLC-MS/MS based assay yields accurate quantification of DHA, adenine, allopurinol, oxypurinol and febuxostat in human plasma. The assay has been implemented to monitor the efficacy of pharmacotherapy and treatment adherence among patients with APRT deficiency.

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P08

Title
Correlating human gut microbiota metabolites and composition in a longitudinal study

Authors (presenting author underlined)
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Abstract text
The gut microbiota consists mainly of prokaryotes, which form highly diverse communities within the human gut. The collective genome of the gut microbiota comprises hundreds of thousands of genes and has a much larger metabolic potential than the human genome alone. This ecosystem creates a large network of metabolic interactions that provide food digestion, essential nutrients, and pivotal molecular precursors for the human host.

Analysing fecal samples is a non-invasive way to study the gut microbiome composition and its metabolites. The aim of this study was to determine the stability of the gut microbiome composition and metabolites over one month. To accomplish this, we measured both the metabolites and the microbial composition of fecal sample that had been taken from 14 healthy individuals over the course of 1 month (4 time points). The metabolic composition of the samples was identified and quantified using NMR spectroscopy. 16S rRNA amplicon sequencing was used to determine the taxonomic composition of the microbial community. This provided a longitudinal multi-omic dataset, which we are using to elucidate how and to which degree changes at one level (i.e. composition) correlate with changes at another level of the microbiome activity (i.e. metabolites).

We observed that each individual displays specific enterotypes that remain constant over the course of one month, despite a diverse and free diet. Like a fingerprint, this microbial signature can be used to identify individuals. The NMR spectra of fecal samples from some individuals also appeared to be correlated to the gut microbiome composition, and remained constant, with group of metabolites retaining similar proportions between each other across different sampling time points. This correlation could allow the use of fecal metabolites as a distinct readout and signature of the gut ecosystem.

P09

Title

Untargeted metabolomics sample treatment strategies for renal tissue: a comparative study of solid phase microextraction (SPME) and homogenization-solid liquid extraction (Homo-SLE)

Authors (presenting author underlined)

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Abstract text

Renal tissue is known for its inherent heterogeneity, encompassing diverse regions with distinct functions and cellular metabolisms. In the context of metabolomics-based investigations of renal diseases, it is imperative to consider the natural complexity of kidney tissue samples. The selection of an appropriate sample treatment strategy plays a crucial role in the metabolomics workflow, determining the extent of compound coverage and the final analytical outcomes. Solid phase microextraction (SPME) is a sample processing methodology with numerous advantages and great potential for untargeted metabolomics of tissue samples. However, ascertaining whether SPME yields comparable metabolome and lipidome coverage and performance to classical methods remains critical. For these reasons, in this study, we have applied SPME and homogenization with solid-liquid extraction (Homo-SLE) methods to murine kidney tissue, followed by comprehensive metabolomics and lipidomics analysis. An additional focus was placed on investigating potential alterations in the renal lipidome and metabolome induced by tissue freezing and storage, as well as the sensitivity of the two sample treatment methodologies to such effects. Thus, analyses were performed on fresh and frozen kidney tissue. Lipidomics analysis revealed the exclusive presence of different structural membrane and intracellular lipids in the Homo-SLE group. Conversely, all annotated metabolites were detected in both groups. Despite some differences, SPME and Homo-SLE have achieved similar coverage in the lipidome and metabolome. Notably, the freezing of the sample mainly causes a decrease in the levels of most lipids species and an increase in metabolites such as amino acids, purines and pyrimidines, and some lipids as certain acylcarnitines, glycerophosphoethanolamines, glycerophosphoserines, ceramides, and triglycerides. These alterations are principally detected in a statistically significant way by SPME methodology, indicating its possible sensitivity to detect tissue damage in situ. Consequently, we underscored the critical nature of freezing and storing samples when working with SPME. Finally, with this work, we have shown that in SPME methodology, as long as the fundamentals of non-exhaustive extraction in a pre-equilibrium kinetic regime, extraction of free analytes in a localized area of the tissue, the chemistry of the fiber coating type, and non-disruption and homogenization of the tissue are taken into account, is an excellent option as a method to use in tissue metabolomics analysis; since this methodology presents an easy-to-use, efficient, and less invasive approach that simplifies the different sample processing steps in analyzing kidney tissue in untargeted metabolomics.

P10

Title
CE-MS-based strategy to assess the metabolic signature of testicular cancer in human seminal plasma

Authors
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Abstract text
Over the past 50 years, the rising incidence of testicular cancer has been accompanied by a worldwide decline in semen quality. Both phenomena appear to be related to industrialized societies and the associated changes in lifestyle and environmental exposure. Seminal fluid is a complex matrix containing sperm cells and fluid from the testes along with secretions from the seminal vesicles, prostate, bulbourethral, and periurethral glands. It can therefore provide biochemical information on the pathologies affecting these organs, while being a non-invasive and easy to obtain clinical sample. As testicular germ cell tumors (TGCTs) remain the most common malignancy in young adult males, the development of simple and accurate diagnostic approaches is a clinical priority to enable appropriate patient management. In the present study, we used capillary electrophoresis coupled with mass spectrometry detection to characterize the polar metabolomic profile of seminal plasma samples obtained from healthy donors and TGCT patients.

Seminal fluid samples were collected from 36 patients with a TGCT and 11 controls without a TGCT. Fifty microliter aliquots of cell-free plasma were subjected to protein precipitation and spiked with effective electrophoretic mobility (μ_{eff}) markers prior to filtration on 3 kDa cut-off centrifugal devices. To gain a broad perspective of the seminal fluid metabolome, an untargeted CE-MS approach comprising two analyses was chosen. Direct polarity separation with positive electrospray ionization was used to profile compounds with proton affinity, complemented by reverse polarity separation and negative ionization to detect molecules with a tendency to form anionic species. Time-of-flight (ToF) data were pre-processed to correct analytical drift, normalize sample content, and filter out unreliable signals before univariate and multivariate analysis. Compound identification was performed by matching accurate mass, in-source fragmentation pattern, and effective electrophoretic mobility value to those obtained from reference standards using the same experimental setup.

Classical supervised multivariate models (OPLS-DA) failed to reliably distinguish the metabolomic profiles of the control and cancer groups. Therefore, Monte Carlo Uninformative Variable Elimination (MCUVE) was used to remove least meaningful metabolites. The resulting models pointed towards differences between the metabolic profiles of the two groups, characterized by the levels of aspartic acid, glutamine, and other energy metabolism-related molecules. This suggests that seminal plasma composition could become a useful sample in the diagnosis of testicular cancer by revealing the metabolic rewiring that takes place in malignant germ cells.

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P11

Title

Automated sample preparation and analysis of steroid hormones, bile acids, perfluoroalkyls, oxylipins and non-steroidal anti-inflammatory drugs in human plasma using UHPLC-MS/MS

Authors

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Abstract text

The analysis of metabolites down to ultra-trace levels (ppt) in biological fluids presents challenges concerning high throughput and low detection limits. Targeted metabolomics approaches can mitigate some of these challenges by providing sensitive and quantitative analyses. This facilitates the exploration of disease-associated metabolic effects, evaluation of drug treatment impacts, and analysis of intervention outcomes. The aim of this study was to a) establish an automated method enabling the simultaneous sample preparation and instrumental analysis of steroid hormones, bile acids, perfluoroalkyls, oxylipins and non-steroidal anti-inflammatory drugs from human plasma and b) to assess potential differences in analyte response between positive and negative electrospray ionization (ESI) with UniSpray ionization. With the assistance of a robotic liquid handler, an automated 96-well plate SPE-based method was developed that enabled sample preparation, extraction, and transfer to LC-vials. The method also allows for dilution of the elution solution with water prior to UHPLC-MS/MS analysis, avoiding an evaporation and reconstitution step. In brief, two analytical set-ups were used. In the first, 10ul of the final sample extracts were injected onto an Acquity Premier UPLC BEH C18 (2.1mm x 100mm) analytical column and enabled the analysis of steroid hormones, perfluoroalkyls, and bile acid. In the second set-up, 10ul of the final extract were injected onto an Acquity Premier UPLC BEH (2.1mm x 150mm) for analysis of oxylipins and related metabolites. The recoveries of 54 isotope labeled internal standards ranged between 54-81% depending on the analyte. The method was reproducible, with most compounds above the limit-of-detection showing inter- and intra-batch variability <25% RSD. We found that the use of UniSpray improved response from 5-19% when compared to electrospray, depending on the analyte. The developed automated method is suitable for exploring a diverse range of analytes in human plasma making it valuable for large cohort studies.

P12

Title

Unraveling Lipidomics Complexity: Overcoming False-Positives after Software Assisted Annotation for Building a comprehensive in-house human plasma MS library for Accurate Lipid Annotation

Authors

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Abstract text

The primary objective of high-throughput lipidomic studies applied to biological samples is to achieve exhaustive and comprehensive coverage of the lipidome. Its advancement, in addition to being supported by analytical techniques is highly dependent on the implementation of specialized computational approaches to facilitate the processing of the large raw datasets generated. These computational tools are crucial for lipid researchers as they enable fast and accurate algorithms to manage deconvolution for peak detection and lipid species identification. Unfortunately, an incorrect handling of either of these two processes as well as not realizing the potential risk of generating unacceptable rates of false-positives can inevitably lead to biological misinterpretation of the data.

This study aims to systematize the most common cases of miss-annotations provided to reduce false-positive annotations in the software-assisted process using UHPLC-ESI(±)-QTOF-MS analysis in addition to the development of an in-house human plasma lipid library, with high confidence annotation level, for its application in high-throughput RP-LC-MS-based lipidomic analysis of plasma samples.

Lipidome data from NIST Standard Reference Material for Human Plasma (SRM 1950) obtained using iterative MS/MS data acquisition - sampled by automated data-dependent acquisition (DDA) experiments - were analyzed using three independent software (Lipid Annotator®, MS-DIAL-4 and LipidHunter). The combined annotations resulted from the software were examined. Although software solutions facilitate the first step in lipid annotation, an appropriate MS1 and MS/MS data handling was performed simultaneously.

After manual inspection of MS1 and MS/MS spectra of the identifications given by software annotation tools several false-positives were observed. A deep examination of RT, *m/z*, adduct profile, isotopic pattern distribution and MS/MS spectral overlap was performed for the systematization of all types of interferences detected to allow and simplify false-positives identification. After the resolution of the miss-annotations and the inspection of MS1 spectra to complete acyl- or alkyl- series for each lipid subclass, an in-house plasma lipidomic MS library was created containing 586 lipid species with high level of confidence on lipid annotation.

As a conclusion, a deep-knowledge on how to solve these possible interferences is decisive prior to the use of software-assisted annotation strategies to ensure proper lipid annotation and a posterior accurate biological interpretation. In addition, the use of in-house lipidomic MS libraries for clinical studies not only leads to high levels of confidence on lipid annotations but also to good-quality signals for the posterior statistical analysis allowing a better understanding of the alterations suffered in a specific pathology.

P13

Title

Chemoselective Metabolomics – New Chemical Biology Tools to Explore Microbiome and Diet Metabolism

Authors

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Abstract text

Metabolites produced by the gut microbiome play a crucial and diverse role on host physiology, which are detectable in a wide range of biological samples including feces, plasma, urine, and brain. Microbiota dysbiosis has been associated with the development of diseases, however, the metabolic link has yet to be detected. The detailed and targeted analysis of these metabolites is important for the discovery of biomarkers and unknown bioactive molecules. Mass spectrometric metabolomics is the method of choice for identification and quantification of these metabolites. Advanced methods at the interface of chemistry and biology coupled with metabolomics analysis are required but still limited. We have therefore developed a unique and multifunctional chemoselective probe with synthetic $^{13}C/^{12}C$ isotopically labelled analogues that allows for comparative and quantitative analysis of metabolites in human samples at low concentrations (1). We have termed this method quantitative **Quant**itative **S**ensitive **CHE**moselective **M**et**A**bolomics (*quant*-SCHEMA). Coupled to magnetic beads, this method allows the straightforward chemoselective extraction of metabolites from human samples (1-3). This isolation procedure of specific metabolite classes from sample matrices led to a substantial increase in mass spectrometric sensitivity by up to sixth orders of magnitude and facilitates the detection of metabolites at femtomolar quantities.

We have recently applied these chemoselective probes for analysis of dietary carbonyl-containing metabolites in human urine samples and SCFAs microbiome co-cultures with pathogens. For the first time, we have applied this methodology for large-scale analysis in a dietary intervention study with 156 samples for nutritional biomarker discovery (2). We have successfully found four potential dietary carbonyl biomarkers, which have not been reported before. Additionally, we have developed a chemoselective probe methodology for the absolute quantification of short-chain fatty acids (3). The targeted SCFA analysis was combined with global metabolomics on gut microbiome co-cultures with *Salmonella* and treated with antibiotics. The successes of these methodologies encourage the general use of this method in metabolomics studies.

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P14

Title
CE-MS-based strategy to assess the metabolic signature of testicular cancer in human seminal plasma

Authors
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Abstract text
Over the past 50 years, the rising incidence of testicular cancer has been accompanied by a worldwide decline in semen quality. Both phenomena appear to be related to industrialized societies and the associated changes in lifestyle and environmental exposure. Seminal fluid is a complex matrix containing sperm cells and fluid from the testes along with secretions from the seminal vesicles, prostate, bulbourethral, and periurethral glands. It can therefore provide biochemical information on the pathologies affecting these organs, while being a non-invasive and easy to obtain clinical sample. As testicular germ cell tumors (TGCTs) remain the most common malignancy in young adult males, the development of simple and accurate diagnostic approaches is a clinical priority to enable appropriate patient management. In the present study, we used capillary electrophoresis coupled with mass spectrometry detection to characterize the polar metabolomic profile of seminal plasma samples obtained from healthy donors and TGCT patients.

Seminal fluid samples were collected from 36 patients with a TGCT and 11 controls without a TGCT. Fifty microliter aliquots of cell-free plasma were subjected to protein precipitation and spiked with effective electrophoretic mobility (μ_{eff}) markers prior to filtration on 3 kDa cut-off centrifugal devices. To gain a broad perspective of the seminal fluid metabolome, an untargeted CE-MS approach comprising two analyses was chosen. Direct polarity separation with positive electrospray ionization was used to profile compounds with proton affinity, complemented by reverse polarity separation and negative ionization to detect molecules with a tendency to form anionic species. Time-of-flight (ToF) data were pre-processed to correct analytical drift, normalize sample content, and filter out unreliable signals before univariate and multivariate analysis. Compound identification was performed by matching accurate mass, in-source fragmentation pattern, and effective electrophoretic mobility value to those obtained from reference standards using the same experimental setup.

Classical supervised multivariate models (OPLS-DA) failed to reliably distinguish the metabolomic profiles of the control and cancer groups. Therefore, Monte Carlo Uninformative Variable Elimination (MCUVE) was used to remove least meaningful metabolites. The resulting models pointed towards differences between the metabolic profiles of the two groups, characterized by the levels of aspartic acid, glutamine, and other energy metabolism-related molecules. This suggests that seminal plasma composition could become a useful sample in the diagnosis of testicular cancer by revealing the metabolic rewiring that takes place in malignant germ cells.

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P15

Title
Global metabolomics and lipidomics in a university hospital setting

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Global metabolomics and lipidomics has gained widespread use in medical research. Its usefulness in biomarker discovery and in unravelling the biochemical consequences of pathological processes is undoubted. At Oslo University Hospital we have during the last decade, based on standardized clinical chemistry principles for medical diagnostics, established robust LC-MS-based platforms for both global metabolomics and global lipidomics and established protocols for analysis of all types of biofluids. Based on this, we were recently approved as Core Facility for Global Metabolomics and Lipidomics at University of Oslo.
Taking research tools into everyday diagnostics is a long journey. It requires identification of the challenges, and putting efforts in overcoming them. Important factors are control of pre-analytical factors, sample logistics, competence in performing the global analysis, competence in quality assurance and analytical interpretation, reporting of results and competence in medical interpretation. We will present and share some of our experiences and thoughts on the long and work-demanding process of building global metabolomics and lipidomics in both clinical research and diagnostics.

