Nordic Metabolomics Society workshop and Throne Holst symposium, October 31st-November 1st 2019

Cardiometabolic disease – pathways, biomarkers, personalised medicine

Abstract-Book

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Abstracts. Oral presentation

Low carbohydrate diet elevates blood sphingomyelin in people with type 1 diabetes

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Background and Aim:

Reduction of carbohydrate intake stabilizes glucose levels in persons with type 1 diabetes (T1D). To maintain similar energy intake, a reduction in carbohydrate must be accompanied by an increase in protein and/or fat intake. However, this dietary change may affect the lipid balance and lead to an increased risk of cardiovascular events. We investigated how blood lipid levels responded to dietary changes and how these changes related to diet-induced changes in metabolic characteristics, such as body mass index (BMI), waist circumference, systolic blood pressure (SBP), glycemic variability and time spent in hypoglycemia.

Materials and Methods: Ten adults with T1D (mean±SD: age 43.6±13.8 years, diabetes duration 24.5±13.4 years, BMI 24.9±2.1 kg, HbA_{1c} 57.6±2.6) using insulin pumps participated in a randomized 2-period crossover study with 12-week intervention periods of Low Carbohydrate Diet (LCD < 100 g carbohydrates/day) or High Carbohydrate Diet (HCD > 250 g carbohydrates/day) respectively, separated by a 12-week washout period. A comprehensive lipidomics analysis was done for fasting plasma samples obtained after each diet, and changes in lipid levels were compared with paired tests.

Results: In total, 245 lipid levels were identified from 9 major lipid classes (Triacylglycerides, Phosphatidylcholines, Phosphatidylethanolamines, Hexosyl Ceramide, Sphingomyelins, Lysophosphatidylcholines, Ceramides, Lactosylceramide, and Lysophoshatidylethanolamine). In the high-resolution lipidomics analysis, phosphatidylcholines and sphingomyelins were elevated after LCD (not significant). A short-chain monounsaturated sphingomyelin, SM(d34:1), was elevated after LCD compared with HCD (p=0.002). Sphingomyelins and phosphatidylcholines were inversely associated with BMI, waist circumference, SBP and glycemic variability (not significant). SM(d34:1) did not show significant association to changes in clinical measurements.

Conclusion: Plasma from persons with T1D showed an increase in a monounsaturated sphingomyelin with LCD compared to HCD. Results from this randomized cross-over study warrant for more investigation on the long-term effects of diet and lipid homeostasis in T1D.

Title: Prospective associations of cardiorespiratory fitness and lipoprotein subclasses in a cohort of Norwegian schoolchildren: the Active Smarter Kids (ASK) study

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Abstract text

Aim: Cardiorespiratory fitness (CRF) is associated with cardiometabolic risk factors in children (1). Associations with traditional measures of lipid metabolism are uncertain (2). We investigated whether higher levels of fitness benefit lipid metabolism by exploring cross-sectional and prospective associations between CRF and a comprehensive lipoprotein profile.

Methods: We used targeted proton nuclear magnetic resonance (^{1}H NMR) spectroscopy to profile 29 measures of lipoprotein metabolism for 903 fifth-grade Norwegian schoolchildren (49.2% girls; mean age 10.2 years). Serum samples were taken on two occasions across the academic year. CRF was measured at baseline using the Andersen aerobic fitness test. We used multiple linear regression adjusted for potential confounders to examine both cross-sectional and prospective (adjusted for baseline lipoprotein measure) associations between CRF and lipoprotein profiles.

Results: Higher levels of CRF were associated with all but one measure of lipoprotein metabolism in the cross-sectional analysis after applying a false discovery rate correction. There were inverse associations with the apolipoprotein B-containing (apo B) lipoprotein subclasses, including cholesterol and triglyceride concentrations. The associations between CRF and the concentration of high-density lipoprotein (HDL) particles were divergent between larger and smaller subclasses. In the prospective analysis, the inverse associations between CRF and the measures of larger apo B-containing lipoprotein subclasses persisted as did all but one of the associations with triglyceride levels. Associations with the other measures were of low magnitude. Additional adjustment for adiposity attenuated most associations in both cross-sectional and prospective models, but an independent effect of CRF remained for certain measures.

Conclusions: Higher levels of CRF are associated with a favourable lipoprotein profile cross-sectionally and prospectively, partly independent of adiposity. Associations tended to be stronger and more consistent over time for the larger apo B-containing lipoprotein measures and those of triglyceride concentrations, suggesting differential effects dependent on the metabolic pathway and/or lipid load.

