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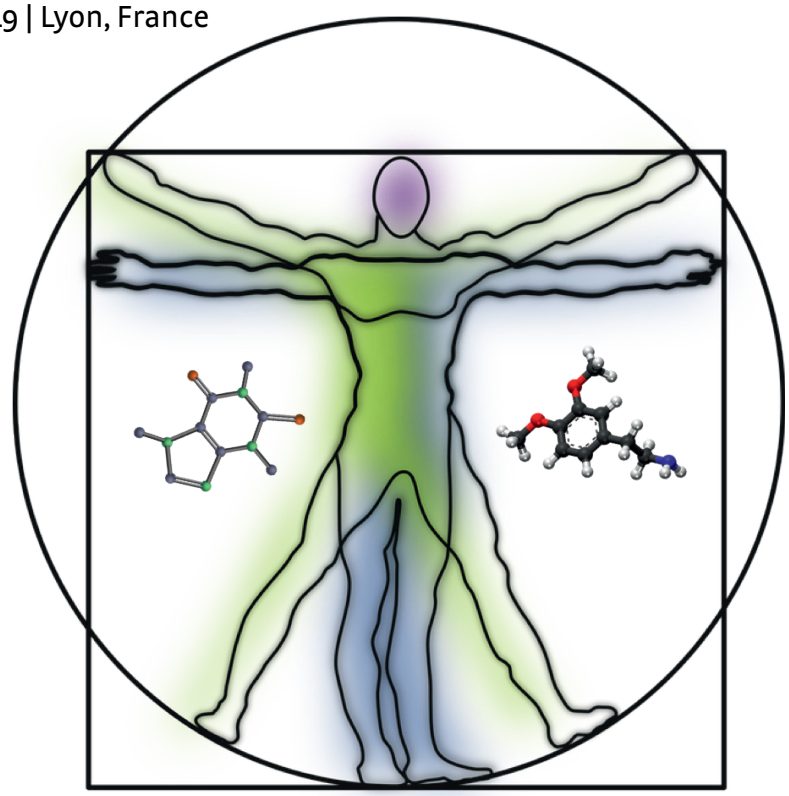
Metabolomics bioinformatics in human health

16-20 September 2019 | Lyon, France



Abstract Book

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EMBO Practical Course: "Metabolomics Bioinformatics in Human Health"

16-20 September 2019, Lyon, France

September 13, 2019

About the EMBO metabolomics course

The EMBO Practical Course "Metabolomics Bioinformatics in Human Health" will be held in the International Agency for Research on Cancer on 16-20 September 2019 and will provide an advanced overview with hands-on practical on key issues and challenges in metabolomics, handling datasets and procedures for the analysis of metabolomics data using bioinformatics tools.

Combining lectures from experts, computer-based practical sessions and interactive discussions, the EMBO Practical Course will provide a platform for discussion of the key questions and challenges in this field, from study design to metabolite identification.

This five-day course is aimed at PhD students, post-docs and researchers with at least one to two years of experience in the field of metabolomics who are seeking to improve their skills in metabolomics data analysis.

This EMBO Practical Course will not only provide face-to-face training, but also offers a unique opportunity for students to speak with experts for the duration of the course. This high-level engagement contributes to the development of a network of scientists working at all levels of the field. Integrating metabolomic data with other "omics" data is increasingly common, but requires a background understanding of the topic to generate high-quality results. Our course now looks to provide instruction for interpretation of complex omics data, allowing researchers to combine multiple dimensions of metabolism in their analyses.

During this course, you will learn about:

- Metabolomics study design and sources of experimental error
- Difference between target and un-target approaches
- Hands-on open source R based programs, XCMS, MetFrag, MetFusion, etc
- Understanding the usage of univariate and multivariate data analysis, data fusion concepts, etc
- Metabolomics downstream analyses: KEGG, BioCyc, and MetExplore for metabolic pathway and network analysis
- Metabolomics standards and data dissemination and deposition
- Metabolomics Flux and Stable Isotope Resolved Metabolomics (SIRM)

After this course you should be able to: Discuss the major principles of metabolomics experimental design and factors that impact upon subsequent analysis. Identify strengths and weaknesses in a variety of metabolomics analytical approaches. Use a range of Bioinformatics software to pre-process, process and analyse metabolomics data. Discuss current trends and challenges in metabolomics.

International Agency for Research on Cancer

World Health Organization

EMBO excellence in life sciences

EMBO Practical Course
"Metabolomics in Human Health"
IARC, Lyon, France - 16-20 September 2019

	Monday 16 September	Tuesday 17 September	Wednesday 18 September	Thursday 19 September	Friday 20 September
08:30	Registration (Karine / Sandrine)				
09:00		Overview of day (5 min)	Overview of day (5 min)	Overview of day (5 min)	Overview of the day (5 min)
09:15					
09:30	Welcome to IARC & Course overview (RS)				Deciphering metabolism using Stable Isotope Resolved Metabolomics (SIRM) using GC-MS (SMM)
09:45		Analysis of LC-MS-based metabolomics data using XCMS (PF)	GC–MS workflow; from data processing to metabolite identification - (MVC)	Simple statistics, QC, data normalisation and scaling - (HW)	
10:00	Flash Oral Poster Presentation (All)				
10:15					
10:30	Coffee Break	Coffee Break	Coffee Break + Group photo	Coffee Break	Coffee Break
10:45					
11:00					
11:15	Flash Oral Poster Presentation (All)	Analysis of LC–MS-based metabolomics data using XCMS (PF)	NMR and Computer-assisted structure elucidation (CS)	Multivariate statistics: only the brave (HW)	Metabolomics flux with applications in Cancer (MCC + IMDM)
11:30					
11:45					
12:00	Metabolomics: From experimental design to data analysis - limitations & avantages (OY)	Exploring LC–MS data matrix dealing with missing values (PF - RS)			
12:15					
12:30					
12:45	Lunch	Lunch	Lunch	Lunch	Lunch
13:00					
13:15					
13:30					
13:45		Poster session (All)	Poster session (All)	Poster session (All)	FAIR Metabolomics, metabolomics resources and reproducibility, are we there? (RS)
14:00					
14:15	Dissecting the multidisciplinary nature if an untargetd matabolomics workflow vs targeted (OY)	The whole story in real–time: MS data processing (SN)	Integrative analysis of metabolomics data through computational modelling (JH)	Data fusion and batch correction (JW)	
14:30					
14:45					Course feedback and wrap-up (IARC Staff)
15:00		The whole story in real–time: MS metabolite identification (SN)			
15:15					End of course
15:30	Tea break		Tea break	Tea break	
15:45					
16:00					
16:15	Experimental design - Group activity (RS)	Tea break		What's Next for Metabolomics? (DW)	
16:30			Metabolomics data analysis in the context of metabolic networks (FJ)		
16:45		Why is lipidomic a world apart in metabolomics ? (JBM)		LC-MS approaches for the measurement of the Exposome (AK)	
17:00					
17:15					
17:30					
17:45					
18:00	Experimental Design - Statistical point of view (PF)				
18:15					
18:30	Poster session (All)	Discussion, short presentation	Discussion, short presentation	Free time	
18:45					
19:00					
19:15	Welcome drinks and Buffet reception (IARC lobby)				
19:30		Dinner at Monplaisir Côté Cour	Dinner at IARC Restaurant	Gala Dinner at Le Vivarais	