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Title
Decontamination of Aflatoxin B1 by Lactic Acid Bacteria

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Abstract text

Climate change is one the most urgent global challenges. The altering climate patterns have raised concerns about the potential escalation of mycotoxin contamination in agricultural products. Mycotoxins are toxic secondary metabolites produced by molds, and among all known mycotoxins, aflatoxin B1 (AFB1) is the most toxic, and is considered carcinogenic to humans (1). Lactic acid bacteria (LAB) have gained increasing attention for their potential in mycotoxin decontamination. Several studies have demonstrated that LAB can reduce AFB1 content through binding or by enzymatically transforming its structure (biotransformation) (2). Prior research has predominantly focused on LAB's ability to reduce AFB1, with limited exploration of the biotransformation products.

The aim of this study was to elucidate the AFB1 reduction mechanism of LAB and evaluate the possible biotransformation products. Initially, 31 different LAB strains were screened for their AFB1 reduction capabilities using a simple binding assay. The study utilized ultra-performance liquid chromatography with fluorescence detection (UPLC-FL), which demonstrated that the binding process led to a significant reduction of up to 65.7% in AFB1. When AFB1 was added to the LAB growth media and incubated for 48 hours, a reduction range of 0.3-18.0% was observed. The further evaluate the reduction mechanism *Lactobacillus helveticus* FAM22155 was selected for further investigations of potential biotransformation products.

UPLC-FL showed the formation of two new peaks eluting after 2.0- and 3.3-min. These peaks were considered as possible biotransformation products. As the amount of the latter compound was very low, it could not be further analyzed. The peak at 2.0 min had a mass-to-charge ratio of 207. Two possible structures for the first compound were proposed, but due to low intensities, further studies to confirm the structure are needed. Ultimately, the main AFB1 reduction mechanism was attributed to binding. This may also explain the low intensities of the potential biotransformation products as the bound toxin may not be available for the enzymatic degradation. Furthermore, bacterial biotransformation products may also be bound which limits their availability for structure analysis.

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P17

Title
Comparison of serum metabolome profiles of four types of solid cancers by MS and NMR approaches

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Abstract text
Metabolic analysis of cancer-specific profiles is a powerful method for understanding complex molecular changes characteristic to the promotion and progression of different types of tumors. In our study, we implemented two analytical approaches: high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) spectroscopy to examine the metabolomic features of four types of solid cancer. We analyzed metabolites presented in sera collected from women patients with breast (n = 35), head and neck (n = 32), lung (n = 35), and colorectal (n = 30) cancers to reveal patterns characteristic for each group of patients.
Metabolic signatures of all four malignancies were compared based on both types of analytical approaches. The metabolic profile of colon cancer patients was the most distinct while the profiles of lung cancer and head and neck cancer were the most similar to each other. Colon cancer was characterized by the lowest levels of lipids (lysophosphatidylcholines, cholesteryl esters, and triglycerides in particular), lipoproteins, and amino acids. On the other hand, breast cancer patients were characterized by relatively high concentrations of lipids (cholesteryl esters and sphingomyelins in particular) and low concentrations of glycans. Noteworthy, only a minor correlation between cancer stages and metabolic patterns was observed, which indicated that cancer-type-specific features might be more important than cancer-stage-specific features when metabolic patterns are observed at the systemic level in patients' serum. We concluded that the analysis of serum metabolome provides new information about molecular differences between different types of solid cancers.

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Title
Spatial multi-omics to uncover prostate cancer heterogeneity

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Metabolites, genes and proteins play key roles within cells and organs, but lack of technology has restricted the spatial analysis within tissues and research has mainly used bulk measurements that lack information on the phenotype of given cell types, and biomarkers may therefore be masked by the average output. Novel imaging-based techniques, such as MALDI MSI, spatial transcriptomics, and laser micro-dissected (LMD) proteomics are emerging, and they allow the spatial analysis of analytes within a single tissue sample. The aim of the ProstOmics project, was to establish a high-quality tissue multi-omics protocol to uncover biomarkers and molecular mechanisms underlying the heterogeneity of aggressive prostate cancer (PCa). We performed new and emerging techniques of spatial multi-omics on the same tissue sample from several samples from each patient.

We searched through a local biobank (N~1000) of patients undergoing radical prostatectomy donating 2-mm whole-organ slices. The two adjacent paraffin-embedded HES-stained sections were fused with the tissue slice and 4-6 targeted samples were selected from 114 patients with consent. Samples (n~500, diameter=3µm) were drilled frozen using live pathology-imaging targeting several cell-types (cancer, close to the cancer and far away from the cancer). From each sample, 10µm sections were cryo-sectioned and laid on specific glass for various omics analyses including MALDI MSI, spatial transcriptomics and LMD proteomics. The rest of the sample were used for various conventional bulk omics analyses (RNA sequencing, MR spectroscopy, DNA methylation, various staining).

A novel and fresh frozen tissue extraction method of prostatectomy tissue was developed to target pathology-defined tissue types such as stroma, cancer epithelium and normal epithelium. Data from spatial gene expression, spatial metabolomics, spatial proteomics, histopathology, and various bulk data of DNA-methylation, RNA sequencing, copy-number and metabolomics were produced for high-quality analyses. Multi-omics data from each sample and from several samples from each patient were produced for global and targeted analysis to assess the heterogeneity of aggressive PCa. We have established an algorithm GreedyFHist that spatially integrate all the omics-data to one data-matrix to study molecular mechanisms within their microenvironment in samples from patients with 15 years of follow-up.

A novel spatial multi-omics protocol was designed for global and targeted analyses of fresh frozen PCa tissue. The integrated data-matrices from patients with both recurrent and non-recurrent cancer after 15 years have already shown interesting results and will further have the potential to advance the understanding of aggressive and indolent biology from cell types within their microenvironment.

P19

Title
Metabolomic study of metabolites in winter damaged soils that can impact plant growth

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Abstract text

In the Nordic countries, an ice cover on golf greens and agricultural grass fields in combination with sudden sunlight heat in the spring often leads to soils depleted of oxygen under the ice layer and subsequent death of the grass and large damages to the fields. The phenomenon is termed *'isbrann'* in Norwegian and it is the most common type of winter damage. It is well known that butyric acid and acetic acid accumulate in the soil under the ice layer during such conditions and that they are highly toxic for the grass plants. But we know much less about other types of metabolites formed in these anoxic stress processes, and their impact on grass plants.

Soil water was centrifuged out of tube core samples from grass plots of creeping bentgrass (*Agrostis stolonifera*) or red fescue (*Festuca rubra*) with and without previous ice-encasement at Kapp, Norway. Soil water samples raw files were acquired in Full MS-ddMS2 mode at *m/z* 60-450 on a LC-Q-Orbitrap-HRMS, followed by tentative identification of 60 metabolites using the software Thermo Fisher Scientific Compound Discoverer™. Butyric acid and acetic acid were determined using HPLC-RI. The metabolites were finally identified by matches to reference standards. The poster presents an overview of the compounds detected, their relative concentrations, and displays the compounds upregulated/downregulated because of soil ice-encasement. The results give us a better understanding of how the grass plants reacts to anoxic stress and the interplay between soil microorganisms and plant roots in an anoxic environment.

P20

Title

In silico expansion of the phospholipidome compositional profile and polar metabolome characterization in *Haemophilus influenzae* Rd KW20 using multiplatform metabolomics and probabilistic modelling.

Authors

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Abstract text

Haemophilus influenzae is a gram-negative bacterium of relevant clinical interest. *H. influenzae* Rd KW20 was the first organism whose genome was fully sequenced, allowing for the generation of the first genome-scale metabolic model (GEM) (1). While current *H. influenzae* GEMs (2, 3) capture an accurate image of the *H. influenzae* global metabolic network and its topology, both the metabolome and lipidome of *H. influenzae* have been poorly characterized by direct instrumental techniques. In this study, we have performed a multiplatform metabolomics approach with broad metabolite coverage, combining gas chromatography, capillary electrophoresis and liquid chromatography, all coupled to high resolution MS. We generated an extensively curated characterization of the metabolome of *H. influenzae* Rd KW20 (4), obtaining direct evidence of 15-20% of the small molecule datasets present in current GEMs (2, 3) and high-quality evidence of 17 metabolites not previously contemplated in such GEMs, including the antimicrobial metabolite cyclo(Leu-Pro). Additionally, we comprehensively characterized and evaluated the quantitative composition of the phospholipidome of *H. influenzae*, allowing us (i) to expand the repertoire of fatty acyl chains known to be bound to *H. influenzae* glycerophospholipids (GPs) (ii) to determine the previously unreported presence of lysophosphatidylethanolamines (LPEs), alongside with phosphatidylethanolamines (PEs) and phosphatidylglycerols (PGs) (iii) to determine that the diacyl-GP acyl chain compositional profile is largely independent of the polar head, (iv) to estimate the PE sum composition abundance profile from the LPE abundance profile and (v) to determine that the probability distribution of phospholipids is mostly related to the conditional probability distribution of individual fatty acyl chains. These findings enabled us to provide a rationale for the observed phospholipid profiles and to estimate the abundance of low-level species via probabilistic modelling, permitting the prediction of 135 diacyl-GP chain isomers, in addition to the 61 PE and 29 PG chain isomers determined experimentally.

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P21

Title
Metabolomics-based drug screening – a pilot study

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Abstract text
Introduction: Whole blood drug screening in forensic toxicology is often performed by LC-HRMS targeting many drugs and related metabolites using either in-house libraries or libraries supplied by the instrument vendors. Several inherent challenges like constant appearance of new psychoactive substances on the illegal market and a need for detecting ingestion of potent drugs after a long time reveal an unmet need for true untargeted drug screening. This pilot study aims to perform untargeted drug screening using a metabolomics approach and simple univariate and multivariate statistics in the search of psychoactive drugs.
Materials and methods: A designed dataset consisting of 50 positive driving under the influence of drugs (DUID) samples (five individual cases, 10 sample replicates/case), 100 "blank" DUID samples (100 individual cases, one sample/case), and 12 clean samples donated by healthy volunteers (12 individual cases, one sample/case) were used in this pilot study and analyzed with LC-HRMS in two consecutive batches. Raw data was preprocessed using XCMS, normalized, and scaled prior to further data processing. Metabolites and drugs were annotated using an in-house library and other external libraries (HMDB, massbank, and many more). All known endogenous metabolites were removed from further analysis. Significant features were discovered through a comparative analysis involving positive DUID samples against both "blank" DUID samples and the clean samples obtained from healthy volunteers.
Results: The comparative analysis, together with the utilization of a Pearson Correlation network, elucidated that the "blank" DUID samples possess a satisfactory level of purity, rendering them suitable for background subtraction during the pursuit of novel drugs in each positive DUID case. By using "blank" DUID samples for background subtraction, the number of significant metabolites were reduced by ~20% - ~60% in the five positive DUID cases compared to using clean blood samples for background subtraction. Utilizing this metabolomics approach, the annotated drugs discovered are in consistency with the results from the standard toxicological analysis and interestingly, several significant unknown features displayed a high fold-change. These features could be direct drug-metabolites not included in the standard toxicological analysis, or new psychoactive substances.
Conclusion: The preliminary results of the pilot study suggest that the metabolomics-based approach holds a potential for performing untargeted drug screening. Additionally, it suggests that a retrospective dataset based on "blank" DUID samples can be used for comparison thereby reducing the number of injections in a routine setting, which needs to be studied further.

P22

Authors
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Abstract text

Breast cancer (BC) is a leading cause of cancer-related death worldwide. BC's diverse nature and heterogeneous biology impose challenges in prognosticating survival as patients with similar diagnoses often respond differently to treatment. Previous studies have employed alterations in transcriptomes, proteomes, or metabolomes for BC characterization. However, relying on a single omics approach does not sufficiently capture the heterogeneous nature of BC. Clinically relevant BC intrinsic subtypes have previously been established and are implemented in the clinic through gene expression profiling. The gene-expression based intrinsic subtypes however deviate from their anticipated clinical trends for long-term survival outcomes. In this study we thus aimed to categorize BC patients into clinically relevant subgroups by integrating transcriptomic, proteomic and metabolomic profiles. We included 335 patients from the Oslo2 study and used unsupervised Multi-Omics Factor Analysis (MOFA+) to integrate omics data from tumor tissues of untreated BC patients. Our analysis revealed three prominent multi-omics subgroups of BC patients with different prognosis ($p = 0.009$), providing novel information beyond the well-established intrinsic subtypes. Our subgroups were well validated in two independent large cohorts, METABRIC and TCGA. Furthermore, by employing a systems-biology approach and integrated pathway analysis, we observed high enrichment of cell-cycle and immune-related pathways between the subgroups with most and least favorable prognosis, offering comprehensive molecular insight into BC heterogeneity.

P23

Title

Lipid removal during sample pretreatment - effective ways to reduce matrix effects during HILIC LC-MS analysis of nucleotides and their derivatives

Authors (presenting author underlined)

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Abstract text

Sample preparation is one of the most important steps in LC-MS analysis. The main benefit is the pre-concentration of analytes and the removal of the complex matrix that causes an undesirable matrix effect in both qualitative and quantitative analysis.

In HILIC LC-MS analysis of nucleotides and their derivatives, the most common sample pretreatment is the precipitation of proteins with organic solvents such as acetonitrile or methanol. A disadvantage of this procedure is primarily the co-precipitation of analytes, losses due to poor solubility of analytes in extraction solvents, and the inability to remove lipids, which also contribute significantly to matrix effects. Lipids - and phospholipids in particular - are present in significant concentrations in all biological samples such as plasma, urine, or tissue extracts and are therefore causing a problem in HILIC mode analyses. They suppress the ionization of the analytes, can coelute with the analytes and thus negatively affect chromatographic separation. In addition, they are often accumulated on the chromatography column and in the ion source of the mass spectrometer. For the separation of lipids in nucleotide analysis, solid-phase extraction (SPE) and liquid-liquid extraction (LLE) appear to be promising sample preparation procedures. With thorough optimization, SPE and LLE are methods capable of removing specific lipids from samples that interfere with targeted nucleotide analytes during LC-MS analysis (1,2).

The aim of the study was to optimize and compare solid-phase and liquid-liquid extraction sample pretreatment procedures for the removal of the lipid components in complex samples designated to HILIC LC-MS analysis. The developed sample preparation methods were compared to a standard protein precipitation procedure. Results were evaluated in terms of extraction efficiency, recovery, and chromatographic separation by using fast HILIC analyses on 5 cm column. The results confirmed, that the extraction procedures are able to remove some lipid classes from samples and improve the analysis of specific nucleotides.

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P24

Title
Mass spectral fingerprinting metabolomics – flexible, high throughput metabolomics for sample screening

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Abstract text

Metabolomics has proven its value across many fields of the biological sciences over the past two decades. Yet sample throughput remains a barrier to adoption of metabolomics in areas outside of research science. Instruments that can measure a metabolite spectrum without prior sample preparation in <10 seconds may be able to fill this gap. In our laboratory we have used rapid evaporative ionisation mass spectrometry (REIMS) and direct analysis in real time-mass spectrometry (DART-MS) to demonstrate the potential of mass spectral fingerprinting as a tool for rapid metabolomics. Application examples include detecting pre-slaughter stress and pasture feed in lamb meat, identification of insect and fungal species, plant infection, Covid-19 infection in saliva samples, and detection of honey origin. To increase the range of samples and speed of throughput, we have adapted a laser to interface with the REIMS, reducing variation and operator fatigue compared to the standard electronic surgical knife and enabling analysis of liquid as well as solid samples. As a proof of concept for 'mobile metabolomics', we have mounted our DART-MS system on a custom-made trolley, enabling easy transport and use away from its home laboratory. These examples demonstrate the flexibility of mass spectral fingerprinting and its promise for translational metabolomics, though there are still several operational aspects to address before mass spectral fingerprinting can be used for routine analysis. Mass spectral fingerprinting has the potential to be a useful complement to other established metabolomics methodologies, especially in cases where screening of many samples in a short space of time is required.