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2. Mintjens S, Menting MD, Daams JG, van Poppel MNM, Roseboom TJ, Gemke RJBJ. Cardiorespiratory Fitness in Childhood and Adolescence Affects Future Cardiovascular Risk Factors: A Systematic Review of Longitudinal Studies. Sports Med. 2018 Nov 1;48(11):2577–605.

Title: Comprehensive metabolic profiling supports that metabolically healthy obese subjects have intermediate-stage cardiovascular disease risk compared with normal weight and at-risk obese subjects

Authors (presenting author underlined)

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Abstract text

The ever-increasing prevalence of obesity constitutes a major health problem worldwide. A subgroup of obese individuals has been described as "metabolically healthy obese" (MHO). In contrast to at-risk obese (ARO), the MHO phenotype has a favorable risk profile. Despite this, the MHO phenotype is still sub-optimally characterized with respect to a comprehensive risk assessment. Therefore, we set out to increase our understanding of the metabolic alterations associated with healthy and at-risk obesity.

In this cross-sectional study, obese (BMI ≥ 30 kg/m^2) or normal weight (NW) subjects (men and women; 18–70 years) were characterized as MHO (n = 9) or ARO (n = 10) or NW (n = 11) according to lipid profile and glycemic regulation. We characterized the obese subgroups and NW subjects by comprehensive metabolic profiling using a commercially available high-throughput proton NMR metabolomics platform. Plasma fatty acid profile (MHO=6, ARO=8), including short chain fatty acids (MHO=9, ARO=10, NW=11), was measured. Dietary intake was assessed by a food dairy (MHO=6, ARO=9).

The fasting particle concentrations of VLDL, IDL and LDL subclasses were overall higher, and particle concentrations of HDL subclasses lower in ARO compared with MHO subjects. VLDL and IDL subclasses and HDL subclasses were higher in NW subjects compared with MHO subjects. We further found that the fasting concentrations of the branched chain amino acids isoleucine, leucine and valine were higher in ARO compared with MHO subjects. In addition, the fasting concentration of aromatic amino acid phenylalanine was lower in NW subjects compared with MHO subjects. Interestingly, NW subjects had lower levels of Gp-acetyls and CRP compared to both ARO and MHO. The fatty acid profile in MHO subjects was overall more favorable compared with ARO subjects.

In conclusion, despite a favorable risk profile, comprehensive metabolic profiling supports that MHO subjects have intermediate-stage cardiovascular disease risk compared with NW and ARO subjects.

LC-MS metabolic profiling of pig heart tissue after whole grain enriched feeding

Authors

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Abstract text

Higher intake of whole grain products has been associated with lower incidence of cardiovascular diseases (1, 2). Molecular mechanisms behind the cardioprotective potential of whole grains are not fully understood to date. Bioactive phytochemicals and micronutrients of whole grain are likely contributing to the beneficial health implications. We utilized non-targeted metabolomics to study how whole grain products impact the metabolite composition of various heart compartments. Total of 30 Ossabaw pigs were on conventional diet enriched with four types of commercial grain products, breads; refined wheat as control (RW), whole grain wheat (WGW), whole grain rye (WGR) and sourdough whole grain rye (WGRSD). After 16 weeks, six types of heart tissues samples (aorta, aortic valve, left and right atrium, left and right ventricle) were collected. Frozen tissues samples were weighted and homogenized with Omni Bead Ruptor homogenizer in 80% methanol. Samples were analyzed by liquid chromatography time-of-flight mass spectrometry UHPLC-qTOF-MS system (Agilent Technologies) with hydrophilic interaction chromatography and UHPLC-Orbitrap system (ThermoFisher Scientific) with reverse phase chromatography. Our preliminary results show that whole grain groups (WGW, WGR, WGRSD) had higher abundances of several phospholipid species (e.g. PUFA-containing LysoPCs) compared to RW both in atriums and ventricles. Various energy metabolism intermediates were affected by the type of grain product. Abundance of AMP was higher in both atriums after WGW enriched diet when compared to RW.

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(2) Seal CJ, Brownlee IA. Whole-grain foods and chronic disease: Evidence from epidemiological and intervention studies. Proc Nutr Soc. 2015 Aug;74(3):313-9.