Faculty Reza Salek (RZ); Oscar Yanes Torrado (OYT); Pietro Franceschi (PF); Steffen Neumann (SN); Justine Bertrand-Michel (JB-M); Maria Vinaixa Crevillent (MVC); Christoph Steinbeck (CS); Johannes Hertel (JH); Fabien Jourdan (FJ); Hermann Wehrens (HW); Johannes Westerhuis (JW); David Wishart (DW); Agneta Kiss (AK) Silvia Marín Martinez (SMM) Marta Cascante Serratosa (MCS); Igor Marín De Mas (IMDM);

Contents

The Abstracts

[R101] CE-MS-based plasma metabolomics for the discovery of biomarkers from a mouse model for acute epileptic seizures

Karen Segers[1,2,3], Wei Zhang[3], Debby Mangelings[1], Thomas Hankemeier[3], Dimitri De Bundel[2], Yvan Vander Heyden[1], Ilse Smolders[2], Rawi Ramautar[3], Ann Van Eeckhaut[2]

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Metabolomics is a powerful tool and is particularly promising for the discovery of novel diagnostic and prognostic disease biomarkers as well as to obtain insight into pathological mechanisms of complex diseases, such as epilepsy. Different analytical techniques are used for metabolic profiling studies. Capillary electrophoresis-mass spectrometry (CE-MS) has gained interest, due to the suitability of analysing low sample volumes. Acute seizures were generated twice using a six hertz (6 Hz) partial seizure mouse model (n=8) [1], respecting a one-week recovery. Before and after each seizure, blood was collected by capillary microsampling in order to obtain 10 μL plasma samples. Liquid-liquid extraction was performed on the plasma samples to isolate the polar metabolites. Finally, these extracted samples were analysed using a conventional CE-MS method with low pH separation conditions to selectively target cationic metabolites. The obtained metabolic profiles were normalized with an internal standard and auto-scaled before unsupervised and supervised analysis. Based on variable importance in projection scores several amino acids showed a significant decrease in plasma concentration after the first seizure. This first study shows the utility of CE-MS in metabolic profiling studies of low volume samples. [1] L. Walrave et al., Validation of the 6Hz refractory seizure mouse model for intracerebroventricularly administered compounds, Epilepsy research, 115, 67-72 (2015).

[R102] Plasma branched chain amino acid levels alteration is driven by gender in human NAFLD

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Objectives: The pathophysiological mechanisms driving Non-Alcoholic Steatohepatitis (NASH) development are still poorly understood, though are closely linked to insulin-resistance (IR) and body mass index (BMI). Current literature indicates that alterations in plasma branched chain amino acids (BCAA) are associated with obesity, IR and Non-Alcoholic Fatty Liver Disease (NAFLD). Due to the close association between NAFLD, BCAA and IR, it is currently unclear whether altered BCAA metabolism may drive NAFLD independently of changes in IR. BCAA could drive NAFLD through alterations of their catabolism, which is closely linked to mitochondrial function and glucose and lipid metabolism. In addition to their potential pathophysiological role, BCAA could be potential markers of the different stages of NAFLD. This study aimed to determine whether BCAA plasma concentrations are associated with the different stages of NAFLD in humans. Methods: We used targeted metabolomics to quantify plasma amino acids using the aTRAQ Kit (Sciex, Framingham, MA, USA) which uses internal standards of isotope-labeled amino acids. Analysis was performed using LC/MS for detection and quantification in a well-characterized patient cohort with histologically assessed NAFLD (n=112 patients). Results: Four groups of patients matched by gender were defined: 23 healthy liver (15 women, 8 men), 30 fatty liver NAFL (15 women, 15 men), 30 NASH (15 women, 15 men) and 29 NASH with advanced fibrosis (F$\geq$ 2) (14 women, 15 men). In addition, patients were selected to obtain homogeneous groups regarding main metabolic parameters ($mean + \backslash - sd$) such as BMI ($40.5 kg/m^2 \pm 6.5$), age ($42.6y \pm / - 12.5$), HOMA-IR (4.1 ± 2.8) and HbA1c ($5.6\% \pm 0.5$) to avoid confounding effects. Our preliminary results indicate that plasma BCAA are correlated with progression of NAFLD stages in a gender-dependent manner, increasing from NAFL to NASH in women, while decreasing in NASH and NASH with fibrosis in men. Conclusion: These results suggest that changes in BCAA metabolism depend on gender and could be involved in the progression from NAFL to NASH independently of altered glucose metabolism. **Keywords:** NAFLD, Branched Chain Amino Acids, Gender, Insulin Resistance, Fibrosis.

[R103] A semi-targeted MS approach to provide better coverage to study oxidative lipidomics in inflammatory disease models

Alpesh Thakker[1], Corinne Spickett[1], Andrew Pitt[1]