P25

Title

Metabolic profiles reflect weight loss maintenance and the composition of diet after very-low-energy diet

Authors

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Abstract text

Plasma metabolite profiles are known to be affected by both diet and weight loss. Yet, the scientific knowledge on how longer-term weight loss maintenance and different weight loss maintenance diets influence the circulating metabolome remains limited. We utilised non-targeted liquid chromatography and mass spectrometry-based metabolomics to analyse plasma metabolic signatures of two isocaloric 24-week weight maintenance diets with differing satiety values in 79 participants (58 women, 21 men; age 49.7 ± 9.0 years; BMI 34.2 ± 2.5 kg/m^2) who had first undergone a 7-week weight-loss period on a very-low-energy diet. Higher satiety food (HSF) group consumed high-fibre, high-protein, and low-fat products, whereas lower satiety food (LSF) group consumed low-fibre products with average protein and fat content as a part of their weight maintenance diets. We also investigated metabolite features that associated with successful weight loss maintenance: features that discriminated participants who maintained ≥10% weight loss (HWM) and participants who maintained <10% weight loss (LWM) at the end of the study were annotated. The associations between metabolite features and anthropometric and food group variables were further assessed.

Altogether, we annotated 126 metabolites that discriminated the HSF and LSF groups and HWM and LWM groups ($p < 0.05$). Compared to LSF group, HSF group had lower levels of several amino acids, short-, medium- and long-chain acylcarnitines (CARs), odd- and even-chain lysoglycerophospholipids, and higher levels of fatty amides. Irrespective of the weight maintenance diet, HWM group showed higher levels of glycerophospholipids with a saturated long-chain and a C20:4 fatty acid tail, and unsaturated free fatty acids (FFAs) in comparison to LWM group. Changes in various saturated odd- and even-chain LPCs and LPEs and fatty amides showed a linkage with the intake of several food groups, with grain and dairy products especially highlighted. Increase in many (lyso)glycerophospholipids was associated with decrease in body weight and adiposity while increase in short- and medium-chain CARs was associated with decrease in body fat-free mass. Our results suggest that amino acid and lipid metabolism are affected by weight maintenance diets differing in dietary fibre, protein, and fat content and that greater weight loss maintenance is linked with increased levels of several phospholipid species and FFAs. Further, in the context of weight reduction and weight management, both common or distinct metabolites for weight-related variables and dietary factors exist.

P26

Title

Rapid and efficient LC-MS/MS diagnosis of inherited metabolic disorders: a semi-automated workflow for analysis of organic acids, acylglycines, and acylcarnitines in urine

Authors

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Abstract text

Urinary organic acid (OA) analysis is an important part of the diagnosis of inherited metabolic disorders (IMDs), treatment monitoring and detecting possible metabolic crisis. Clinical manifestations of IMD are usually non-specific, thus laboratory analysis of specific biomarkers is crucial for the differential diagnosis. Routinely, OA analysis is performed by GC-MS, which has certain advantages, however, it also has certain shortcomings such as the time-consuming sample preparation and data evaluation and a low sensitivity for OA conjugates. Therefore, an LC-MS/MS method has been developed covering a total of 146 metabolites from a range of urinary organic acids, acylglycines and acylcarnitines. Sample preparation only includes urine dilution to same creatinine concentration and addition of internal standards. Analytes are separated in 26 minutes on the Acquity UPLC HSS T3 C18 (1.8 µm, 100 x 2.1 mm) Waters column using Exion LC (Sciex) and analysed by QTRAP 6500+ (Sciex) in scheduled MRM mode. Raw data processing in is quick and easy as well as the actual data evaluation and diagnostics. A robust standardised value calculation as a data transformation was applied for easy evaluation of complex data. Multiple variables plots in GraphPad Prism software were introduced for simple and automatic data visualisation. The workflow also includes plotting the IMD metabolic network created in Cytoscape software, which can be used to monitor changes in biomarker levels in individual patients and to evaluate dysregulations in their metabolism. The method has been analytically and clinically validated, more than 800 clinical urine samples have already been analysed and 34 IMDs have been correctly diagnosed by this platform. The combination of three groups of biomarkers and the fact that all isomers can be separated allows to diagnose more than 80 IMDs. This work was published in May this year in Q1 journal[1].

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P27

Title
Quantification of steroids in stool samples using LC-MS

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Abstract text

Steroids are lipophilic signaling molecules that regulate metabolism, growth and reproductive functions of the body. Quantitation of steroids in different matrices is necessary in order to study the role of steroid hormones in health and disease. Steroids are present in low concentrations in most biological matrices, for example 100 pmol/L-10nmol/L in blood for majority of steroids with several abundant exceptions [1]. As far as we are aware steroids profiling was researched thoroughly in human blood and human urine, but for human stool samples there is very little information. Additionally, the ionization efficiencies of steroids are different and structure dependent. In general, the ionization efficiencies are low [2], meaning that sensitive instruments are required in order to avoid having to perform derivatization. For extraction of steroids various Liquid-liquid extraction (LLE) techniques are widely used, since these provide cleaner extracts and better recoveries than for example protein precipitation.

An LC-MS method was developed to quantify steroids in stool samples collected using OMNImet®-GUT (ME-200) - kits for collection of fecal samples for metabolome studies. LLE was used to extract steroids from homogenized stool samples. Normalization of the results was performed by dry weight of the samples. However due to high variability of dry content there will be new ways of normalization tested.

Out of 21 steroids analyzed, 9 were detected: Cortisone (E), 11-ketotestosterone (11-KT), 11-Keto-Dihydrotestosterone (11-KDHT), Testosterone / Epi-testosterone (T/Epi-T), Dihydrotestosterone (DHT), Androstenedione (A4), Progesterone (P4), Estradiol (E2) and Estrone (E1). The highest concentrations were measured for DHT (4000 ± 4000 pM) and the lowest for 11-KT (85 ± 80 pM). Concluding there has been established a quantitative LC-MS assay, which could be used for steroid profiling in human stool samples

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P28

Title
Metabolomics assessment of colistin induced toxicity

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Abstract text

Colistin is the last resort antibiotic, used for the curation of infections caused by multidrug-resistant bacteria. The drug is effective even against Gram-negative bacteria by disrupting their bacterial cell membrane. Despite its beneficial action, the use of the drug is compromised by the high percentage of toxicity incidents that causes, particularly in kidney and neuronal cells. Colistin is administered in humans usually in the form of colistimethate sodium (CMS), and the recommended doses are 2.5–5 mg/kg/day. The toxicity onset is correlated with the higher doses or the long-time administration. The action of CMS induced toxicity is not fully understood, and there is limited literature on CMS nephrotoxicity. The existing *in vivo* toxicity studies use doses > 10-fold higher than those administered to humans. Despite that there are evidence indicating CMS-induced liver deregulation, there are no metabolomics studies focusing on this aspect. However, it is intriguing to investigate the impact and the metabolic response for low doses of CMS, where there is no clinical evidence of toxicity. Furthermore, as the liver plays significant role in drug metabolism, the impact of CMS to this organ should be investigated as well.

This study stimulated *in vivo* the effects of the human doses in order to investigate the metabolic alterations in blood, kidney, and liver. Two doses of CMS, Low (1 mg/kg), and High (1.5 mg/kg) versus a Control (Normal saline), were administered to mice. RP-HRMS-based protocol was developed for the metabolomics analysis. Multivariate and univariate methods were combined to detect potential trends and to identify statistically important biomarkers. The PLS-DA methods separated the three groups in all datasets. The PLS-R analysis showed that the metabolic alterations were linearly correlated to the dose.

Phenylacetic acid levels were decreased in kidney by the increased of CMS, whereas 2,8 dihydroxyadenine, N-methylsalsolinol and dopamine-4-sulphate were decreased at the same tissue. The study showed for the first time that the drug impairs the liver metabolome, identifying suberylglycine and spermine as important biomarkers. In the case of kidneys, uremic toxins as the hypoxanthine and 4-hydroxynonenal were dysregulated even in the Low Dose. Additionally, the metabolic pathways of purine and phenylalanine metabolism were altered in the kidneys of mice injected with the Low Dose.

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Title
LC-MS method development for analysis of vitamins, hormones, and neutransmitter

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In clinical research and diagnosis, it's vital to accurately measure the exact levels of biologically active substances like vitamins, hormones, and neurotransmitters. Typically, these substances are assessed using different methods. However, a more efficient approach is to analyze them together using a single method. This study aims to create a technique using ultra-high-performance liquid chromatography with mass spectrometry (UHPLC-MS). This technique separates a complex mixture of standard solutions containing vitamins, hormones, and neurotransmitters, and systematically examines their chromatographic characteristics. Initially, the method development involved preparing isoconcentrated standard solutions. For this, the most suitable solvent was chosen for each substance. After this initial step, the prepared isoconcentrated solutions were combined into one mixture and diluted with methanol. In the early stages of the method development, various HPLC parameters were tested, including the pH of the mobile phase, the chemistry of the stationary phase, and the gradient elution profile. Detection was carried out using the Orbitrap Exploris 120 mass spectrometer, operating in full-scan mode with a resolution of 30000. The scan range spanned from 100 to 800 m/z. Electrospray ionization (ESI) was used both positive and negative ionization mode. Throughout the method development process, the main goals were to analyze and compare the results of the separated compounds using different methods. Each method underwent slight parameter adjustments, including changes to mobile phase acidity, polarity, buffer concentration, and gradient conditions. Additionally, factors like column length, pore size, diameter, and chemical structure were considered. Initial results indicated that some compounds exhibited better ionization in positive mode, while others were more suited to negative mode ionization. This suggests that the final method will likely use a dual polarity ionization approach. Interestingly, some compounds could be ionized in both positive and negative modes, hinting that the pH of the mobile phase could influence the ionization mode. Furthermore, modifying the gradient showed that the selectivity of the separated compounds changed based on the gradient slope. This underscores the importance of optimizing these parameters for accurate and effective compound separation.

P30

Title
Serum metabolome profiling in early detection of lung cancer

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Abstract text

Serum metabolome and components of extracellular vesicles (EVs) present in the blood are promising sources of molecular biomarkers that could support the early detection of lung cancer in screening programs based on low-dose computed tomography. Several panels of metabolites present in serum end serum-derived small EVs (exosomes) that differentiate lung cancer patients and healthy individuals were reported, yet none of them were validated in the population at high risk of developing cancer. Here we analyzed metabolome profiles in participants of two lung cancer screening studies: MOLTEST-BIS (Gdańsk, Poland; n=369), and SMAC (Milano, Italy; n=93). Three groups of screening participants were included from each cohort: patients with screen-detected lung cancer, individuals with benign pulmonary nodules, and those without any lung alterations. Concentrations of about 400 metabolites (lipids, amino acids, and biogenic amines) were measured by a mass spectrometry-based approach. We observed a reduced level of lipids, in particular cholesteryl esters, in the sera of cancer patients. However, despite several compounds showing significant differences between cancer patients and healthy controls were detected within each study, only a few cancer-related features were common when both studies were compared, including reduced levels of LPC(18:0). A large heterogeneity of serum metabolomes was observed, both within and between studies, which impaired the accuracy of classifiers based on specific metabolites. The average AUC values of three-state classifiers were 0.60 and 0.51 for the test (MOLTEST) and validation (SMAC) cohorts, respectively. Therefore, a hypothetical metabolite-based biomarker for early detection of lung cancer would require adjustment to lifestyle-related confounding factors that putatively affect the composition of serum metabolome. Moreover, the lipid profiles of the total fraction of small EVs obtained from the sera of participants of the MOLTEST study were analyzed. A few lipids whose levels were different between compared groups were detected, including ceramide Cer(42:1) upregulated in vesicles from cancer patients. On the other hand, the contribution of phosphatidylcholines with poly-unsaturated acyl chains was reduced in vesicles from lung cancer patients. However, high heterogeneity of lipid profiles of small EVs was observed, which impaired the performance of classification models based on specific compounds (the average AUC value of the three-state classifier was 0.58 in the test subset). Hence, the data obtained do not support the concept of using the lipidome components of the total mixture of extracellular vesicles present in serum as biomarkers for early lung cancer detection.

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Title

Diet (habitual Western vs Mediterranean) and food type (organic vs conventional) significantly affects different groups of plasma metabolites, a randomized, controlled intervention trial.

Authors

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Observational studies have linked Mediterranean Diets (MedDiet) and organic food consumption with positive health outcomes, and recent dietary intervention studies suggest that this may be explained by higher mineral micronutrient and phenolic intake with MedDiets and lower dietary exposure to toxic compounds with organic food consumption. To determine the effects diet and food type (organic versus conventional) on the plasma metabolic profiles and identify correlations between metabolites that are significantly affected by diet and/or food type, and urinary makers for pesticide exposure and micronutrient intake.We randomly allocated healthy, adult participants to a conventional (n=14) or an intervention (n=13) group. The intervention group consumed a MedDiet made entirely from organic foods, while the conventional group consumed a MedDiet made from conventional foods during a two-week intervention period. Before and after the intervention period, both groups consumed their habitual Western diets made from conventional foods. The primary outcome was plasma metabolic profiles. In addition we monitored urinary excretion of selected pesticide, mineral micronutrients, toxic metals and phenolic markers and diets using food diaries. The participants were aware of study group assignment, but the study assessors were not. Highly significant effects (at $P \leq 0.000001$) of diet and food type were detected for 68 and 54 metabolites, respectively, but there was no overlap between the group of metabolites affected by diet and food type. Six of the metabolites affected by diet (at $P \leq 0.000001$) were identified as glycine betaine, α-tocopherol, isovalerylcarnitine, 1- aminocyclohexane carboxylic acid and trimethylamine N-oxide and all six were higher with the MedDiet. In contrast, testosterone and L-α-hydroxyvaleric acid were significantly higher with the habitual Western Diet ($P=0.000002$). None of the 54 metabolites affected by food type (at $P \leq 0.000001$) could be identified, but 52 were higher with conventional food consumption and only 2 with organic food consumption. Correlations between metabolites significantly affected by food type and urinary pesticide markers.The finding that diet and food type affected different groups of plasma metabolites suggests that the mechanisms responsible for positive health impacts associated with changing to a MedDiet are substantially different to those linked to organic food consumption in observational studies.

P32

Title
Establishing appropriate levels of internal standards in quantitative targeted metabolomics research: profiling lipid mediators.