Title: Metabolomics approaches in non-alcoholic fatty liver disease

Authors (presenting author underlined)

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Abstract text

Nonalcoholic fatty liver disease (NAFLD) is a major risk factor leading to chronic liver disease and type 2 diabetes. Non-invasive diagnostic techniques for the different stages of NAFLD, such as steatosis, nonalcoholic steatohepatitis (NASH) and fibrosis, are currently unavailable and thus are an unmet medical need. In our previous studies, we successfully identified specific serum molecular lipid signatures which associate with the amount of liver fat[1] as well as with NASH[2].

Here, we go further by analysing both lipidomic and polar metabolomic profiles of individuals (n = 627) from the European project: Elucidating Pathways of Steatohepatitis (EPoS). The EPoS cohort comprises individuals at various stages of NAFLD, including NASH and fibrosis. In line with previous studies[1], we found that steatosis grade was strongly associated with (1) an increase of certain triglycerides with low carbon number and double bond count as well as (2) a decrease of specific phospholipids. As NAFLD progresses from an earlier steatosis state to a later, more severe fibrotic stage, fibrosis grades are also used as a clinical measure for assessing progression to and severity of NASH. Further, network analysis, as we provide here, can offer some insight into patterns of lipid and metabolite changes that may underlie NAFLD as a disease process.

As invasive biopsy is required to provide steatosis and fibrosis grades as major indicators of disease progression, lipidomic / metabolomic markers may provide an alternative method of patient classification and intervention recommendation. Machine learning approaches can offer a way to harness computational power to classify patients as per these clinical grades of disease. Using this cohort as a proof-of-concept, we demonstrate current progress in tuning the accuracy of neural network and random forest approaches with a view to predicting various subtypes of NAFLD patient using a minimal set of lipidomic and metabolic markers.

In summary, our findings suggest that dysregulation of lipid metabolism in progressive stages of NAFLD is reflected in circulation and may thus hold diagnostic value as well as offer new insights about the NAFLD pathogenesis. Both lipidomic and metabolomic markers alone and in combination show promise for prediction of clinical outcomes in NAFLD.

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Title: Differences in metabolic profiles in venous and arterial cord blood

Authors (presenting author underlined)

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Abstract text

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 Random Forest (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.

Abstract: poster presentation

Title: Determination of plasma amino acids using Triple Quad 5500 LC-MS/MS: Results from a controlled randomized diet intervention

Authors

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Abstract text

Quantification of amino acids (AA) and sulphur amino acids including methionine (met) and cysteine (cys) have been consistently linked to obesity and metabolic disruptions. In this pilot study, we aimed to study the effects of a 7 d diet restricted in met and cys on body weight and markers related to lipids and glucose metabolism. We have also evaluated the effects of the diet on plasma concentrations of sulphur amino acids and related metabolites. For quantification of AAs, we developed a chromatographic method. The plasma concentrations of 28 amino acids including total sulphur amino acids, taurine and creatinine were measured by liquid chromatography–tandem mass spectrometry. Briefly, isotopically-labelled internal standards were added to plasma followed by reduction of disulphides using dithioerythritol and then protein precipitation using 5-sulfosalicyclic acid. The extracts were diluted with an aqueous solution of formic acid [0.5%] and heptafluorobutyric acid (HFBA) [0.3%] prior to analysis. LC-MS/MS was carried out using a Shimadzu LC-20ADXR Prominence LC system coupled to a Sciex QTRAP5500 mass spectrometer with a Turbo V ion source and TurboIonspray probe. Run time was 8 min.

Results showed that the body weight decreased more in the low met/cys group. In conclusion, results from this pilot study suggest that a diet low in met and cys may have beneficial effects on parameters related to metabolic risk. In addition, there were effects on plasma markers of sulphur amino acid metabolism, indicating that altered metabolism of these compounds may be related to the observed beneficial effects. The results from this study will be used in the design and planning of a full-scale dietary intervention.

(1). Elshorbagy AK, Curr Opin Clin Nutr Metab Care. 2012

(2). Dong Z, Ann. N.Y. Acad. Sci 2018

Title FBOnto: An ontology to represent food intake data and associate it with metabolomic data

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Abstract text

MOTIVATION: Nutrition research can be conducted by using two complementary approaches: 1) traditional self-reporting methods or 2) via metabolomics techniques to analyze food intake biomarkers in biofluids. However, the complexity and heterogeneity (e.g. information provided by participants in nutritional studies about what they have eaten) of these two very different types of data often hinder their analysis and integration. To manage this challenge, we have developed a novel ontology that describes food and their associated metabolite entities in a hierarchical way. This ontology uses a formal naming system, category definitions, properties and relations between both types of data.