1. School of life and health science, Aston University

Phospholipid oxidation by adventitious damage generates a wide variety of products with potentially novel biological activities that can modulate inflammatory processes associated with various diseases such as Malaria, Acute Pancreatitis and Type 2 diabetes. To understand the biological importance of oxidised phospholipids (OxPL) and their potential role as disease biomarkers requires precise information about the abundance of these compounds in cells and tissues. There are many chemiluminescence and spectrophotometric assays available for detecting oxidised phospholipids, but they all have some limitations. Mass spectrometry coupled with liquid chromatography is a powerful and sensitive approach that can provide detailed information about the oxidative lipidome, but challenges still remain owing to detection of low abundance OxPL species in biological samples. The aim of this work is to develop improved methods for detection of OxPLs, for example by optimising the chromatographic separation by testing several reverse phase columns and solvent systems, and using targeted mass spectrometry approaches (precursor ion [PIS] and neutral loss [NL] scanning. Initial experiments were carried out using oxidation products generated in vitro from a commercially available phosphatidylcholine (PC) and phosphatidylethanolamine (PE) mixture in order to optimise the chromatography separation parameters and mass spectrometry parameters. We have evaluated the chromatographic separation of oxidised phosphatidylcholines (OxPCs) and oxidised phosphatidylethanolamines (OXPEs) using C8, C18 and C30 reverse phase, polystyrene – divinylbenzene based monolithic and mixed – mode hydrophilic interaction (HILIC) columns, interfaced with mass spectrometry. Our results suggest that the monolithic column was best able to separate short chain OxPCs and OxPEs from long chain oxidised and native PCs and PEs. Targeted mass spectrometric approaches for the selective identification of short chain OxPCs using PIS for m/z 184 Da and NL for m/z 141 Da for identification of OxPEs were tested on OxPL mixture and it enabled identification of low abundant oxidation products such as: γ-hydroxy alkenals and alkenoates and saturated aldehydes. Quantitative differences in oxidised products were observed in malarial components.

[R104] Combination of MCR-ALS and K-means information for mass spectrometry imaging data analysis

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The characterization of cancer tissues by matrix-assisted laser desorption ionization-mass spectrometry images (MALDI-MSI) is of great interest because of the power of MALDI-MS to understand the composition of biological samples and the imaging side that allows for setting spatial boundaries among tissues of different nature based on their compositional differences. In tissue-based cancer research, information on the spatial location of necrotic/tumoral cell populations can be approximately known from grayscale images of the scanned tissue slices. This study proposes as a major novelty the introduction of this physiologically-based information to help in the performance of unmixing methods, oriented to extract the MS signatures and distribution maps of the different tissues present in biological samples. Specifically, the information gathered from grayscale images will be used as a local rank constraint in Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) for the analysis of MALDI-MSI of cancer tissues. The use of this constraint, setting absence of certain kind of tissues only in clear zones of the image, will help to improve the performance of MCR-ALS and to provide a more reliable definition of the chemical MS fingerprint and location of the tissues of interest.

[R105] Metabolic phenotyping reveals potential biomarkers of diet-modifiable individual susceptibility to coronary heart disease

Stefania Noerman[1], Marietta Kokla[2], Ville M Koistinen[1], Tarja Nurmi[1], Jyrki K Virtanen*[1], Kati Hanhineva*[1,3]

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The healthy Nordic diet, as assessed by the Baltic Sea Diet Score (BSDS), seems protective against risk factors of metabolic diseases, but the differences between responders and non-responders may confound the association. In this study, we aim to highlight the metabolic intermediates and pathways involved in the association between the healthy Nordic diet and the risk of coronary heart diseases (CHD), including the ones predictive for differences in dietary responses and CHD resistance. From the sample collection of Kuopio Ischaemic Risk Factor Study, we analyzed the serum samples from the subcohort of 364 participants: 1) 88 subjects with high BSDS who did not develop CHD during the mean follow-up of 21 years (controls), 2) 94 subjects with high BSDS who develop CHD during follow-up (cases), 3) 93 CHD cases with low BSDS, and 4) 89 controls with low BSDS. The non-targeted metabolite profiling was performed with high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) analysis. While the compound annotation is still ongoing, the preliminary results show some lipophilic molecules, including eicosanoids, steroid derivatives, and phospholipids, as the differential metabolites between cases and controls, and between groups with high and low BSDS. These findings hence may suggest that lipid metabolism may potentially be involved in the association between healthy Nordic diet, individual dietary responses, and susceptibility of CHD.

[R106] Quantification of 30 tryptophan pathway metabolites in serum by HPLC-MS/MS method

Dai Long Vu[1,2], Anna Bauer[1,2], Ke Pu[1,2], Andras Balogh[1,2,3,4], Hendrik Bartolomaeus[1,2,3,4], Jennifer Kirwan[1,2]

1. Berlin Institute of Health, Berlin, Germany. 2. Max Delbruck Center for Molecular Medicine, Berlin-Buch, Germany 3. Charité - Universitätsmedizin Berlin 4. Experimental and Clinical Research Center, Berlin, Germany

Tryptophan and its metabolites play key roles in heart and brain health. The eventual fate of tryptophan involves an interplay between host and gut microbiome. Quantification of the tryptophan pathway metabolites provides important information about the eventual fate of tryptophan in the body which may be important for the study of certain diseases. Although current methods exist for the quantification of a subset of these metabolites, methods for simultaneous quantification of a broad coverage are still missing. We demonstrate a robust, accurate, reproducible method for the quantification of 31 compounds related to the tryptophan pathway using an ultrahigh performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) technique. Three other metabolites can be detected but peak shape does not allow accurate and reproducible quantification

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[R107] Syringol metabolites as biomarkers of smoked meat intake

Roland Wedekind[1], Pekka Keski-Rahkonen[1], Nivonirina Robinot [1], Vivian Viallon[1], Pietro Ferrari[1], Erwan Engel[2], Marc J. Gunter[1], Inge Huybrechts[1], Augustin Scalbert[1] on behalf of the EPIC study.

1. Nutrition and Metabolism Section, International Agency for Research on Cancer, 150 cours Albert Thomas, Lyon, France. RW, PK-R, NR, VV, PF, MG, IH, AS). 2. MASS Group, INRA UR370 QuaPA, Saint-Genès-Champanelle, France (EE)

Background: Processed meat is associated with higher risk of colorectal cancer but the estimation of intake of this heterogeneous food group in epidemiological studies is challenging because of the lack of sufficient details in dietary questionnaires. Objective: To identify novel biomarkers for processed meat intake using metabolomics. Design: An untargeted metabolomic approach based on LC-MS was applied to processed meat products previously digested in vitro, and to urine and plasma samples from a randomized crossover dietary intervention in which 12 volunteers consumed successively 3 processed meat products and two other control foods during 3 days. The identified biomarkers were then measured in urine from 474 subjects from the European Prospective Investigation into Cancer and nutrition (EPIC) cross-sectional study for which a 24h dietary recall and food frequency questionnaires were available. Results: Syringol and four derivatives of syringol were found to be characteristic of digests of smoked meat products. The same compounds present as sulfate esters in urine showed increased levels following consumption of smoked meat products in the intervention study. The same syringol sulfates were also positively associated with recent or habitual consumption of smoked meat products in urine samples from participants of the EPIC cross-sectional study. These markers showed good discriminative ability for smoked meat intake with receiver operator characteristic areas under the curve up to 0.86 and 0.79 for short-term and habitual intake, respectively. Conclusions: The biomarkers of smoked meat intake identified in this study may be used to improve assessment of smoked meat intake in epidemiological studies.