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Abstract

The use of matching stable isotope labeled internal standards is the best approach for chromatographic quantification of a wide spectrum of biological samples. The inclusion of dedicated internal standards for all analytes has some practical benefits. For instance, they compensate for changes in extraction efficiency, changes in detector response due to sample loss during the sample preparation steps, or changes in detector response due to different flow rates. However, variations in the results have been observed in cases where internal standards were added into the samples, which may indicate: *i*) potential interactions between the targeted metabolites and their corresponding internal standards; or *ii*) inappropriate amounts of internal standards. Unfortunately, experimental procedures on how to select the optimal amounts of internal standards for quantitative targeted metabolomics are rarely considered in scientific publications. The lack of literature could be attributed to the inherent complexity of the typical metabolomics settings that involve the analysis of dozens or even hundreds of analytes. As a result, the inclusion of matching internal standards for every analyte can be challenging in terms of the strategies behind the selection of their optimal concentration levels, cost, and availability [1].
Based on practical experience, a successful liquid chromatography tandem mass spectrometry quantification of pro- and anti-inflammatory lipid mediators (e.g., prostaglandins, leukotrienes, resolvins), derived from omega-6 and omega-3 polyunsaturated fatty acids (PUFAs), relies on the addition of internal standards. In this respect, we have proposed the use of a uniform shell design (aka Doehlert matrix) for selecting appropriate amounts of internal standards (PGE_2-d_4, LTB_4-d_4, RvE_1-d_4, RvD_1-d_5, RvD_2-d_5, RvD_3-d_5) to be used in immunological studies, more specifically exploring the impact of different factors (e.g., PUFAs, nutritional polyphenols, lipopolysaccharides, bacteria) on the quantitative production of eicosanoids (PGE_2, PGE_3, PGD_2, LTB_4, LTB_5. RvE_1, 5,12,18-*tri*-HEPE, 5,6,18-*tri*-HEPE) and docosanoids (RvD_1, RvD_2, RvD_3, RvD_4, RvD_5) in human and fish cell cultures. Mathematical models were computed for every internal standard to visualize regions where the response factors remain constant over a wide range of analytical concentrations. The levels of eicosanoids and docosanoids in the samples were reported in absolute units (ng/mL). In addition, polyphenols, omega-6, and omega-3 PUFAs were also determined in the samples and their concentration levels reported in relative units (%).

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P33

Title
Plasma NMR metabolites of psoriasis and common immune-mediated inflammatory diseases in HUNT and UK Biobank

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Abstract text
Psoriasis is a common and chronic immune-mediated inflammatory disease (IMID) in the skin, caused by the interplay between multiple genetic and environmental risk factors. Although it is closely associated with systemic metabolic aberrations, insight into the metabolic profile of psoriasis is still hampered. Existing studies analyzing metabolomic profiles of psoriasis are limited in sample size (less than 1000 psoriasis cases) and often lack independent validation cohorts. To address these limitations, we aim to discover metabolites associated with prevalent psoriasis using two large population biobanks, the third wave of the HUNT Study (N = ~50,000) and UK Biobank (N = ~500,000). Here, we present the preliminary results from UK Biobank, with available plasma nuclear magnetic resonance (NMR) data from ~118,000 participants. The measurements cover 249 metabolites, including lipoprotein subfractions, fatty acids, and small-molecular metabolites. Outcomes were defined using the first occurrence of diagnosis based on either self-report, inpatient hospital data, primary care, or death record. Patients with a diagnosis date after the blood sampling date were excluded. For each metabolite measure, association analysis was conducted using multivariate logistic regression against the scaled measures and adjusted for age, sex, and body mass index. We then identified significant associations with a Bonferroni corrected p-value threshold of 0.001. Our results show 46 metabolites with significant associations with psoriasis. The three largest associations are the monounsaturated fatty acid percentage of total fatty acid (OR = 1.125, 95% CI = 1.078-1.174), degree of fatty acid unsaturation (OR = 0.88, 95% CI = 0.843-0.917), and glycoprotein acetyls (OR = 1.118, 95% CI = 1.073-1.165). To further investigate metabolites shared between psoriasis and other common IMIDs, we compared significant metabolite associations in psoriasis to atopic dermatitis, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, and ulcerative colitis. All metabolites significantly associated with psoriasis also exhibit significant associations with at least one other IMID, with many overlapping associations showing similar directions. Among the six IMIDs assessed, Crohn's disease is the disease with the largest number of significant associations (148 significant associations) and atopic dermatitis with the least number of significant associations (4 significant associations). Our results shed light on the metabolic profile of psoriasis and other common IMIDs, with the potential of using these metabolic measures as metabolic markers for developing treatment. Further validation in HUNT is ongoing.

This research has been conducted using the UK Biobank Resource under application number [40135].

P34

Title: Quantitative NMR serum spectroscopy deciphers metabolomic and lipidomic heterogeneity in endometriosis and pelvic inflammatory disease

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Abstract text (395 words)

Background: Endometriosis (EMT) affects around 190 million reproductive-age women globally, posing a substantial economic burden equal to or surpassing other chronic conditions. Pelvic inflammatory disease (PID), like EMT, causes pelvic pain, requiring antibiotic therapy. EMT pain can only be temporarily eased by painkillers, ovulation, menstruation inhibition, or surgery. It induces infertility and a 50% ovarian cancer risk rise. Further research is required to uncover pathophysiological mechanisms, enhance prevention, early diagnosis, and disease management.

Methods: We investigated a cohort of 230 serum samples of which 73 were received from PID and 157 from EMT. All the serum samples were validated and phenotyped by ^{1}H-NMR-based metabolomics with in vitro diagnostics research (IVDr) standard operating procedures generating quantitative data on metabolites and lipoprotein parameters. Quantitative metabolite and lipoprotein data of 129 healthy serum samples was enrolled as a healthy control. Uni- and multivariate statistics were applied to identify NMR-based alterations.

Results: Both lipoprotein and metabolites parameters show differences between EMT and the healthy group. Glucose and pyruvic acid level were significantly higher, while glycine, glutamine, glutamic acid, and methionine which are involved in the glutathione synthesis decreased significantly in PID and EMT compared to the healthy group. Tyrosine, N, N-dimethylglycine (DMG), and trimethylamine-N-oxide (TMAO) were also significantly lower in PID and EMT. Additionally, lipoprotein parameters were significantly differentiated between PID and EMT. Lipoprotein subfractions of TPCH, TPAB, TBPN, and LDL significantly decreased in EMT when compared to healthy and PID groups individually. HDL levels were elevated in the EMT compared to the healthy group and decreased compared to the PID.

Conclusion: High glucose could lead to an overproduction of reactive oxygen species (ROS), damaging mitochondria, and promoting inflammation. This also would result in the formation of advanced glycation end products (AGEs), which trigger inflammatory responses. Metabolic syndrome or mitochondrial disorders may explain the increase in pyruvic acid levels while glutathione precursors can help protect tissue from oxidative damage, suggesting glutathione supplementation as a potential therapy. Stress from pelvic pain might change gut microbiota and influence TMAO production.

Tyrosine and DMG, linked to neurotransmitters, are connected with mood and stress responses, pointing towards mental well-being in EMT and PID patients. A noticeable increase in LDL-receptor expression is prominent in endometriosis. This lowers LDL levels and eliminates circulating apolipoprotein b-100. Of note, LDL is mostly composed of cholesterol. TPCH, TPAB, TBPN, and LDL could potentially differentiate PID and EMT non-invasively.

P35

Title
Altered plasma metabolite levels can be detected years before a glioma diagnosis

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Abstract text
Genetic and metabolic changes in tissue and blood are reported to occur several years before glioma diagnosis. As gliomas are currently detected late, a liquid biopsy for early detection could impact the quality of life and prognosis of patients. Here, we present a nested case-control study of 550 pre-diagnostic glioma cases and 550 healthy controls, from the Northern Sweden Health and Disease study (NSHDS) and the European Prospective Investigation into Cancer and Nutrition (EPIC) study. We identified 93 significantly altered metabolites related to glioma development up to eight years before diagnosis. Out of these metabolites, a panel of 20 selected metabolites showed strong disease correlation and consistent progression pattern towards diagnosis in both the NSHDS and EPIC cohorts, and separated favorably future cases from controls independently of biological sex. The blood metabolite panel also successfully separated both lower grade glioma and glioblastoma cases from controls, up to eight years before diagnosis in NSHDS (glioma AUC=0.85, P=3.1e-12; glioblastoma AUC=0.85, P=6.3e-8), and up to two years before diagnosis in EPIC (glioma AUC=0.81, P=0.005; glioblastoma AUC=0.89, P=0.04). Pathway enrichment analysis detected metabolites related to the TCA-cycle, Warburg effect, gluconeogenesis, cysteine-, pyruvate- and tyrosine metabolism as the most affected.

P36

Title

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

Authors

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Abstract text

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 (1). When available, analyzing 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.

To this end, 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 mass spectrometers, after metabolite separation via liquid chromatography (LC), including the novel Orbitrap™ Astral™ mass spectrometer to evaluate the SQUAD metabolomics workflow.

SQUAD analysis on Orbitrap IQ-X™ and Ascend™ Tribrid instruments, for example, utilized the sensitive and wide dynamic range linear Ion-Trap (i.e., 6 orders of magnitudes dynamic range, with LLOQ of 5 femtomoles and LLOD of 0.5 femtomoles) for PRM quantitation of the analytes in human plasma. 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 MS^2 level for a higher annotation rate and enough points across the peak on the Orbitrap MS^1 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, e.g., Orbitrap Exploris series, showing promising capabilities of the new metabolomics workflow. Fast polarity switching on these Orbitrap Hybrid instruments enables wider coverage of the metabolome by securing sufficient scans per peak at high MS^1 resolution (i.e., 120k).

(1) Amer B, et al. Simultaneous Quantitation and Discovery (SQUAD) Analysis: Combining the Best of Targeted and Untargeted Mass Spectrometry-Based Metabolomics. Metabolites. 2023 May 10;13(648).

P37

Title: High-throughput metabolite exchange across organs provides unique insights to understand underlying metabolic perturbations in progressive obesity and insulin-resistance in minipigs

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Abstract text

The specific role of organs on type 2 diabetes onset remains to be elucidated. Indeed, the available biomarkers are whole-body biomarkers that provide little information about organs' metabolism. We therefore developed an innovative arteriovenous (AV) metabolomics approach aiming to define early organ-specific signatures in insulin-resistant (IR) and obese minipigs. Minipigs were fed either a normal-fat (NFC) or a high-fat high-sugar diet during 2 (HF2M), 4 (HF4M) or 6 (HF6M) months. All HF groups developed similar IR and progressive obesity up to a morbid stage. By performing LC-HRMS metabolomics on 9 vessels and computing ratios of metabolites levels between blood going in and out of organs, we established the uptake and release profiles of 9 organs: head, muscle, intestine (small, large and total), liver, pancreas, kidney and spleen. Interestingly, regarding the 4 main organs involved in obesity and IR onset (liver, pancreas, muscle, intestine) we observed that the profiles of organ-specific metabolite exchanges shifted from release to uptake as early as in HF2M group for 5, 10, 7 and 1 metabolites respectively. Thus, several metabolites were actively up-taken in the HF2M animals, including methionine, cystathionine and 3-hydroxypentanoic acid by the liver hydroxyoctadecadienoic acid and eicosapentaenoic acid by the pancreas and pyroglutamic acid by the muscle. Some of these metabolites are known to be related to oxidative stress potential and lipotoxicity and deserve more investigation. In conclusion, this organ-specific AV approach is promising for the investigation of the metabolism regulation during obesity and IR onset at the organ and inter-organ exchanges levels.

P38

Title
Using labeling probes and isotope tagging for detection and quantification of short chain fatty acids by LCMS in biological samples

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Abstract text
Introduction
Short chain fatty acids (SCFAs) have emerged as important physiological metabolites that may mediate effects of microbiota on human health (1). The analysis of SCFAs is often used to monitor the efficacy of gut-health interventions (2). However, quantification of SCFA in biological samples is challenging due to their low concentrations in blood, and the ubiquitous nature such as volatility, requiring precise standardization procedures during the sample preparation process to allow fast, sensitive, and robust analysis of SCFA in different matrices.

Methods
We have developed a UPLC/multiple-reaction monitoring-mass-spectrometry-probe method combined with isotope tagging to quantify SCFAs in biological samples. This method is an extension of the method reported by Han et al (3). Briefly, SCFAs in plasma are extracted with 10ul 75% MeOH. Samples were derivatized with $^{12}C/^{13}C$ 3-nitrophenylhydrazine (3NPH) to convert SCFAs quantitatively to their 3NPH derivatives. We obtained chromatographic separation of 9 common SCFAs (formic, acetic, propionic, isobutyric, butyric, succinic, isovaleric, valeric and caproic acid). Matrix effects (ionization suppression or enhancement) are often encountered in electrospray ionization and it affects precision and accuracy using LCMS techniques (3). To overcome such problems, we used Atmospheric Pressure Chemical Ionization in the negative-ion mode with no solvent modifiers.

Preliminary data
By using 75% methanol during the derivatization process we obtained reproducible results from just 10 ul plasma. We have obtained excellent sensitivity and chromatographic separation of SCFAs in plasma by using the QTRAP 6500+ LCMS system (LOQ 2 nM and LOD 0.1 nM). Our sample workup and derivatization procedure are facile, fast, and long-term reproducible in aqueous-organic medium. Background levels of formic acid and acetic acid were detected in sample blanks after derivatization and these levels increased over time. Addition of a quenching step after the derivatization to consume excess reagent ensured stability of the response for up to 1 week for all SCFAs. Nevertheless, plasma as a matrix is tricky because of the large protein content. By the use of stable isotope-labeled internal standards we can compensate for ionization suppression or enhancement as well as chromatographic issues. Isotope label coding was performed using 13C-3NPH to create an internal standard for each SCFA. The internal standard solution could be used for at least 3 months when it was stored in a −20°C freezer.
This novel method was applied to quantify SCFAs in plasma samples from several clinical intervention studies, preliminary data will be presented.

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P39

Title
Comprehensive plasma steroidomics in patients with different stages of prostate cancer disease

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Abstract text
The steroid submetabolome, or steroidome, is of particular interest in prostate cancer (PCa) as the dependence of PCa growth on androgens is well known and has been routinely exploited in treatment for decades. Nevertheless, the community is still far from a comprehensive understanding of steroid involvement in PCa both at the tissue and at systemic level. In this study we used reversed-phase liquid chromatography hyphenated with orbitrap high resolution mass spectrometry backed by a dynamic retention time database DynaSTI to obtain a readout on circulating steroids in a cohort reflecting a progression of the PCa. Principal component analysis performed on the resulting data revealed only subtle alterations of the systemic steroidome in the study groups. A supervised approach allowed for a differentiation between the healthy state and any of the stages of the disease. Two groups of steroids were responsible for this outcome: one consisted primarily of the androgens, whereas another contained corticosterone and its metabolites. The androgen data supported the currently established involvement of a hypothalamic-pituitary-gonadal axis, whereas the role of corticosterone group remained elusive. As 22 currently unknown steroid isomers were highlighted in course of this study, we also discuss steroid annotation problematic. It will be demonstrated, how established collision-induced dissociation techniques and novel electron-activated dissociation approach can help to reveal otherwise hardly accessible identities of steroid-like compounds.

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Title
Analysis and prediction of postprandial metabolic response to multiple dietary challenges using dynamic mode decomposition

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Abstract text

Background: In the field of precision nutrition, predicting high dimensional metabolic response to diet and identifying groups of differential responders are two highly desirable steps towards eveloping tailored dietary strategies. However, proper data analysis tools are currently lacking, especially for complex settings such as crossover studies. Current methods of analysis often rely on matrix or tensor decompositions, which are well suited for identifying differential responders but lacking in predictive power, or on dynamical systems modelling, which may be used for prediction but typically requires detailed mechanistic knowledge of the system under study.
Objectives: To remedy these shortcomings, we aimed to explore dynamic mode decomposition (DMD), which is a recent, data driven method for deriving low-rank linear dynamical systems from high dimensional data.
Methods: To allow integration of complex data from several dietary inputs to the metabolic stem, we combine parametric DMD (pDMD) with DMD with control (DMDc). The resulting method allows i) to predict the postprandial metabolic response of a new diet given only the metabolic baseline and dietary input, and ii) to identify inter-individual differences in metabolic regulation, useful in determining metabotypes, i.e., metabolic phenotypes in dynamic data. To our knowledge, this is the first time DMD has been applied to metabolomics data.
Results: pDMDc enabled data driven construction of low dimensional dynamical models, able to capture the underlying dynamics of the metabolome after three dietary challenges. We demonstrate the utility and accuracy of the model in a cross-over study setting on both measured and simulated data. Using simulated data, metabolic response to a new diet was accurately predicted having trained on 6 diets, with an average cosine similarity score of 0.71 (SD=0.39). In measured data, we could identify previously published metabolic groups with 100% overlap and compare the results with using the tensor decomposition PARAFAC.
Discussion: Accurate predictions via pDMDc requires data from several dietary exposures with large variation, which can be costly to collect to confirm the efficacy of the method. A possible remedy is to share data among individuals using the mixed effects framework. Tensor decompositions may be more intuitive for metabotyping but employing pDMDc paves the way towards using control theory to approach PN by estimating the optimal input given a target metabolite trajectory.

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Title
Unraveling the chemical ecology of successful monoculture farming in termites using LC-MS metabolomics

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Abstract text (Max 400 words)

Human monoculture farming faces a number of challenges and especially the lack of genetic diversity makes these crop systems vulnerable to pathogens. In contrast, some insects have evolved the ability to successfully maintain genetically uniform farming systems. One of these are the fungus-farming termites (Macrotermitinae) that dominate many tropical ecosystems. For 30 million years, these termites have engaged in an obligate symbiosis with the basidiomycete fungal genus *Termitomyces* that they maintain in gardens within their nests as a nutritious source of food. Despite foraging in pathogen-rich environments, termite fungus gardens appear to have remarkable low prevalence of non-*Termitomyces* fungi.[1] Only when the colony is dying, does a specialist stowaway ascomycete fungus *Pseudoxylaria* appear. Understanding the fundamental mechanisms that safeguard termite monocultures could lead to new tools for crop protection in human farming systems.

Termitomyces produces chemical compounds with potential roles in suppressing *Pseudoxylaria* and other competitor fungi within the termite gardens.[2] We hypothesized that compounds serving key defensive functions would be upregulated when *Termitomyces* was challenged by other fungi. To test this hypothesis, we grew *Termitomyces* and *Pseudoxylaria* in mono- and co-cultures and collected media plugs for chemical profiling by UHPLC-QTOF-MS/MS. To mimic variations in growth conditions in healthy and declining colonies, we grew the fungi on media with different nutrient compositions and CO_2 levels characteristic of healthy (5%) and compromised (atmospheric) colonies.

Our results revealed complex regulation of *Termitomyces* and *Pseudoxylaria* metabolite production influenced by both co-cultivation and growth conditions. We identified a series of sesquiterpenes from *Termitomyces* and a series of tetracyclic peptides (pseudoxylallemycins) from *Pseudoxylaria*. Both groups of compounds have potential defensive functions but their regulation followed very different patterns. Cultivation at high CO_2 for example favoured the production of *Termitomyces* sesquiterpenes while atmospheric CO_2 levels reflecting the decaying nest when the antagonist takes over favoured *Pseudoxylaria* peptides. Our results point to sesquiterpenes playing a role in maintaining fungus gardens antagonist free and that high CO_2 supresses *Pseudoxylaria* chemical defences that are only triggered when the colony is in decline. Uncovering the identities and regulation of chemicals governing interactions between *Termitomyces* and *Pseudoxylaria* is important to assess the role that the crop fungus plays in its own and consequently colony defence.

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P42

Title
Metabolic biomarkers on the surface of cutaneous melanoma

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Skin cancer is among the five most common cancer types worldwide (WHO, 2020). Currently the gold standard for skin cancer diagnosis is based on visual inspection followed by biopsy. Skin biopsy is an expensive, time-consuming procedure, involving surgery which comes with its own risks and discomfort. Therefore, the aim of our study was to test the feasibility of detecting skin cancer non-invasively by analyzing metabolites collected from the surface of cutaneous melanoma.
Sixteen patients with suspected melanomas were enrolled in this study at the Department of Dermatology Skåne University Hospital, Sweden. Seven suspected skin lesions were diagnosed as malignant melanomas, 6 – melanomas in situ, 2 – dysplastic nevi and 1 – seborrheic keratosis. Sampling from the patients was performed by tape stripping (3 consecutive tape strips) of suspected skin lesions and adjacent healthy skin sites. Metabolites extracted from the tape strips were analyzed by LC-MS/MS and GC-MS.
Tyrosine (tyr), phenylalanine (phe), tryptophan (trp) and kynurenine (kyn) were quantified by LC-MS/MS, due to the expectation of altered levels of these metabolites in tumors overexpressing indoleamine 2,3-dioxygenase (IDO1) [1], which converts trp to kyn [2]. Based on our previous studies, these molecules have similar diffusion rates through the skin, and can be successfully collected from the skin surface [3-5]. The results from our pilot study shows that in all samples collected from malignant melanoma lesions (*n=7*) the amounts of tyr, phe, trp and kyn are 2- to 5-fold higher than in the adjacent healthy skin. In cases of melanoma in situ, 3 patient samples showed a similar trend as malignant melanoma, whereas 3 cases were more similar to dysplastic nevi and seborrheic keratosis samples. Additionally, targeted LC-MS/MS analysis of 31 metabolites (amino acids, lactate, glutathione, succinate *etc*.) and untargeted metabolomics were performed on the collected samples.
Preliminary results from this pilot study indicates that metabolite levels differ between cancerous and healthy skin surfaces. Further data analysis is ongoing.

This study was funded by the Interreg Öresund-Kattegat-Skagerrak foundation via the project ‘’Consortium for Intelligent Diagnostics of Skin Cancer (CINDIS)’’ and by Knowledge Foundation via the project “Biobarriers - Health, Disorders and Healing”.

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P43

Title

Biochemical composition of soy-based meat alternatives examined using non-targeted metabolomics approaches.

Authors

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Abstract text

As the global population grows, so does the need for increased sustainable food production and processing. To address this challenge, changes in dietary patterns are needed and more . In the future, meat protein needs to could be replaced with plant-based protein sources such as soy. Dietary patterns based on protein-rich plant-based foods, are associated with healthier and more sustainable lifestyles and therefore have become increasingly popular among people.

The health-beneficial aspect of plant-based diets is evidenced *e.g.* by the fact that people consuming plant-based diets have decreased risk for cardiovascular diseases (1). Health benefits from plant products derive from *e.g.* dietary fiber, phytochemicals, vitamins, and minerals, although molecular mechanisms behind beneficial effects are not fully understood. Plant phytochemicals, *e.g.* flavonoids and phenolic acids, are known to have multiple health-promoting benefits such as anti-inflammatory and antioxidative properties (2) Until today, food and nutrition research has typically focused on a narrow range of nutrients, even single compounds, and the comprehensive readout across the wide polarity scope of the thousands of biochemicals present in any food has not been disclosed. The current understanding of the health implications of food is based on <200 basic nutrients, even when foods contain tens of thousands of potentially bioactive biochemicals, and this pool of compounds should be more carefully assessed (3).

Meat analogues, *i.e.* products made from plant-based raw materials using various processing technologies to resemble meat, have become extremely popular during the past years. These products usually contain a mixture of different plant protein extracts to achieve better amino acid composition or to improve organoleptic properties. Processing technologies vary from drying to heavily refined products containing only the extracted protein part of the plant. Heavily refined products may therefore have poorer nutritional composition compared to the raw material, caused by the loss of nutritionally important components such as fiber and bioactive compounds during processing. In our study, we used non-targeted metabolomics approaches, in order to evaluate the biochemical composition of soy-based products and meat alternatives and determine the impact of how different processing technologies can affect it. Our results indicate that the level type of technological processing can drastically affect the phytochemical composition.

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Title

Understanding the role of matrix polysaccharides of cell wall in altering aspen cuticle chemistry integrating mass spectrometry with multivariate tools.

Authors

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Abstract

Cuticle, a hydrophobic layer covering all aerial plant surfaces, plays multifaceted roles in organ growth and development and in plant resistance to biotic and abiotic stresses. Cuticle modification is therefore an important target for crop improvement. Chemically, the cuticle is a complex polyester made of C16 and C18 fatty acids; α,ω-dicarboxylic acids; small amount of glycerol, hydroxycinnamic acids (including ferulic and p-coumaric acids), flavonoids and other phenolics. Moreover, cuticle is linked with cell wall polysaccharides howbeit the mode of these linkages is still unknown. This study aimed at developing optimized depolymerization and analysis methods for hybrid aspen (Populus tremula L. X tremuloides Michx., clone T89). As the hybrid aspen is a model hardwood species, such methodology would enable molecular genetic studies using this system to tackle many biologically relevant questions about cuticle architecture and functions. In particular, by using hybrid aspen lines with modified cell wall matrix polysaccharides, we would like to test how these polysaccharides affect cuticle structure. We used enzymatically isolated leaf cuticles, removed wax and extractives, and tested three different depolymerization methods: the calcium oxide – based catalysis (CO) and sodium methoxide – based catalysis (SM), as reported by Graça et al. (2002), or acetyl chloride - based catalysis (AC; new method), followed by gas chromatography-mass spectrometry (GC-MS) analysis, to select the method detecting most expected cuticle monomers. The AC method was thus selected, and it was subsequently applied to transgenic hybrid aspen with reduced 4-O-methyl glucuronic acid substitution in glucuronoxylan. Such modification was previously observed to affect suberin-like lipids bound to cell walls in the xylem tissue (Derba-Maceluch et al., 2023), but the importance of the 4-O-methyl glucuronic acid in binding cuticle to cell wall in epidermis has not previously been investigated. Our optimized method of cuticle analysis allowed us to tackle this question providing a proof-of-concept that transgenic lines of T89 with altered structure of cell wall polysaccharides can now be used to determine the different roles of these cell wall polysaccharides in cuticle development and function.

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P45

Title
Chloroplastic ascorbate level may regulate arginine metabolism through ascorbate – protein interactions

Authors
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Abstract
Ascorbate plays various roles in a wide range of organisms, from reactive oxygen scavenging to the regulation of gene expression. However, how and to what extent ascorbate modulates metabolism is largely unknown. To address this in plants, we explored the implications of chloroplastic and total cellular ascorbate deficiencies by analysing mutant lines of *Arabidopsis thaliana*. Under regular growth conditions, neither ascorbate-deficiencies led to oxidative damage. In contrast, metabolomics analysis revealed a global and largely overlapping metabolome rewiring in both ascorbate-deficiencies. We observed significant alterations in arginine metabolism in particular. Moreover, proteome-wide analysis of thermostability revealed that ascorbate may interact with arginine aminohydrolase enzymes, involved in arginine turnover. Overall, our results suggest that, independently of oxidative stress, chloroplastic ascorbate interconnects diverse metabolic pathways, at least partly through ascorbate protein interactions.

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Title
Global LC-MS multi-omics for investigating the effects of High Intensity Training (HIT)

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Abstract text

Physical activity is generally accepted to improve the health and well-being, and is included in the recommended therapy for many diseases. The metabolic changes induced by physical activity occur both during and after the exercise. The effects of physical activity is well studied in context of metabolism. High Intensity Training (HIT) has gained more popularity over the years. Recent studies has shown that HIT-training reduces the overall mortality significantly. However, the metabolic effects exercise has on a global scale is not fully explored. For better understanding the global effects of exercise, approaches such as global metabolomics and lipidomics can be applied. However, the two omics techniques are rarely combined in the same studies, removing the possibility to study multiple aspects of the metabolome under identical circumstances.

In this study global LC-MS lipidomics and metabolomics (1) were included for understanding the metabolic effects of a single 10 minute HIT session. Five volunteers participated in a HIT session and 4 volunteers acted as a control group. Dried blood spot (DBS) samples were collected from the participants at the same time over a 3-day period. All participants followed identical diets. The two different omics approaches were applied to all DBS samples. The global metabolomics approach revealed significant metabolic alterations in energy catabolism, specifically adenine-nucleotide catabolism, and hypoxia responses. The global lipidomics approach did not reveal these responses. Likewise, with the lipidomics approach significant inflammatory responses and significant alterations in lipolysis where observed that were not picked up with the metabolomics approach. Our study thus shows that applying multiple omics technologies in one study will provide a more complete picture of the metabolome and the changes induced. In addition, two unidentified features where found to be highly significantly associated with HIT, one with the metabolomics approach (*m/z* 157, level of confidence 5) and one with the lipidomics approach (C22 lipid, with confidence level 3). These two features are possible biomarkers for HIT, and revealing their identity and role in the biochemical processes involved in HIT-training will be our next step. Combining the two different omics techniques provides a wider understanding of how the whole metabolome is affected as a result of conducting a single HIT session.

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Title
Clinical metabolomics and lipidomics: what we have done and where we are going

Authors
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Abstract text
In recent years, omics techniques at the level of low molecular-weight analytes have found their place in the search for biomarkers of known diseases as well as in routine diagnostics. As methods for metabolite and lipid analysis continue to improve and are used in the study of various diseases, it is becoming clear that metabolic disruption plays a key role in many pathophysiological and pathobiochemical processes. Targeted and non-targeted approaches then provide a new comprehensive view of metabolic profiles corresponding to alterations in a wide range of diseases. In the field of inherited metabolic disorders, the rapid implementation of metabolomics in the form of multicomponent analysis of small molecules is saving a large number of children. Recently, metabolomic and lipidomic studies have shown promising new biomarkers for inflammatory conditions, or to predict the risk of coronary heart disease and acute coronary syndrome, obesity and hyperlipidemia, cancer, Alzheimer's disease, and others. Evaluation using multivariate statistical methods and subsequent application of bioinformatics tools is an essential part of this, allowing us to gain a comprehensive view. This presentation will present new findings in the above areas and summarize the current trends in both metabolomics and lipidomics and results of studies that offer potential in the field of laboratory diagnostics.

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Title
Metagenomic study of the human gut microbiome

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Abstract text

Due to its fundamental impact on overall health, the gut microbiome has emerged as one of the most popular research subjects over the last decade. It is believed that there are associations between gut microbial perturbation and several disorders, such as obesity, diabetes, and cancer. Surprisingly, gut microbiota might also play a role in non-digestive disorders such as depression and autism. Therefore, it is crucial to analyze the microbial composition of a healthy person in order to comprehend how this complex ecology preserves human health.

The longitudinal study showed that overall participants' phyla distributions were dominated by Firmicutes, followed by Bacteroidetes with each participant's gut microbiota containing between 300 and 650 species. Diversity analysis reveals that the majority of participants had stable diversity over 28 days, with each participant having a different microbial composition that can vary greatly from one healthy person to another. Venn diagram revealed that after one month, most participants seem to have maintained around 60 and 80% of their genera. Based on our findings, the more prevalent the taxa, the more their abundance varied over a period of 28 days. Clostridia, Bacteroidia, Actinobacteria, and Erysipelotrichia were the four classes assumed to be the active members of the gut microbiota.

Taken together, longitudinal studies show that most individuals can maintain the stability of their gut microbiota composition, though some microbe abundance varies over a one-month period.

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Title

Network analysis reveals systematic alterations in lipidome profiles in early-onset hyperuricemia, gout, and the effect of urate-lowering treatment

Authors (presenting author underlined)

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Abstract text

Background: Gout is the most common type of inflammatory arthritis, characterised by chronic deposition of uric acid crystals in the joints. Currently, it is not possible to predict whether patients with hyperuricemia will develop gout, to evaluate the risk of recurrent attacks, or joint damage.

Objectives: Our study aimed to evaluate differences in plasma lipidome between patients with asymptomatic hyperuricemia (HUA) detected ≤ 40 years (HUA≤40) and > 40 years, gout patients with disease onset ≤ 40 years (Gout≤40) and > 40 years, and normouricemic healthy controls.

Methods: Plasma samples were collected from 94 asymptomatic HUA (77% HUA≤40) subjects, 196 gout patients (59% Gout≤40), and 70 normouricemic controls. A comprehensive targeted lipidomic analysis was performed to semi-quantify 608 lipids in plasma. Univariate and multivariate statistics and advanced visualizations were applied.