[R108] Monitoring in vivo NAD+ metabolism and its precursors by liquid chromatography-mass spectrometry-based metabolomics

Maria Pilar Giner[1], Stefan Christen[1], Mathieu Membrez[1], Mikhail V. Makarov[2], Marie Migaud[2], Carles Canto[1], Jérôme Feige[1], Sofia Moco[1]

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Nicotinamide adenine dinucleotide (NAD+) and its reduced form (NADH) are coenzymes widely employed in metabolic processes, such as glycolysis or fatty acid oxidation. In addition, NAD(H) can serve as a substrate for multiple enzymes including sirtuins and poly(ADP-ribose) polymerases (PARPs). Given the pivotal role of NAD(H) in health and disease, studying the NAD+ biosynthesis has become an attractive strategy to monitor related metabolic changes and therapies. Yet, the low stability of redox cofactors offers a challenge to obtain robust direct measurements. Here, we present a strategy, which includes sample preparation and a qualitative liquid chromatography-mass spectrometry (LC-MS) method, for the analysis of the NAD+ metabolome and its precursors. Hydrophilic interaction chromatography was able to separate in total 13 closely NAD related metabolites, without using ion pairing or derivatisation agents. Our method was used to investigate NAD+ metabolism in vivo, as mice were administered with isotopically labelled nicotinamide riboside (NR) by either gavage or intraperitoneal injection. To assess label incorporation and tissue distribution after 2 hours, the 'NAD+ metabolome' was analyzed in urine, blood, liver, and skeletal muscle. The main detected metabolites in tissues were nicotinamide (Nam), nicotinic acid adenine dinucleotide (NAAD), NAD+, NADH, nicotinamide dinucleotide phosphate (NADP+) and NADPH, as well as the methylated by-products MeXPY. Interestingly, from all the compartments, intact NR was only detected in urine, suggesting a rapid degradation to Nam in circulation. Nevertheless, NR treatment provoked a pronounced increase of NAD+ in the liver, while being less effective in the muscle. To further explore whether NR or its degradation product Nam contributed to increased NAD+ levels in the liver, the labeling patterns of Nam, NAAD and NAD+ were analyzed. These showed that both, NR and Nam, contributed to NAD+ biosynthesis. Moreover, our data suggested that NR-derived Nam does not only participate in the salvage pathway of NAD+ but also contributes to the de novo biosynthesis pathway, which gives rise to NAAD. Overall, the combination of stable isotopes and metabolomics effectively allowed us to follow differential activities of in vivo NAD+ metabolism upon treatment with NAD+ precursors, such as NR.

[R109] Pre and postnatal exposure to environmental pollutants alters lipid and polar metabolites profiles in non-obese diabetic mice

Tim Sinioja[1], Johanna Bodin[2], Aidan McGlinchey[3], Daniel Duberg[1], Hubert Dirven[2], Hanne Friis Berntsen[5,6], Karin Zimmer[6], Unni C. Nygaard[2], Matej Orešič[3,4], Tuulia Hyötyläinen[1]

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According to the World Health Organization, chronic non-communicable diseases, such as heart disease, stroke and diabetes, are causing up to 60 % of annual global mortality. The incidence of these diseases is not only due to genetic factors, but also to, or in combination with, the environmental factors, which comprise both external and internal exposures. External exposure, for instance to environmental contaminants, affects the internal exposure, i.e. biological factors, such as metabolism, which in turn mediate risks of various diseases. However, little is known how the exposure to chemical pollutants affects the metabolome. In this project, we explored the lipid and metabolic changes in mice following exposure to a mixture of persistent organic pollutants (POPs) containing organochlorides, organobromides, and per- and polyfluoroalkyl substances. Prior to blood collection, non-obese diabetic mice were pre- and postnatally exposed to two different concentration levels of POPs. Lipidomic profiling of mice blood serum samples was carried out on UPLC-Q-TOF/MS, while polar metabolite profiling was conducted on GC-Q-TOF/MS. Significant changes in lipid and polar metabolite profiles were observed between mice in the control and treatment groups. The largest difference in metabolite regulation was detected between control and the high exposure group. In particular, many phospholipids and several triglycerides containing polyunsaturated fatty acids were down regulated, while tricarboxylic acid cycle metabolites were upregulated. Our findings suggest that alteration of the lipid profile in mice exposed to the POP mixture is similar to previously reported metabolic changes associated with higher risk of type 1 diabetes.

[R110] Metabolic differences in venous and arterial umbilical cord blood

Olle Hartvigsson[1], Malin Barman[1], Carl Brunius[1], Agnes Wold[2], Ann-Sofie Sandberg[1], Alastair Ross[1]

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Analysing umbilical cord blood is of importance for assessment of neonatal health and development and could help identify e.g. biomarkers for various diseases. The easiest and probably the most common way to collect umbilical cord blood is to squeeze blood out of the cord after it is severed. Hence a mixture of venous (from mother to child) and arterial (from child to mother) blood is collected. This study aimed to determine key differences in metabolite profiles between venous and arterial cord blood plasma.

The metabolome of venous, arterial and mixed squeezed umbilical cord blood was analysed from 50 children using a combination of targeted and untargeted GC-MS/MS. Data was analysed by multilevel Partial Least Squares (ML-PLS) in a repeated double cross validation framework incorporated with unbiased variable selection.

In pairwise analysis of arterial and venous blood, approximately 75% of the samples were correctly classified ($p=0.0078$). Arterial cord blood had higher concentrations of glucose, sorbose and galactose than venous cord blood which contained higher levels of e.g. α-ketoglutaric acid, L-glutamic acid and homocysteine. Mixed blood had a metabolic profile that was in-between the arterial and venous blood, but could not be classified properly by multivariate models. Our results clearly show that cord blood sampling with non-systematic mixing of arterial and venous blood induces undesirable variability in metabolomics analyses. We therefore conclude that control of the sampling procedure is imperative during metabolomics analyses, especially when monosaccharides and amino acids are relevant for the research question.

[R111] Comprehensive evaluation of a one-step sample preparation for global LC-MS lipidomics of cancer cell cultures

Giovanny Rodriguez-Blanco[1], Victor H. Villar[1], Rachel Harris[1], Gillian Mackay[1]

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Lipidomics aims to provide a global analysis of all lipid species present in biological samples. Lipids play an important role in cell membrane integrity, energy storage, and cell signalling, all of which are relevant in cancer development and progression. Standard methods in lipidomics profiling of cell cultures involve biphasic separation using organic solvents such as chloroform or methyl tert-butyl ether to extract the lipids, followed by evaporation and reconstitution in an LC-MS compatible solvent. Some technical issues can arise using this biphasic extraction and reconstitution: we experienced issues with reproducibility between biological replicates and inaccuracy in our normalisation to protein content, an important parameter when comparing cells growing at different rates. In this study, we propose a rapid single step procedure involving simultaneous lipid extraction and protein precipitation from cell culture plates using either isopropanol (IPA) or a mixture of 1:1 butanol/methanol (BuMe). To evaluate the extraction efficiency of IPA and BuMe compared with chloroform/methanol, HepG2 (liver cancer) cells were grown in a standard medium, in a lipid-rich medium and after treatment with a DGAT inhibitor, inhibiting triacylglycerol biosynthesis. Our LC method and mass spectrometry parameters were addressed, as were various methods and software for data analysis. We compared global changes in lipid classes, differences in lipid intensities and the effect of the DGAT inhibitor, with the three different extraction procedures.

[R112] Investigation of urine metabolic profiles in newborns with prenatally diagnosed unilateral urinary tract dilatation using 1H NMR spectroscopy and metabolomics analysis

Yohann Clement[1], Aurélien Scalabre[2], Elodie Jobart[1], Delphine Demede[3], Pierre Mouriquant[3], Bénédicte Elena-Hermann[4], Pierre Lanteri [1], Pierre-Yves Mure[3]

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The prenatal finding of unilateral Urinary Tract Dilatation (UTD) can be transient or represents a significant urinary flow impairment that would lead to a progressive deterioration of renal function. Identifying urinary biomarkers could help to differentiate uropathy requiring surgical management from transient dilatation at an early stage.

[R113] Salmonella metabolism inside host cells

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Intracellular pathogens like Salmonella Typhimurium need to adapt their metabolism for survival and replication inside host cells. While genetic and proteomic studies suggest that Salmonella gets access to a selected but wide range of nutrients, direct measurements of the bacterial metabolism inside host cells have been unfeasible so far. Here, we have developed a novel isotopic labeling approach based on the differential metabolism between the two species and hydrophilic interaction liquid chromatography (HILIC) coupled to high-resolution mass spectrometry. After supplementation of 13C-mannitol to a macrophage host cell line (RAW 264.7) infected with Salmonella, we rapidly separate bacteria from the host cell cytoplasm by filtering to improve detection and quantification of bacterial metabolites. Our measurements of bacterial metabolites detect 13C label in central carbon metabolism, nucleotide and glutamate metabolism, and in selected amino acids, indicating that intracellular Salmonella are actively using the corresponding biosynthesis pathways. Interestingly, we also detect 13C label in specific metabolites present in the cell culture medium. The complete inability of the host to metabolize mannitol therefore suggests that Salmonella exchanges metabolites with the host and may contribute to the host metabolism. We are currently extending our method to different host cell lines (epithelial cells, primary cells) and conditions.

[R114] Use of breath analysis, gas chromatography and chemometrics tools to improve the diagnosis of pulmonary diseases

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Breath analysis is a powerful and very promising technique for evaluating respiratory diseases with the advantages of the non-invasive nature, unlimited sample supply and the potential to facilitate early diagnostics. Breath analysis is carried out today by diverse techniques and for untargeted analysis, GC-MS is considered the gold-standard. Furthermore, the type of data generated by GC-MS is especially challenging in this case due to the presence of many noisy variables, confounding (intrinsic and extrinsic) factors and usually small sample conditions leading to the 'curse of dimensionality' problem. The aim of this work was to identify the main challenges to develop robust predictive models in omics sciences, more specifically in breath analysis, from breath collection until to data processing, and propose analytical chemistry and chemometrics solutions. The exhaled air from health cases was collected in the hospital using Tedlar® bags and carried out to the lab. The volatile organic compounds were extracted using Solid Phase Micro Extraction (SPME) and GC-MS analysis was performed. From an analytical point of view, the actual changes done in the sample collection step were the inclusion of biological filters, replace 10 L Tedlar bags for 3L Tedlar bags, and include one step for cleaning the bags before reusing it. It was also possible to verify that Tedlar bags present a considerable background on GC-MS analysis and depending on the aim of the work this type of bags would not be the best option to use. For the data processing, the software PARADISe , was used for deconvoluting the peaks and obtain the peak tables for further chemometrics analysis. The results

were compared with AMDIS and all the steps for using the two programs were explored. It was possible to verify some pros and cons of both programs, for instance, PARADISe is very intuitive and easy to use, however previous knowledge on PARAFAC2 models is needed. On the other hand, on AMDIS is not necessary to define intervals, however there is a high number of parameters to optimize before using. These first results show that analytical chemistry and chemometrics are essential for the improvement of breath analysis techniques. Further experiments include the use of experimental design to optimize SPME extraction and development of a complete workflow to breath analysis that will be used to evaluate the susceptibility of chronical disease patients on developing bronchial infections.

[R115] Metabolomics in preclinical studies on metal based anticancer drugs

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In this work metabolic perturbations upon exposure to metal based anticancer drugs were investigated with LC-MS-based metabolomics in hypoxic 3D cell culture models and 2D (monolayer) cultures. A targeted workflow with 133 metabolites combined with protein quantification resulted in absolute concentrations normalized to total protein content in the cultures. Multivariate statistical analysis and pathway analysis reveals that not only the different metal-based drugs (Pt vs Ru-based), but the choice of model (normoxic 2D vs hypoxic 3D) have distinct metabolic patterns.

[R116] Serine-threonine phosphatase PGAM5 knockout cells accumulate several small phosphopeptides

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PGAM5 is a member of the phosphoglycerate mutase enzyme family. PGAM5 has been shown to have phosphatase activity on several proteins. Using an untargeted metabolomic analysis in PGAM5 knockout cells, we found a reproducible increase in several metabolites, which we identified as small phosphorylated di- and tripeptides. These suggest hitherto unidentified targets of PGAM5.

[R117] Phenolic metabolites of purple potatoes in healthy men

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The purple hue of the coloured potatoes is derived from natural polyphenolic colorants called anthocyanins. Anthocyanins have a flavylium cation structure substituted with mono- or oligomeric sugars and in case of acylated anthocyanins, aliphatic or aromatic acids. Interestingly, anthocyanin-rich foods, such as berries, may be beneficial for health[1, 2]. Our recent study showed that purple potatoes may decrease human postprandial blood glycaemia compared to yellow potatoes [3].