Results: Both HUA and gout patients showed similar changes in lipid profiles with the highest upregulation of phosphatidylethanolamines (median fold-changes and p-values were 2.9/3.4 and p=1.5×10-16/p=5.5×10-21 for HUA/Gout, respectively) and downregulation of lysophosphatidylcholine plasmalogens/plasmanyls (LPC O-/P-, median fold-changes and p-values were 0.3/0.5 and p=4.8×10-21/p=2.2×10-21 for HUA/Gout, respectively). More profound changes were observed in HUA≤40 and Gout≤40. Moreover, a shift in the lipid profile towards controls was apparent in HUA≤40 and Gout≤40 during urate-lowering treatment. Multivariate statistics differentiated HUA≤40 and Gout≤40 groups from controls with an overall accuracy of > 94%.

Conclusions: Our results point to the involvement of multiple glycerophospholipid classes, for instance lysophosphatidylcholines, LPC P- and LPC O-, in gout pathobiochemistry. The upregulation of lysophosphatidylcholine acyltransferase 3 (LPCAT3), which was already previously described in hyperuricemic mouse liver, could serve as the explanation for our results. The most significant glycerophospholipid dysregulation was found in HUA≤40 and Gout≤40 patients, together with a correction of this imbalance with urate-lowering treatment. These results will help to further understand the pathobiochemical mechanism of gout and hyperuricemia, with the potential for early detection of patients.

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P50

Title
Metabolic impact of whole grain diets on brain regions in a pig feeding trial

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Abstract text

Diets rich in whole grains (WG) have been associated with reduced risk of various diseases, most likely due to fibre, vitamins and bioactive phytochemicals. The metabolism of different WG components by microbiota is a decisive moment giving rise to the array of dietary metabolites with bioactivity entering circulation. However, there is a lack of knowledge on the dietary metabolites that reach the tissue level and how they are contributing to the various health effects associated with these diets. Furthermore, it is fundamental to understand the metabolism of dietary compounds by gut microbiota since it's involved in the bi-directional communication between the brain and gut, the gut-brain axis, where a single metabolite can affect immunological, neuronal, and endocrine signalling. Given this, there is a rising need to explore the diverse metabolites originating from gut microbiome from different WG-rich diets and found in brain tissue. (143 Words)

To achieve this, a pig feeding trial was performed with four groups of Ossabaw pigs subjected to isocaloric bread-supplemented diets (refined wheat, WG rye, WG rye sourdough, WG wheat) and consequently compared to a non-supplemented control diet for 6 weeks. Tissues were collected from the striatum, prefrontal cortex, anterior cingulate cortex and hippocampus regions of the brain. The non-targeted metabolomic profiling was performed using reversed-phase and hydrophilic interaction LC coupled with QTOF-MS. Peak picking and alignment were performed with MS-DIAL software, followed by data pre-processing and statistical analysis with R and Notame R-package. (92 Words)

Multivariate analysis revealed differences between brain regions, especially between the striatum and hippocampus regions, as well as marked differences between control and treatment groups. These metabolites belonged to various classes of compounds related to energy or amino acid metabolism. Noticeably, threonine was significantly increased by all bread-supplemented diets, while other amino acids such as proline and ornithine showed diet specific behaviour towards refined wheat and WG rye despite different structural side chains of the compounds. Furthermore, linoleyl carnitine (CAR 18:2) decreased, and adipoyl-L-carnitine (CAR 6:1; O2) increased with the refined wheat diet when compared to control, thus displaying opposite behaviours. Overall the amino acids and carnitines showed the biggest difference with the refined wheat diet, and most lysophosphatidylcholines demonstrated differences affecting mostly the hippocampus and anterior cingulate cortex. These preliminary results demonstrate that the metabolic impact of bread-supplemented diets extends to the brain tissue, and there are different patterns observed in the different brain compartments even for compounds of the same class. (162 words) (397/400 words)

P51

Title
An integrated understanding of the metabolic benefits of a novel double-targeted intervention using genetically engineered probiotic expressing aldafermin with dietary changes on NAFLD

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Abstract text

Lifestyle changes toward a healthy diet and increased physical activity are the cornerstone interventions in the treatment of non-alcoholic fatty liver disease (NAFLD), the most common liver disease worldwide. However, due to its increased prevalence, new therapeutic approaches targeting the gut-liver-axis such as the use of microbial therapeutics and gut-hormonal interventions have been suggested. The present study introduces a seven-week double-targeted intervention using genetically engineered probiotic Escherichia coli Nissle 1917 to continuously express aldafermin (a non-tumorigenic analog of a human intestinal peptide hormone, fibroblast growth factor 19) along with dietary change (EcNA). The safety, efficacy, and mechanisms of action of the EcNA intervention were demonstrated using a high-fat-diet-induced NAFLD mouse model. The beneficial effects of the EcNA intervention were evidenced by the decrease in body weight, liver steatosis, and plasma concentrations of aspartate aminotransferase and cholesterol. Comprehensive integrated transcriptomics and non-targeted metabolomic analyses further revealed alterations in NAFLD-related genes and metabolites from the host and gut-microbial origin; along with a switch in amino acid, lipid, and their associated receptor signaling pathways. These results suggest the potential efficacy of EcNA in ameliorating NAFLD by decreasing insulin resistance, steatosis, oxidative stress, and maintaining gut-liver axis homeostasis;

and highlight the potential of exploring multi-targeted interventions combining microbial therapeutics with the diet for NAFLD.

P52

Title
Spatial multi-omics to uncover prostate cancer heterogeneity

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Metabolites, genes and proteins play key roles within cells and organs, but lack of technology has restricted the spatial analysis within tissues and research has mainly used bulk measurements that lack information on the phenotype of given cell types, and biomarkers may therefore be masked by the average output. Novel imaging-based techniques, such as MALDI MSI, spatial transcriptomics, and laser micro-dissected (LMD) proteomics are emerging, and they allow the spatial analysis of analytes within a single tissue sample. The aim of the ProstOmics project, was to establish a high-quality tissue multi-omics protocol to uncover biomarkers and molecular mechanisms underlying the heterogeneity of aggressive prostate cancer (PCa). We performed new and emerging techniques of spatial multi-omics on the same tissue sample from several samples from each patient.

We searched through a local biobank (N~1000) of patients undergoing radical prostatectomy donating 2-mm whole-organ slices. The two adjacent paraffin-embedded HES-stained sections were fused with the tissue slice and 4-6 targeted samples were selected from 114 patients with consent. Samples (n~500, diameter=3µm) were drilled frozen using live pathology-imaging targeting several cell-types (cancer, close to the cancer and far away from the cancer). From each sample, 10µm sections were cryo-sectioned and laid on specific glass for various omics analyses including MALDI MSI, spatial transcriptomics and LMD proteomics. The rest of the sample were used for various conventional bulk omics analyses (RNA sequencing, MR spectroscopy, DNA methylation, various staining).

A novel and fresh frozen tissue extraction method of prostatectomy tissue was developed to target pathology-defined tissue types such as stroma, cancer epithelium and normal epithelium. Data from spatial gene expression, spatial metabolomics, spatial proteomics, histopathology, and various bulk data of DNA-methylation, RNA sequencing, copy-number and metabolomics were produced for high-quality analyses. Multi-omics data from each sample and from several samples from each patient were produced for global and targeted analysis to assess the heterogeneity of aggressive PCa. We have established an algorithm GreedyFHist that spatially integrate all the omics-data to one data-matrix to study molecular mechanisms within their microenvironment in samples from patients with 15 years of follow-up.

A novel spatial multi-omics protocol was designed for global and targeted analyses of fresh frozen PCa tissue. The integrated data-matrices from patients with both recurrent and non-recurrent cancer after 15 years have already shown interesting results and will further have the potential to advance the understanding of aggressive and indolent biology from cell types within their microenvironment.

P53

Title
The optimization of the endocannabinoids' measurement method for LC-MS

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Abstract text

Endocannabinoids (ECs) are endogenously produced lipid-based compounds that can activate cannabinoid receptors. Current knowledge regarding the endogenous ECs is mostly limited to the two major ECs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). These two ECs are known to act at both CB1 and CB2 receptors. In addition to these two molecules there is a chemically diverse set of related compounds more broadly known as long chain N-acylethanolamines (NAAs) and 2-monoacylglycerols. A large body of evidence points that endocannabinoid system plays an important role in various diseases (e.g. traumatic brain injury (TBI)).

Here, we aimed to develop a method for ECs, NAAs and cannabinoids measurements using the UHPLC-QTRAP-MS (7500 QTRAP, AB Sciex Inc., Framingham, MA, USA) with the Kinetex® C18 (Phenomenex, Torrance, CA, USA). The development was based on the LC conditions described by Casati et al. (1) and MS conditions used previously in our laboratory with the XBridge BEH C18 column (Waters Corp, Milford, MA, USA). First, we tested the original LC and MS conditions. Then, the optimization of the method was performed, including: shortening of the separation time by changing the starting gradient, decreasing the flow rate, and tuning MS method. For low abundant samples, like extracellular vesicles, an on-line solid phase extraction method was developed.

Results obtained with different conditions were compared in terms of the dynamic range as well as the resolution of the peaks (defined as the width at the 50%) of each of the 18 analyzed compounds. The method with shorter separation time, lower flow rate and tuned MS conditions was chosen. The dynamic range with lower quantitation limit was obtained for four of the compounds (2-AG, 2-arachidonyl glyceryl ether (2-AGe), AEA and cannabidiol (CBD)). Peaks with better resolution were achieved for 2-AG, AEA and CBD (the width of the peaks was decreased about 27%, 7% and 5%, respectively).

The optimized method will be applied for the analysis of the serum samples from the CENTER-TBI project where we aim to determine the differences in serum ECs profile between patients suffering from traumatic brain injury (TBI) and healthy controls to better understand the role of ECs in TBI outcomes which may help develop new method of treatment.

1. Casati S, Giannasi C, Minoli M, Niada S, Ravelli A, Angeli I, Mergenthaler V, Ottria R, Ciuffreda P, Orioli M, Brini AT. Quantitative Lipidomic Analysis of Osteosarcoma Cell-Derived Products by UHPLC-MS/MS. Biomolecules. 2020 Sep 9;10(9):1302. doi: 10.3390/biom10091302.

P54

Title

Reproducibility and Data Pooling for large scale studirs – A Interlaboratory comparison of metabolomics analyses of plasma using biocrates kit technology

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Abstract text

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 is required. Moreover, the size of cohort studies is increasing and poses a challenge to sustainability, quality management and quality control for metabolite profiling. Kit-based metabolomics assays with standardized workflows have the potential to meet these requirements. We investigated the analytical performance of the MxP® Quant 500 kit (biocrates life sicence ag.) across 14 labs worldwide, performed on various models and manufacture type of LC-MS/MS instruments. The kit allows the determination of up to 634 metabolites. Each participating laboratory was provided with a set of 12 identical samples including human and rodent serum and plasma. Preprocessed data (µmol/L) were collected and submitted to joint statistical analyses. Out of 561 normalized metabolites with values above LOD in more than three laboratories we observed good reproducibility of measurements across laboratories with CV less than 25% for 400 (rat plasma) to 494 (NIST 1950) metabolites. The results of this project will facilitate study design and multicenter studies by provision of quality data and metabolite coverage in different matrices.

P55

Title
Circulating lipoprotein subfractions and microRNAs as potential biomarkers for improved risk prediction of myocardial infarction: the HUNT study

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Abstract text
The current ability of risk prediction models to detect high-risk individuals of myocardial infarction (MI) is limited. Circulating biomarkers associated with coronary atherosclerosis may have the potential to improve risk prediction of MI. We therefore aimed to investigate the association between future percutaneous intervention (PCI) or MI and circulating biomarkers in apparently healthy participants with a predicted low 10-year risk of MI, calculated by the risk prediction model NORRISK 2, and to assess the biomarkers' performance as potential predictors. Blood samples from 300 participants from the Trøndelag Health Study were analyzed for lipoprotein subfractions and microRNA (miRs) with NMR spectroscopy and qPCR, respectively. Within 10 years, cases developed severe atherosclerosis that required PCI or caused fatal or non-fatal MI, while controls remained apparently healthy. Elastic net combined with bootstrapping were used to analyze the association between the circulating biomarkers and future coronary event, as well as to perform variable selection in different sets of predictors. 10-fold cross-validated area under the receiver operating curve (AUC) was used to assess the predictive performance of the selected set of variables from elastic net. miR-424-5p and triglycerides in low-density lipoprotein 4 (LDL-4) were the circulating biomarkers most strongly associated with a future coronary event. For every unit increase in miR-424-5p and triglycerides in LDL-4, there were an estimated 39% and 21% increased odds of coronary event, respectively. When stratified by sex, Family history of MI and triglycerides in LDL-4 were found to be the predictors most strongly associated with future coronary event among women and men, respectively. The combination of miR-424-5p, triglycerides in LDL-4, and Apolipoprotein A2 (Apo-A2) in high-density lipoprotein 4 (HDL-4), selected as the best subset among all predictors, showed a moderate predictive performance (AUC 0.67, 95% CI 0.59 to 0.74). The best subset of predictors in women included miR-424-5p, miR-201-3p, and family history of MI (AUC 0.66, 95% CI 0.54 to 0.78), and the best subset in men included triglycerides in LDL-4, Apolipoprotein A1 (Apo-A1) in HDL-1, and the ratio Apolipoprotein B/Apo-A1 (AUC 0.65, 95% CI 0.55 to 0.75). In conclusion, these biomarkers may reflect subclinical atherosclerosis and hold the potential to improve risk prediction algorithms for MI. This should be investigated in larger studies, and the possible enhanced importance of lipoprotein subfractions as risk markers for men, and miRs for women, should be further explored.

P56

Title

Immobilized Enzymes on Magnetic Beads for Separate Mass Spectrometric Investigation of Human Phase II Metabolite Classes

Authors

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Abstract text

The clearance of xenobiotics in the human body is a multistep process consisting of phase I and phase II modifications. These biotransformations require a series of enzymes that convert the non-endogenous metabolites into compounds with higher hydrophilicity. Xenobiotics are important for diverse research fields such as biomarker discovery, toxicology, nutrition, doping control, and microbiome metabolism. The two major phase II modifications, sulfation and glucuronidation, have been linked to microbiota-human host co-metabolism. Furthermore, the importance of the microbiome metabolism on the conversion of dietary compounds has been revealed to impact human physiology (1).

One of the major challenges in this field has been the separate investigation of these two phase II modifications and their corresponding unconjugated aglycons. We have now developed a new methodology utilizing an immobilized arylsulfatase and an immobilized β-glucuronidase to magnetic beads for treatment of human urine samples obtained from a dietary intervention study (2). After the selective conversion of sulfated and glucuronidated metabolites using these immobilized recombinant enzymes, the samples were subjected to UHPLC-MS/MS analysis. The obtained raw data were then processed with R using the XCMS metabolomics framework to selectively identify metabolites with a sulfate and glucuronide moiety (3). The metabolite structure was either validated via authentic standards or MS/MS fragmentation analysis.

The separate mass spectrometric investigation of each metabolite class in a single sample was successfully applied to obtain the dietary glucuronidation and sulfation profile of about 100 compounds. This methodology allows for the identification of the metabolite structure for all three metabolite forms (unconjugated, glucuronidated and sulfated) in the same urine sample. Our new Chemical Biology strategy provides a new tool for the investigation of metabolites in biological samples with the potential for broad-scale application in metabolomics, nutrition and microbiome studies.

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(2) Tsiara I, et al. Immobilized Enzymes on Magnetic Beads for Separate Mass Spectrometric Investigation of Human Phase II Metabolite Classes. Analytical Chemistry. 2023. doi: 10.1021/acs.analchem.3c02988.
(3) Ballet C, et al. New enzymatic and mass spectrometric methodology for the selective investigation of gut microbiota-derived metabolites. Chemical Science. 2018;9(29):6233-9.