The health effects of anthocyanin-rich foods may be contributed by their phenolic metabolites. However, the metabolic fate of dietary anthocyanins is still relatively unknown. Non-acylated anthocyanins are reported to be converted into glucuronide and sulphate conjugates and small phenolics[4]. The metabolites of acylated anthocyanins have been scarcely studied.

Therefore, a cross-over clinical study was organized to investigate the phenolic metabolites in plasma and urine of healthy men after a meal rich in purple potato acylated anthocyanins. The samples were cleaned using solid-phase extraction, and the targeted metabolites were detected using tandem mass spectrometry. The anthocyanins were mainly acylated petunidin and peonidin glycosides. Our results suggest that purple potato anthocyanins are metabolized into phenolic metabolites, such as phloroglucinaldehyde and hydroxycinnamic acids, and to their glucuronides and sulfates. [1] Li D et al (2015) J Nutr. 145, 742–748. [2] Törrönen R et al (2010) Br J Nutr. 103, 1094–1097. [3] Linderborg KM et al (2016) Int J Food Sci Nutr. 67, 581–591. [4] deFerrars RM et al (2014) Mol Nutr Food Res. 58, 490–502.

[R118] Comprehensive evaluation of pulmonary tuberculosis progression in mice using a multiplatform metabolomics data fusion workflow

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Metabolomics enables the study of modulations on the host metabolome induced by pathogen infection to clarify metabolic mechanisms of onset and progression of mycobacterial diseases. Metabolites isolated from uninfected and tuberculosis (TB)-infected lung tissues at 4 and 9 weeks after infection from a mouse model of active TB were analyzed using a multiplatform approach with GC-QTOF/MS, CE-TOF/MS and LC-QTOF/MS, all high-resolution mass spectrometry platforms. The potential of consensus orthogonal

partial least squares discriminant analysis (cOPLS-DA), a multiblock omics data fusion algorithm, was assessed to track global metabolic changes detected by multiple platforms and highlight metabolic pathways altered in infection. Samples clustered according to the length of infection trajectory. By the inference of metabolic changes occurring in the lungs of TB-infected mice and previous literature, we suggest that cOPLS-DA is capable of determining alterations in a system of highly-interconnected metabolic processes, particularly related to the central carbon metabolism, oxidative stress, proteolysis, inflammation, immunomodulation, and lipidome regulation.

[R119] Measuring relative perceived exertion in blood, as determined one drop at a time

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We observed an elite runner and obtained samples while he ran 13 km in 60 min. We obtained a drop of blood every 2 km. At 6 km, our subject reached his relative perceived exertion (RPE) maximum, termed "hitting the wall". Nevertheless, he continued, felt better, and finished his run. Lactate levels had increased stably by 2 km, ketoacids rose gradually until the end, while the hypoxia marker 2,3 bisphosphoglycerate peaked at maximum RPE. We next performed exercise studies in normal men and women who performed a steady, predicted, and then increased workload at sea level or simulated 3000 m, and observed highly increased glucose levels at high RPE. Changes in lactate, pyruvate, β hydroxybutyrate, α hydroxybutyrate, and 2,3 bisphosphoglycerate were not identical but similar to our elite athlete. We suggest that glucose availability is not the limiting factor, as it rose strongly towards exercise end in highly exerted subjects, but rather that the tricarboxylic acid→oxphos pathway, lactate clearance, and thus and the oxidative capacity are the defining elements in hitting and overcoming the wall. The metabolomics data were determined from drops of capillary blood and indicate our technical advances. We suggest that our findings and methods could guide future ventures in therapeutical exercise decision making.

[R120] A novel integrative strategy to prevent colorectal cancer within the diet-host-microbiota triangle: from organoids to human in vivo reality

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Colorectal cancer (CRC) is one of the most common cancers in the western world. Several hundreds of thousands people are diagnosed annually with CRC and over half of patients die or have comorbidities. Research has suggested that dietary patterns, dysbiosis and gut microbial metabolites may play a pivotal role in, leading to increasing interest among scientists. However, despite the fact that gut microbial metabolites play a crucial role in many biological cases, adequate tools for deciphering the relationship between diet-microbiome-host are not yet available. TRIANGLE aims to provide new insight into the mechanisms by which gut microbial metabolites may prevent CRC. The first objective is targeted at designing in vitro models mimicking human organogenesis and tumorigenesis to evaluate the role of gut microbial metabolites. Human intestinal organoids capture most, if not all, of the cellular diversity present in the native intestinal tissue, mirroring structural alterations, mutational signatures and gene expression between patient tissues and 3D intestinal organoids. The second objective is to identify gut microbial metabolites that can act as cancer-preventive agents. If gut microbial metabolites are commercially available, they are purchased. Otherwise, a gastrointestinal model able to simulate the digestion and colonic fermentation releases these metabolites. Lastly, intestinal organoid responses to gut microbial metabolites are studied combining metabolomics analysis and live cell imaging. Preliminary results have provided valuable new insights into the mechanisms by which nutrient-gene interaction influences colon stem cell niche and CRC, and will open up new possibilities for CRC understanding and prevention.

[R121] Phytochemical evaluation of Cotinus coggygria and Fragaria x ananassa callus cultures by UPLC-MS

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Strawberry (Fragaria x ananassa) extracts are known to protect human dermal fibroblasts against UV-A radiation and hydrogen peroxide oxidative damage, but less is known about their anticarcinogenic effect. Previous results obtained within this project showed significant antiproliferative effects of extracts obtained from callus cultures of Cotinus coggygria and Fragaria x ananassa on human epidermoid carcinoma cell lines. Therefore, our objective was to obtain a phytochemical characterization of those extracts, in order to better understand the pathways underlying the observed effects. The chosen analytical approach for the phytochemical evaluation was liquid chromatography coupled with high resolution mass spectrometry.

[R122] Metabolomics reveals harmful effects of Thirdhand Smoke exposure in animal models and humans

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Thirdhand smoke (THS) is a poorly understood pathway of tobacco exposure that is produced by the deposition and ageing of tobacco smoke particles and toxicants on surfaces, becoming progressively more toxic. Recent studies revealed the harmful health effects of THS exposure, however, its molecular effects have not been studied yet. In this study we present targeted and untargeted metabolomics studies to characterize the effects of THS exposure in *in-vivo* models, such as mice (kidney and urine) and zebrafish embryos; and in humans (urine) using a multiplatform approach (GC and LC coupled to QTOF and QQQ). In murine models, we found differences between control and THS-exposed mice. Kidney and urine samples showed altered levels of betaine, intermediates of tryptophan metabolism, and metabolites from pyrimidine metabolism, biosynthesis of amino acids and alanine, aspartate and glutamate metabolism suggesting an onset of kidney damage. Lipidomics analysis of mice kidney showed depleted levels of different acylcarnitines. Interestingly, not all the metabolites altered as a result of THS exposure returned to near-control levels after an antioxidant treatment, indicating that the harmful effect of THS cannot easily be reverted. Zebrafish embryos exposed to tobacco compounds showed metabolic and phenotypic alterations. These effects were increased by increasing the concentration of tobacco compounds. Furthermore, preliminary results in a cohort of children's urine showed higher levels of some neurotransmitters in children with smoking parents. The results summarized here demonstrated that THS is a new way of tobacco exposure with harmful consequences at molecular levels in different organisms.