P57

Title
Comparison of serum metabolome profiles of four types of solid cancers by MS and NMR approaches

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Abstract text
Metabolic analysis of cancer-specific profiles is a powerful method for understanding complex molecular changes characteristic to the promotion and progression of different types of tumors. In our study, we implemented two analytical approaches: high-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) spectroscopy to examine the metabolomic features of four types of solid cancer. We analyzed metabolites presented in sera collected from women patients with breast (n = 35), head and neck (n = 32), lung (n = 35), and colorectal (n = 30) cancers to reveal patterns characteristic for each group of patients.
Metabolic signatures of all four malignancies were compared based on both types of analytical approaches.
The metabolic profile of colon cancer patients was the most distinct while the profiles of lung cancer and head and neck cancer were the most similar to each other. Colon cancer was characterized by the lowest levels of lipids (lysophosphatidylcholines, cholesteryl esters, and triglycerides in particular), lipoproteins, and amino acids. On the other hand, breast cancer patients were characterized by relatively high concentrations of lipids (cholesteryl esters and sphingomyelins in particular) and low concentrations of glycans. Noteworthy, only a minor correlation between cancer stages and metabolic patterns was observed, which indicated that cancer-type-specific features might be more important than cancer-stage-specific features when metabolic patterns are observed at the systemic level in patients' serum. We concluded that the analysis of serum metabolome provides new information about molecular differences between different types of solid cancers.

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P58

Title

Biochemical profiling of porcine burn wound healing following treatment with acellular skin grafts

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Abstract text

Biomaterials are commonly used to treat wounds resulting from chronic disease and injury. Among them, acellular fish skin graft (AFSG) and fetal bovine skin graft (FBS) are used due to their molecular and structural properties that support wound healing resulting in less scar tissue formation and pain modulation. While the benefits of treatment are clear, how AFSG modulates the biochemical profiles of wound healing is not understood but of importance for understanding AFSG mechanism of action and for advancing wound care. We are working towards understanding how AFSG influences lipid, metabolite and protein profiles of burn wound healing using a mass spectrometry based data driven approach.

Partial (PTBW) and full (FTBW) thickness burn wounds (n=4 for each type) were created on Yorkishire pigs (n=4). The PTBW were treated either with AFSG or with fetal bovine dermis grafts and the FTBW were treated either with AFSG or cadaver skin initially and followed by a split thickness skin graft. Punch biopsies were collected over time (day 7, 14, 21, 28 and 60) and extracted in order to measure approximately 50 derivatives of EPA, DHA, arachidonic acid (AA), linoleic acid (LA) metabolic pathways by targeted UPLC-MS/MS. Untargeted metabolomics analysis using UPLC/Q-TOF-MS, and label free quantitative shotgun proteomics by UPLC/Orbitrap-MS of tissue biopsies were also performed.

In the partial thickness burn wounds, EPA and DHA derivatives, including 18-HEPE and 17-HDHA, were significantly increased at day 7 in the AFSG treated wounds. A similar but non-significant trend was observed in full thickness wounds. Prostaglandin F2a and its 15-keto derivative from the AA pathway along with 13-HODE and 13-HOTrE from the LA pathway increased significantly at day 7 independent of treatment. Untargeted metabolomics of tissue biopsies revealed differences in amino acid, nucleoside and carbohydrate metabolic profiles only at day 7 irrespective of wound type. Changes in proteomics profiles during healing were primarily characterized by the downregulation of proteins involved in keratinization and the immune response in both partial and full thickness burn wounds.

Our results demonstrate that burn wound treatment with AFSG can lead to the earlier formation of lipid mediators involved in the resolution of inflammation that is in part driven by changes to protein and/or cellular composition of the wound.

P59

Title

Integration of proteomics and metabolomics data in a case-control study of Graves disease

Authors

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Introduction

Graves disease (GD) is a common autoimmune disease. Most patients have hyperthyroidism, but normal and low levels of thyroid hormone my also exist. In a recent report we described the assosiation of GD with a panel of 92 inflammation related protein biomarkers in 100 patients and 120 healthy controls. In a follow up study we related the same GD patients to a panel of metabolite biomarkers + 4 protein markers measured by Bevital AS. The purpose of this work was to combine data from the two studies to explore how deeper mechanistic insights might be gained from integration of different omics data.

Methods

The combined data represents 100 GD patients and 63 controls. Quantitative protein biomarker data measured by Olink with their proximity extension assay (PEA) was provided as relative values (log2 transformed). We applied the same transformation to metabolomics and protein data measured by Bevital AS. Data was combined in a concatenated/horizontal fashion and the aggregated biomarkers formed a common basis for missing values and outlier treatment. The main analysis was performed using a hierarchial analysis strategy: Factor analysis was performed on a subset of 16 Bevital metabolite + 4 Bevital protein biomarkers and a solution with two well characterized factors (uncorrelated weighted linear combinations of biomarkers) was used to predict both Graves status and protein biomarkers.

R*esults*

One of the factors from the factor analysis exhibited a metabolite profile/signature consistent with inflammation mediated activation of the Trp catabolic pathway (i.e. strong loadings for three key metabolites in that pathway). This factor, labeled KP-activation, was used to predict other variables in the combined dataset. KP-activation predicted Graves disease more strongly than single biomarkers and exhibited strong associations with a subset of the protein biomarkers measured by Olink.

Conclusion

Metabolomics data demonstrate that Graves disease is linked to activation of the Trp catabolic pathway (also known as kynurenine pathway, KP) which produces a number of bioactive intermediates related to pro and anti-inflammatory functions. The same metabolite profile was associated with the subset of protein biomarkers that most strongly predicted GD in univariate analyses. The nominal description of several of the Olink protein biomarkers points to a role in Th1 cellular immunity activation. The results from this study indicates which of those are particularly associated with kynurenine pathway activation, thus, adding a layer of information above that obtained by analysing proteomics or metabolomics data on their own.

P60

Title:
Longitudinal associations of sleep duration, vitamin D, and grain intake with serum fatty amides measured by LC-MS from childhood to adolescence

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Abstract:

Lifestyle factors, such as diet and sleep, play a significant role in development of cardiometabolic risk factors in children and adolescents. In addition, poor diet quality and shortened sleep duration have been linked to an increased risk of cardiometabolic diseases later in life. Studies suggest that fatty amides may play an important role in cardiometabolic health; higher circulating levels of some fatty amides have been associated with a lower risk of cardiovascular disease in individuals with type 2 diabetes, while other fatty amides have been negatively associated with diet-induced obesity, insulin resistance, and fatty liver disease in mice. Thus, there is a need for continued research to better understand the role of these lifestyle factors in cardiometabolic diseases, and to develop effective prevention and treatment strategies. We investigated the associations of sleep duration, vitamin D intake, and intake of low-fiber grain products with serum fatty amides measured with LC-MS in a general population of children aged 6-9 years followed-up for two and eight years until adolescence (n = 504 at baseline). Linear mixed-effects models adjusted for sex, age, time, and body mass index were used to analyze these longitudinal associations using the R software. Sleep duration and vitamin D were positively associated with 11 fatty amides and negatively with two fatty amides docosanamide and N-undecanoylglycine ($p<0.05$). Vitamin D intake was negatively associated with 11 fatty amides ($p<0.05$), while low-fiber grain product intake was positively associated with nine fatty amides ($p<0.05$). Sleep duration, vitamin D intake and low-fiber grain product intake were associated with capsiamide, linoleamide, myristamide, oleamide, palmitic amide, and palmitoleamide ($p<0.05$). These findings suggest that sleep duration, vitamin D, and low-fiber grain product intake are associated with serum fatty amides at younger ages, highlighting the importance of addressing these factors to reduce cardiometabolic risk since childhood.

P61

Title
Isotope-guided metabolomics dissects kidney arginine metabolism

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Abstract text

Background-Aim: L-arginine is a key amino acid for organism detoxification, protein metabolism, and kidney and cardiovascular health. In metabolism, ammonia is incorporated into urea through the transformation of arginine to ornithine and urea. Due to the prevalence of this reaction in the liver, its occurrence in extrahepatic tissues, such as the kidney is often neglected, even though can be associated with the increased load of kidney in cases of injury. This study aims to detect the arginine uptake and its metabolic fate in kidney *in vivo* and *ex-vivo* models by metabolic flux analysis. **Method:** Healthy mice (n=22) were fed with normal and $^{13}C_6$-$^{15}N_4$-labelled Arginine diet. Kidneys were collected and separated to cortex and medulla. The extracted metabolites were analyzed with UHPLC/QQQ-based mass spectrometry targeting metabolites of the urea cycle (arginine, citrulline, ornithine, urea), the polyamines pathway (agmatine), and the nitric oxide (NO) synthesis ($^{13}C_6$-Citrulline). In addition, isolated glomeruli, cortical tubules and micro-dissected nephron segments from healthy mice were incubated with $^{13}C_6$-labeled arginine and analyzed for the same pathways. **Results:** Arginine was taken up by all cortical segments except for the proximal straight tubule. It was mainly metabolized towards ornithine and urea, excluding the thin ascending limb of the Henle's loop. Urea was only detected in the distal convoluted tubule. It also contributed to the polyamines' synthesis and NO pathway in the proximal convoluted tubule and the collecting duct. In the glomeruli, the polyamines synthesis was primarily observed. Methylated forms of Arginine were detected as well. **Conclusion:** This study allowed for the detection of the basic arginine's metabolites in the renal compartments and offers the potential to explore a detailed isotope-based arginine metabolic flux. The role and in vivo fate of arginine in kidney disease models will be determined next.

P62

Title
Analysis of biological samples by fast HILIC separations of nucleotides, and RPLC separation of 3-NPH derivatized carboxylic acids with LC-MS/MS

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Abstract text

Nucleotides, deoxynucleotides, and coenzymes comprise a wide range of phosphate-containing metabolites of zwitterionic nature and high polarity. Together with small carboxylic acids they constitute a family of compounds that participate in key metabolic pathways, such as glycolysis, pentose phosphate pathway, tricarboxylic acid cycle and synthesis of nucleic acids. Due to their physical-chemical properties, separation, and sensitive quantification by LC-MS/MS without using ion-pairing reagents remain to be challenging. We developed HILIC methods that enable separation of 42 nucleotides and other phosphorylated metabolites within a 15-min run time on a 5-cm column and adopted similar approach to quantify some of the nucleotides on short columns. At the same time, 3-NPH derivatization proved to be a sensitive complementary technique for quantification of small carboxylic acids.
In HILIC mode, the optimization of separation conditions resulted in a gradient elution with mobile phase composed of (A) 10 mM ammonium acetate with 5 µM medronic acid in water of pH 6.8 and (B) 10 mM ammonium acetate in 90% acetonitrile. The HILIC methods enable targeted analysis of 42 phosphorylated metabolites, for example coenzymes, mono-, di-, and triphosphates of adenosine, guanosine, uridine, and cytosine. Specifically, these methods were used for analysis of bacteria, liver, muscle, human plasma, and white adipose tissue. By optimization of separation on 3-cm columns (iHILIC-P Classic and BEH Amide), we were able to reach faster separation and analyse phosphorylated metabolites in the samples within a 5.5-minute run with acceptable accuracy. Similarly, 3-cm column (iHILIC-Fusion) was used for separation of NAD, NADH, NADP, and NADPH within a 4-minute analysis time. Extraction of the NAD cofactors was optimized with various extraction solvents, finally the NAD cofactors were quantified in 10 mg of mouse liver after extraction with 0.1 M formic acid in 80% MeOH.
The 3-NPH derivatization was used for analysis of TCA carboxylic acids in C2C12 myotubes and in human plasma. Although 3-NPH derivatization is well known and has been described before, we found out that the derivatization forms various species of polycarboxylic and oxoacids. By discovering the most abundant species of citrate and isocitrate and optimizing their MRM transitions, we managed to improve the sensitivity and separation. Unfortunately, most of the tested acids were detected in derivatized blanks (mainly lactate, succinate, pyruvate), which was also confirmed by HRMS measurement using LC-QTOF.

P63

Title
Dynamics of gut metabolome and microbiome maturation during early life

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Abstract text

Early-life gut microbiome-metabolome crosstalk has a pivotal role in the maintenance of host physiology. However, our understanding on early-life gut microbiome-metabolome maturation trajectories in humans remains limited. This study aims to explore the longitudinal patterns of gut metabolites during early life, and how they are related to gut microbiota composition in birth cohort samples of n = 670 children collected at 2.5 (n=272), 6 (n=232), 14 (n=289), and 30 months (n=157) of age.
Factor analysis showed that breastfeeding has an effect on several metabolites including secondary bile acids. We found that the prevalent gut microbial abundances were associated with metabolite levels, especially in the 2.5 months-olds. We also demonstrated that the prevalent early colonizers *Bacteroides*, *Escherichia* and *Bifidobacterium* abundances associated with microbial metabolites bile acids especially in the breastfed infants.

Taken together, our results suggests that as the microbiome matures during the early-life there is an association with the metabolome composition in an analogous fashion to how the genome information mature during early life.

P64

Title
Multi-omics fingerprint of *in vitro* bioengineered heart-on-chip platform

Authors
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Abstract text

In the last decades there is an increase demand to clearly define the effects of pollutants in the ecosystems and living organisms, especially those without regulation. Contrarily to this raising of environmental awareness, chemical toxicity is addressed by animal testing. To avoid this particular paradox, and to incorporate the 3R's principle in toxicology, tissue engineering is being used to develop more innovative approaches to test toxicological effects and identify the risk assessment of potentially harmful chemicals present in the environment. The use of different scaffolds, materials and gel-based matrices to grow and maintain the cells are the main cores on the tissue bioengineering.

However, although some determinations in these systems are already developed and rutinary analyzed (cell viability, oxidative stress…), any multi-omics approach is conducted, and only scarce applications on individual omics analysis (some proteomics studies or targeted transcriptomics by PCR) can be found. Two main problems can be encountered when facing omics analysis using organ-on-chip: 1) the obtention of the embedded cells in the matrix maintaining intact their integrity to, afterwards, extract the molecules of interest; and 2) the limited amount of sample to perform different omics analysis (especially critical in metabolomics).

Here, we developed and optimized the protocols to analyze, under a multi-omics perspective (metabolomics, proteomics and transcriptomics), samples derived from a gelatin methacryloyl-based matrix (GelMA) heart-on-chip platform. The optimization was performed not only for the analytical methodologies, but also for the cell harvesting and the extraction protocols to obtain the maximum coverage of metabolites, proteins and RNA.

The first step was to test different enzymatic and non-enzymatic commercial methods to harvest cells from GelMA hydrogel. The enzymatic methods based on collagenase were the most suitable for a high-throughput sample preparation in terms of speed, easiness, and low interference with all the omics analysis.
Secondly, extraction protocols were selected to maximize the number of molecules and avoid their degradation. In this case, the simplest extraction procedures were the most suitable. Thus, a commercial kit to purify RNA (transcriptomics), tryptic digestion and isobaric TMT labelling (proteomics) and methanolic extraction of metabolites (metabolomics) were used.
Finally, the methodologies adopted to analyze the samples were RNA-seq by Illumina platform for transcriptomics, nanoLC-Orbitrap-MS for proteomics and LC-QqQ/QTOF-MS for metabolomics.

The above methodologies detected around 20000 genes, 500 proteins and 200 metabolites involved on glycolysis, Krebs cycle, biosynthesis of amino acids, lipogenesis/lipolysis, calcium signaling pathway, cardiac contraction and cell adhesion, among others.

P65

Title
Metabolite changes during curative treatment of Prostate Cancer

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Abstract text

High-risk prostate cancer patients are often treated with a combination therapy consisting of androgen deprivation therapy (ADT) and radiotherapy (RT) to the prostate. Our aim is to investigate significant differences in metabolite levels during this two-step treatment and to correlate the changes to the outcome of the disease. To this end, blood samples obtained in the UCAN-project (1), were sent to Metabolon for analysis (LC/MS).