[R123] Metabolic changes in autism, and their evolution

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Autism spectrum disorder (ASD) is a common neurodevelopmental disorder with yet incompletely uncovered molecular determinants. Alterations in the abundance of low molecular weight compounds (metabolites) in ASD could add to our understanding of the disease. Indeed, such alterations take place in the urine, plasma and cerebellum of ASD individuals. In this work, we investigated mass-spectrometric signal intensities of 1,366 metabolites in the prefrontal cortex grey matter of 32 ASD and 40 control individuals.

15% of these metabolites showed significantly different intensities in ASD and clustered in 16 metabolic pathways. Of them, ten pathways were altered in urine and blood of ASD individuals (Fisher test, $p<0.05$), opening an opportunity for the design of new diagnostic instruments. Furthermore, metabolic measurements conducted in 40 chimpanzees and 40 macaques showed an excess of metabolite intensity differences unique to humans, supporting the hypothesized disruption of evolutionary novel cortical mechanisms in ASD.

[R124] Multiplatinum resistance and metabolic plasticity in metastatic castration resistant prostate cancer (CRPC) and colorectal cancer (CRC)

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Platinum chemotherapy is of capital importance for cancer therapy. Still, many tumors circumvent the multitarget antineoplastic effect of platinum drugs by increasing drug efflux, DNA damage repair rates, drug detoxification mechanisms, and suppressing apoptotic stimuli[1]. All these processes are necessarily engaged to a significant reprogramming of metabolic pathways that enables an increased synthesis of nucleotides for DNA damage repair, alters active transport and redox balance, or nurtures the synthesis of drug-metabolizing and antioxidant machinery. For this, in this work, we aimed to investigate the metabolic reprogramming that arises in metastatic solid tumors as a response to long term treatment with platinum compounds. We generated multiplatinum resistant CRPC and CRC models along with their age-matched controls, allowing us to uncouple the effects of aging from acquired platinum resistance, and revealing the metabolic alterations that can be genuinely ascribed to each variable. Even if our CRPC and CRC models are in origin radically opposed in metabolic terms, we attempted to match the metabolic profiling performed for each of them, seeking to unveil a common metabolic signature of platinum resistance across radically different types of metastatic tumors.

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[R125] The search for clinically useful biomarkers of complex disease: preterm birth as a paradigm

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Metabolomics LC-MS datasets from serum samples at 15- and 20-weeks' gestation from a cohort of approximately 50 cases and 55 controls were analysed for candidate biomarkers predictive of SPTB. Lists of the top ranked candidate biomarkers from both multivariate and univariate analyses were produced using strategic data analysis methods that respected the heterogeneity of the data and the disease. At the 20 weeks' GA time-point these lists

had high concordance with each other (85%). A subset of 4 of these features produce a biomarker panel that predicts SPTB with a partial Area Under the Curve (pAUC) of 12.2, a sensitivity of 87.8%, a specificity of 57.7% and a p-value of 0.0013 upon 10-fold cross validation using PanelomiX software.

[R126] Individual variability and coregulation of plasma metabolome in Indian type 2 diabetes patients

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Blood metabolites represent physiological end-points of unique gene-environment interactions in an individual. We analyzed untargeted plasma metabolome of 29 Type 2 Diabetes (T2D) and 30 normoglycemic individuals. We obtained Coefficients of Variation (CV = SD/mean) for 1959 plasma metabolites of 59 subjects. Our analysis shows that metabolite CVs and abundances show minimal correlation overall, however, the least abundant metabolites also have low CVs. Majority of the dysregulated metabolites in T2D patients have low CVs in controls indicating their tight regulation, some of which show sharp increases in variability in T2D patients. Correlation-based clustering identified 14 metabolite modules, of which 4 associated with T2D status involving pathways like linoleic acid metabolism. CV analysis of modules highlight strictly controlled pathways including phenylalanine metabolism and phenylalanine, tyrosine and tryptophan biosynthesis. Module-trait association analysis identified distinct modules associating with Waist-to-Hip ratio and Glycemic indicators. Study of metabolite variability along with their abundance derives great insights for understanding metabolic regulation in disease conditions.

[R127] Nicotinamide deficiency in Primary Open-Angle Glaucoma (POAG)

Judith Kouassi Nzoughet[1,2], Juan Manuel Chao de la Barca[1,3], Khadidja Guehlouz[4], Stéphanie Leruez[4], Laurent Coulbault[5], Stéphane Allouche[5], Cinzia Bocca1, Jeanne Muller[4], Patrizia Amati-Bonneau[1,3], Philippe Gohier[4], Dominique Bonneau[1,3], Gilles Simard[3], Dan Milea[6], Guy Lenaers[1], Vincent Procaccio[1,3] Pascal Reynier[1,3]

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To gain further insights into primary open-angle glaucoma (POAG) pathophysiology, we performed a non-targeted metabolomics analysis on plasma from POAG patients (n= 34)

and age- and sex-matched control individuals (n= 30), using liquid chromatography coupled to high resolution mass spectrometry. One hundred and sixty metabolites were accurately detected. A data mining strategy was performed, combining univariate and multivariate analysis. This strategy highlighted a set of nine relevant metabolites, characterizing the plasma metabolomics signature of glaucoma. Our findings open up therapeutic perspectives based on the identified markers, in terms of diagnosis and treatment. A proposal for the initiation of a clinical trial based on metabolite supplementation is in preparation.