The blood samples were taken at four different time points: before ADT, between ADT and RT, after RT and finally a follow-up sample taken 5 - 10 months after RT. In total 143 samples from 35 patients were analysed. After appropriate processing of the raw data, different data analysis methods were applied. In order to make paired comparisons of metabolite levels for the different stages of treatment OPLS-EP (2) models were calculated. In addition, Volcano Plots were produced in order to reveal interesting metabolites.

The OPLS-EP models performed well ($R2 > 0.9$, $Q2 > 0.65$) indicating the existence of significant changes in metabolite levels during treatment of prostate cancer. Further investigation into the metabolite levels revealed different types of patterns, for example for some metabolites the concentration levels went down during the whole course of treatment while for others the levels went down during ADT but up during RT. Identified top metabolites were defined based on appropriate cutoff-values for the p-values (paired t-test) and the Fold Change (FC). Some of the top metabolites have been reported elsewhere in connection to prostate cancer or other diseases; however some metabolites were unique for this study.

Further investigation into the top metabolites as well as correlating the differences in metabolite levels with the outcome of the disease is underway.

(1) Glimelius B, et al. U-CAN: a prospective longitudinal collection of biomaterials and clinical information from adult cancer patients in Sweden. Acta Oncologica. 2018, 57:2, 187-194
(2) Jonsson P, et al. Constrained randomization and multivariate effect projections improve information extraction and biomarker pattern discovery in metabolomics studies involving dependent samples. Metabolomics. 2015, 11: 1667 - 1678

P66

Title
An integrated molecular networking based non-targeted PFAS analysis workflow enables the identification of novel targets in NIST plasma.

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Abstract text

PFAS are a pervasive group of enduring toxic chemicals that have attracted the attention of health and regulatory agencies globally. However, conventional regulatory monitoring for PFAS is limited due to the need for reference standards and doesn't cover testing in humans, where PFAS bioaccumulation is known. Unlike traditional targets where reference standards are available, less than 200 PFAS standards exist for the more than 9,000 known PFAS, underscoring the need for a non-targeted workflow. Traditional non-targeted PFAS analysis workflows rely on direct spectral library matches, screening for signature fragments, homologous series with progressive retention times tied to chain length, negative mass defect (MD), and CF2 Kendrick MD. These conventional approaches are individually successful at identifying PFAS but aligning their outputs and exploiting them via integration with emerging techniques in complex matrices such as human plasma remains challenging.
This poster describes the comprehensive integration of emerging and conventional PFAS analysis techniques into a singular Compound Discoverer™ software workflow optimized for analyzing NIST plasma, a widely available reference material. In addition, built-in data reduction approaches will be demonstrated, including fragmentation-based target filtering leveraging similarity searches via the mzCloud™ spectral library, Fluoromatch Suite database containing over 700 PFAS signature fragments, and molecular networking. These approaches circumvent the lack of authentic standard availability, sparse coverage in spectral libraries, and limitations with negative mode in-silico fragmentation.
Additional data reduction tools, including extensive mass lists of known and theoretical PFAS, MD filtering thresholds specific to fluorine-containing compounds, CF2 Kendrick MD, and orthogonal MS1 PFAS discrimination plots, ensure the retention of only targets exhibiting PFAS characteristics. Finally, a molecular network from all the preserved annotations in NIST plasma is showcased, containing clusters of known and previously unidentified PFAS neglected by conventional techniques. These findings and workflow increase accessibility for plasma-based PFAS analysis

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Title

Effects of FODMAPs and gluten on irritable bowel syndrome, from self-reported symptoms to molecular profiling

Authors

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Irritable bowel syndrome (IBS) is a complex disorder of gut-brain interactions. The diagnosis of IBS is based on subjective reporting of abdominal pain and altered bowel habits. Dietary regimens for symptom management include a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) diet and a gluten-free diet. However, scientific evidence supporting these dietary recommendations is weak: Trials have been non-blinded and underpowered. Mechanistic understanding and objective markers of response remain scarce. Therefore, the aim of this thesis was to conduct a large double-blind study to investigate the effect of FODMAPs and gluten on symptomatic and molecular data (including untargeted metabolomics and 16S rRNA analysis of the gut microbiota), both at a group and subgroup (differential response) level.

Trial data revealed that gluten caused no symptoms and FODMAPs triggered only modest symptoms of IBS. FODMAPs increased saccharolytic microbial genera, phenolic-derived metabolites and 3-indolepropionate, but decreased bile acids. Specifically, the genera *Agathobacter*, *Anaerostipes*, *Fusicatenibacter*, and *Bifidobacterium* correlated with increased plasma concentrations of phenolic-derived metabolites and 3-indolepropionate, i.e, metabolites related to decreased risk of incident type 2 diabetes and inflammation. Indeed, among FODMAP-related metabolites, only weak correlations to IBS symptoms were detected, as in the case of 3-indolepropionate to abdominal pain and interference with quality of life. Gluten had only a modest effect on unidentified lipid metabolites, but with no interpretable link to health.

No molecular markers of a differential response were found, despite a comprehensive exploration with multiple analytical approaches. This could be explained by the absence of baseline variables, such as other omics layers or psychological factors.

In summary, the results indicate that gluten does not cause IBS symptoms. Moreover, the minor effect of FODMAPs on IBS symptoms must be weighed against their potential beneficial health effects. The complexity of IBS likely explains the absence of molecular evidence for differential responses.

P68

Title
Global metabolomics and lipidomics in a university hospital setting

Authors
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Global metabolomics and lipidomics has gained widespread use in medical research. Its usefulness in biomarker discovery and in unravelling the biochemical consequences of pathological processes is undoubted. At Oslo University Hospital we have during the last decade, based on standardized clinical chemistry principles for medical diagnostics, established robust LC-MS-based platforms for both global metabolomics and global lipidomics and established protocols for analysis of all types of biofluids. Based on this, we were recently approved as Core Facility for Global Metabolomics and Lipidomics at University of Oslo.
Taking research tools into everyday diagnostics is a long journey. It requires identification of the challenges, and putting efforts in overcoming them. Important factors are control of pre-analytical factors, sample logistics, competence in performing the global analysis, competence in quality assurance and analytical interpretation, reporting of results and competence in medical interpretation. We will present and share some of our experiences and thoughts on the long and work-demanding process of building global metabolomics and lipidomics in both clinical research and diagnostics.

P69

Title
Metabolomic and lipidomic approaches in mindfulness-based intervention for health-care students

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Abstract text

Anxiety, stress, and manifestations of depressive cases are the most prevalent psychiatric disorders and affect an increasingly large proportion of the population worldwide. In the case of health-care professionals, high levels of stress are not only impacting their personal life but can also lead to a decrease in empathy and compassion, which translates to worse patient outcomes and increased rates of professional errors. In this context, strategies such as mindfulness-based intervention (MBIs) are a promising approach to stress management. Benefits related to MBIs have already been reported in several studies, however, the biological and physiological effects of these interventions on stress reactivity are not well understood. We therefore aimed to characterize the biochemical responses to MBI in a cohort of 28 health care students in Geneva, Switzerland. The intervention was organized over 8 weekly sessions and students were sampled longitudinally before and after the program as well as 6 months later, with the control group was undergoing the MBI after the third sampling. Blood serum was collected and analyzed using untargeted metabolomics and lipidomics platforms and stress was evaluated using several well-established protocols, such as the perceived stress scale and the prosocialness scale in adults. A total of 124 metabolites and 774 lipids were annotated. Metabolic profiles were varied with MBI impacting amino acid and nucleoside in females, while males displayed increased acetylneuraminic acid after the intervention. In terms of lipids, lower triglycerides and phosphatidylethanolamines (PE) and higher etherPE were associated to MBI in male volunteers.

P70

Title
A quantitative method for analysis of the oat specific compounds avenanthramides and avenacosides in human plasma samples.

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Abstract text

Oats is a rich source of avenanthramides (AVAs) and avenacosides (AVEs). These compounds are unique to oats and have been shown to have interesting bioactive properties that may contribute to the well-established cardiometabolic effects of oats. Due to their unique presence in oats, they could potentially serve as specific biomarkers of oat intake. In order to evaluate their potential as biomarkers, an accurate and applicable quantitative method for their analysis in human plasma samples is warranted. Currently, no method is available for simultaneous quantification of both AVAs and AVEs. The aim of this study was therefore to establish a rapid and sensitive method for robust analysis of most common AVAs and AVEs in human samples and test the applicability of the method on samples from a pharmacokinetic study with single and repeated intakes of two different oat products. Human plasma samples were analyzed with an LC-MS/MS method adapted from a previously established method for analysis of AVAs in plasma (1) to allow quantification of low concentrations of AVAs and AVEs simultaneously. Total AVAs and AVEs were extracted with acetonitrile after enzymatic cleavage of the glucuronide moiety and a clean-up step with SPE increased significantly the recovery of AVE A. The established method has a linear range of 0.7- 133.7 nmol/L AVA 2p, 0.7-121.5 nmol/L for AVA 2f, 0.6-121.5 nmol/L for AVA 2c and 1.2-37.6 nmol/L for AVE A and 1.6-32.6 nmol/L for AVE B. The LLOQ for AVA 2p is 0.7 nmol/L, 0.3 nmol/L for AVA 2f and AVA-2c and 1.2 and 1.1 nmol/LAVA A and B respectively. Average extraction recoveries were 82% for AVE A and AVA 2c, 83% for AVA 2p and AVA 2f and 71% for AVE B. Additionally, the less abundant AVAs 2pd and 2fd can be analyzed in a semi-quantitative manner, using calibration curves of structurally close AVAs. The method is applied on an ongoing fit-for-purpose intervention study with average intra-batch variation is at 7% for all compounds and the in-progress assessment of inter-batch variation.

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P71

Title
System suitability testing of LC-IMS-HRMS for metabolomics applications

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Abstract text

Liquid chromatography-mass spectrometry (LC-MS) produces a vast amount of data from complex samples, particularly in the diverse chemical space of metabolomics. Extracted information required for analysis includes LC retention times, accurate masses, isotope patterns, MS/MS fragmentation patterns and, for systems with ion mobility spectrometry (IMS), collisional cross section (CCS) values. Ultimately, these parameters are used to confidently annotate and quantify metabolites of interest in a given study.
It is challenging, and often overlooked, to monitor system performance for accuracy and precision during the long experimental sequences and multiple batches without substantial manual data curation. To ensure downstream metabolomic data analysis will be meaningful and comparable across a sample cohorts, instrument performance should be appropriately qualified prior to and during data acquisition using standards relevant to the sample cohort – in this case small molecule metabolites.
Here we propose a System Suitability Test (SST) using an aqueous metabolite reference mixture and quality control method that validates system performance, in this case a timsTOF Pro 2, specifically for metabolomics experiments. For this purpose, 20 reference standards were selected from literature with the prerequisites of being relevant to endogenous metabolism, water soluble, and non-hazardous. The chromatographic method was developed with the aim to deliver reliable separation of leucine and isoleucine and mobility separation of melezitose and maltotriose at a total runtime of less than 7 minutes. When used as a QC mixture, the SST mixtures will be able to discover degrading performance trends or acute hardware issues in advance.

P72

Title
Non-targeted metabolomics of urine to characterize benign and malignant canine mammary cancer subtypes,

Authors
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Abstract text

Background: Cancer is an enormous burden to society and afflicts dogs and humans similarly. It is estimated that every third dog will develop cancer during its lifespan and the incidence of canine mammary cancer (CMC) is particularly high. Depending on a variety of factors, different subtypes of canine mammary tumours (CMTs) can occur, exhibiting varying levels of risk depending on their location, their growth and ability to metastasize. The metabolomic signatures of different CMT subtypes have not been characterized and compared in urine, a non-invasive type of biosample. Modifiable risk factors that influence CMC incidence and what type of CMTs will form, are not well recognized, though factors such as aging and certain genes have been identified. Diets, a highly modifiable risk factor, and further research is needed on CMC's relationships to different forms of feeding and their nutritional qualities. Variation in diets may also confound the metabolomic signatures given from differing CMT subtypes and we aimed to explore this relationship. Hence, in the present study our goal was twofold: to characterize the differences in the metabolomic profile of urine of dogs with various forms of both benign and malignant CMTs, and to determine whether and to what extent the dogs' general diet in adult life confounds the metabolic differences observed as a result of CMT characteristics.
Materials & Methods: Urine was collected from dogs (n=134) with malignant CMTs (n=58), benign CMTs (34), and healthy controls (n=52) and their metabolic profile was analysed using non-targeted LC-MS. The urine samples of dogs with CMTs were collected on the same morning prior to surgical tumour removal. The tumours were characterized by veterinary pathologists and histopathological diagnosis was verified by one of the authors (AEL), who specializes in CMTs. Dogowners were asked to fill in a FFQ regarding their dogs diet types (commercial kibble, canned food, raw food, and homecooked food) and comorbidities during the urine collection. Urine creatinine was quantified using NMR and used as a normalization factor. Metabolomics data was processed and statistical analyses of the urine profiles was performed in order to evaluate how differentiable the CMT subtypes are and to what extent the diet played an effect.

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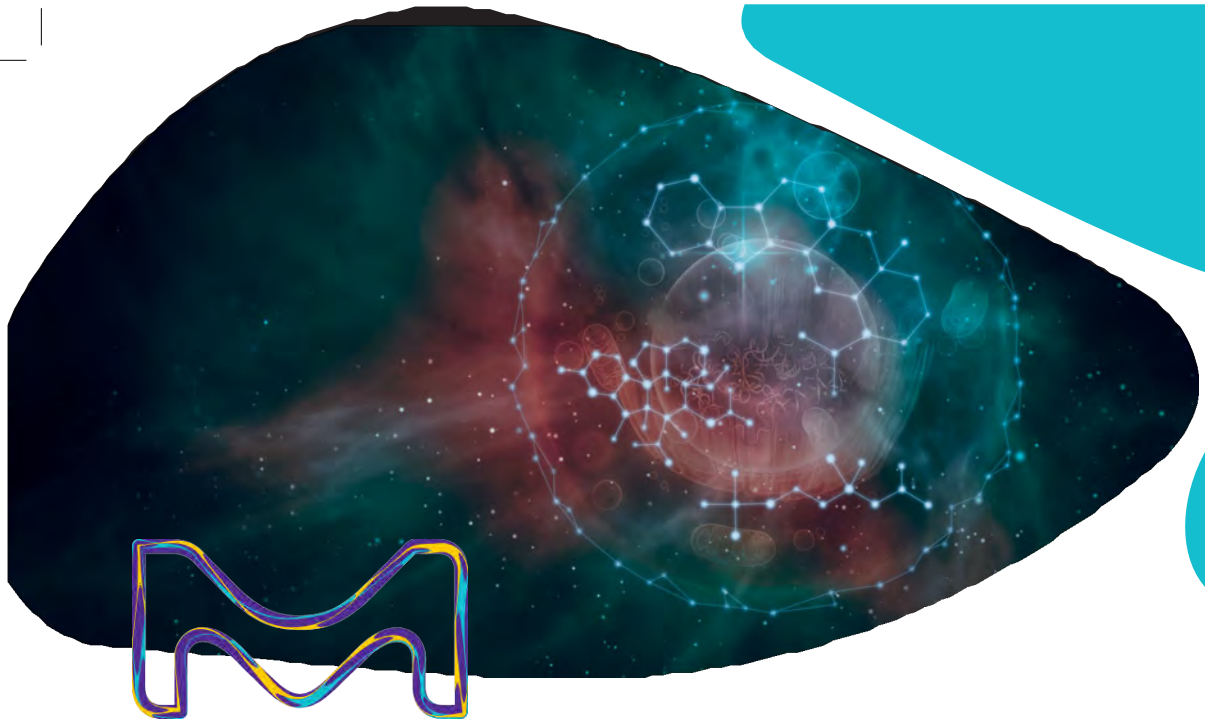

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