[R128] Viral and bacterial infection elicit distinct changes in plasma lipids in febrile children

Xinzhu Wang[1], Ruud Nijman[2], Stephane Camuzeaux[3], Caroline Sands[3], Marieke Emonts[4,5], Jethro Herberg[2], Ian Maconochie[6], Matthew Lewis[3], Michael Levin[2], Myra McClure[1] on behalf of EUCLIDS consortium

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Fever is the most common reason that children present to Emergency Departments (EDs) in the UK. Clinical signs and symptoms suggestive of bacterial infection are often non-specific, and no test exists for the accurate diagnosis of infection. As a result, many children with viral infection are prescribed antibiotics unnecessarily, while others with serious bacterial infections are not treated in a timely manner and progress to sepsis. In recent years, the 'omics' approaches to identifying biomarkers from the host-response to bacterial infection are proving promising. In this study, lipidomic analysis was carried out with plasma samples obtained from febrile children with confirmed bacterial infection (n=20) and confirmed viral infection (n=20). We show for the first time that bacterial and viral infection elicit distinct changes in the host lipidome. Glycerophosphoinositol, sphingomyelin, lysophosphotidylcholine and cholesterol sulate were increased in the confirmed virus infected group, while fatty acids, glycerophosphocholine, glycerophosphoserine, lactosylceramide and bilirubin were increased in cases of confirmed bacterial infection. A combination of 20 metabolites increased diagnostic performance and achieved the AUC value of 0.853 (95% CI, 0.672 - 0.995). This pilot study demonstrates the potential of metabolic biomarkers to distinguish bacterial from viral infection in febrile children, to facilitate effective clinical management and to limit inappropriate use of antibiotics.

[R129] Differential Lipid Profiling of Mycobacterium tuberculosis Strains by Liquid Chromatography Mass Spectrometry

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Rationale: Detection and identification of strain-specific lipid-based signatures as part of routine diagnostic TB testing could be used to direct treatment and as well as for monitoring prognostic outcomes. This is particularly important as it has been shown that certain strains of M. tuberculosis are more transmissible, more pathogenic and can readily acquire drug resistance than others. Objective: Through LC-MS based assays, we interrogated crude Mycobacterium extracts from clinically relevant strains to identify strain-specific discriminatory lipid profiles. Methods: M. tuberculosis genotypic strains, the vaccine strain M. bovis BCG and non-tuberculous mycobacteria were cultured in triplicate. Cells were harvested, crude global lipids were extracted and analysed through LC-MS. sPLSDA and GENAS analyses were respectively used to assess Mycobacterium strain clustering as well as biological correlation. Pairwise comparisons were performed to identify significantly enriched molecular features (fold change $\geq 2, logodds \geq 4.6$) between strains. Database matching was also performed for compound identity prediction. Results: Statistically dysregulated lipidomic bio-signatures were detected. Sets of M. tuberculosis lipids that may be candidate bio-signatures for phenotypic profiling of M. tuberculosis strains were also identified through sPLSDA. Additionally, multivariate discriminatory analysis revealed independent clustering of the laboratory H37Rv strain, co-clustering of the W-Beijing strains as well as a lipid-signature based link between the W-Beijing and H37RvMA and H37RvJO. Global lipidomic profiles of Mycobacterium and M. tuberculosis genotypic strains could therefore be delineated through shotgun LC-MS and bioinformatics workflows. Conclusion: We show the value of using projection-based bioinformatics and statistical platforms for Mycobacterium strain discrimination and detection of differentially enriched lipid species across Mycobacterium strains. The current work together with previous studies shows variability in lipid profiles of different strains of mycobacteria and M. tuberculosis and among pathogenic and non-pathogenic mycobacteria that may be implicated in strain virulence.

[R130] A metabolomics application in an organic diet intervention trial: Preliminary results

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The interest into dietary interventions is increasing in the fields of health care management and public health. The ORGANIKO intervention trial evaluated the effectiveness of a systematic organic diet (40-days) on the magnitude and variability of biomarkers of oxidative stress/inflammation and also to biomarkers of exposure to pesticides in primary school children (n=191). However, there is little data on the use of metabolomics in elucidating biological mechanisms behind a demonstrated effectiveness of an organic diet in

beneficially altering key health outcomes. The objective of this preliminary study was to set out the metabolomics protocol and its characteristics using urine samples from the ORGANIKO trial. In effect, derivatized urine samples were analyzed on an INTUVO 9000 GC-system which was coupled to a 5977B MSD Quadrupole MS detector of Agilent. Metabolites were identified and deconvoluted using AMDIS and data integration and extraction were executed using MassOmics software in R. The obtained metabolome was cleaned up before doing internal standard normalization and batch removal by systematic error removal using a random forest (SERRF) method. Firstly, the metabolomes of the baseline of conventional diet and the three sample points of organic diet period were compared using principal component analysis (PCA). The PCA did not generate a clear separation by dietary treatment. A partial least square-discriminant analysis was applied on the same dataset in an effort to supervise the separation between the conventional diet baseline point and the organic diet sampling points. It is warranted that this preliminary work will pave the way for the complete characterization of the organic dietary treatment's metabolome using the ORGANIKO trial dataset.

[R131] Metabolomics study of the anti-proliferative activity against HT-29 colon cancer cells of a withanolides-rich extract from Physalis peruviana calyx

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In recent years, the prevention of human diseases by means of the proper diet control and the intake of functional food or nutraceutical products is becoming an emerging trend in medicine, food and bioscience fields. The potential health benefits that some food components may confer has drawn the attention over dietary agents of interest (e.g polyphenols, terpenoids) that can reverse, suppress or prevent carcinogenic colorectal progression. Previous research works on the genus Physalis reported the isolation a particular family of C28ergostane-type steroids (withanolides) of great interest from the pharmacological point of view, as they were reported to have anti-inflammatory, antitumor, cytotoxic, hepatotoxic and antimicrobial activities. In this context, metabolomics was shown to be a powerful tool to understand the interaction of bioactive compounds from diet at molecular level, as well as to provide better scientific evidences of the benefits that food bioactive components can have on human's heath.

In this work, a Metabolomics study was carried out to investigate the changes induced at metabolite expression levels on HT29 colon cancer cell lines upon treatment with a bioactives-enriched extract from goldenberry calyx. Differentially expressed metabolites in control and HT-29 colon cancer cells treated with P. peruviana calyx extracts were identified. Metabolomics data revealed altered cellular redox homeostasis, due to the upregulated levels of reduced glutathione (GSH) in treated cells, evidencing the chemopreventive response of cells to the treatment with the bioactive extract. The obtained results also revealed alteration on relevant metabolic processes, suggesting inactivation of aminoacyl tRNA charging pathway, dysfunction on carnitine shuttle and beta-oxidation of fatty acids, and pyrimidine ribonucleotide interconversion impairment. These observations are in line with functional analysis and anti-proliferative activity results, where the viability of HT-29

colon cancer cells was notably reduced after 48h treatment without affecting the viability of normal human colon fibroblast cells.

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