METHODS: This ontology has been created using Protégé and is available in OWL (Web Ontology Language) and OBO (Open Biomedical Ontologies) formats at the project's Github repository (https://github.com/pcastellanoescuder/FoodBiomarkerOntology).

RESULTS: The ontology presented is called FBOnto (Food-Biomarker Ontology) and it is composed of two interconnected sub-ontologies. One is a "Food Ontology" consisting of raw foods and prepared foods while the second is a "Biomarker Ontology" containing food intake biomarkers classified by their chemical classes. These two sub-ontologies are conceptually independent but interconnected by different properties. This allows data and information regarding foods and food biomarkers to be visualized in a bidirectional way, going from metabolomics to nutritional data or vice versa. Potential applications of this ontology include the annotation of foods and biomarkers using a well-defined and consistent nomenclature, the standardized reporting of metabolomics workflows (e.g. metabolite identification, experimental design), or the application of different enrichment analysis approaches to analyze nutrimetabolomic data.

Keywords: Ontology, Metabolomics, Nutrition, Bioinformatics.

Title: Body weight associated metabolites in plasma from *ad libitum* fed Gottingen Minipigs – an animal model for obesity and metabolic syndrome

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Abstract text

The metabolic responses to the intake of high-fat diets supplemented with either fructose or high-amylose maize starch were measured in a long-term intervention study using Gottingen Minipigs (GM) as a model for human obesity and metabolic syndrome. Fifteen animals were allotted to each treatment and the two experimental diets were provided *ad libitum* for a period of 5 months. Jugular blood plasma was collected at week 4, 12 and 20 of the dietary intervention. Samples were analyzed using non-targeted metabolomics with UHPLC coupled to an ImpactHD Quadrupole Time-of-Flight (QTOF) mass spectrometer. Sparse Partial Least Squares Regression (sPLSR) was used for the prediction of body weight using metabolomics data. The longitudinal experiment revealed a rapid increase in body weight (BW) and signs of obesity irrespective of the two diets. No overall differences between the groups were observed in terms of BW and thusly data was combined to focus only on the development of obesity over time. Correlations between BW and plasma in positive electrospray ionization were the strongest compared negative ionization mode and therefore only positive ions are presented here. Several amino acids (leucine, tryptophan, phenylalanine) and amino acid derivatives such as N-(1-deoxyl-1-fructosyl)-leucine/isoleucine and N-(1-deoxyl-1-fructosyl)-valine were found to be positively correlated with BW. An intermediate metabolite of the arginine and proline metabolism, γ-glutamyl-γ-aminobutyraldehyde and its [M+Na], [M+K] and [2M+Na] adducts were positively associated with BW. Several compounds were observed to respond differently and quaternary ammonium compounds (betaine, choline, betaine aldehyde) or acetylcarnitine were negatively correlated to BW, as well as 4-hydroxyisoleucine and hypoxanthine. This regression analysis revealed metabolites that previously have been observed in human experiments to be associated with an increased incidence during obesity development. The presence of BCAA (leucine, isoleucine, and valine) and other amino acids has been previously linked to the development of diabetes, and serve as potential biomarkers for disease.

Title: Plasma methylmalonic acid and risk of acute myocardial infarction in patients with coronary heart disease

Authors)

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Abstract

BACKGROUND: Elevated levels of methylmalonic acid (MMA) is reported in patients with established coronary artery disease, and is considered a specific marker of B12 deficiency, which in turn has been associated with increased risk of cardiovascular disease. Moreover, MMA-dependent reactions have been linked to alterations in mitochondrial function and cellular energy metabolism, the common characteristics of failing heart and its associated state (1). We therefore examined whether MMA may prospectively predict risk of new cardiovascular events in patients with coronary heart disease (CHD). METHODS AND RESULTS: Baseline plasma MMA was measured by gas chromatography/tandem mass spectrometry. By Cox modelling, we explored the association between plasma MMA and risk of acute myocardial infraction (AMI) in two independent cohorts of patients who either underwent elective coronary angiography for suspected stable angina pectoris (SAP) (n=4156) or were hospitalized for acute myocardial infarction (AMI) (n=3733). Median follow-up time was 7.5 and 3.1 years among patients with SAP and AMI, respectively. MMA predicted the risk of AMI in both cohorts (age and gender adjusted hazard ratios (HRs) [95% confidence intervals, 95%CIs] per 1-SD increment of log-transformed MMA 1.17 [1.09-1.26] and 1.16 [1.10-1.23] among patients with SAP and AMI, respectively). The relationship between MMA and AMI was only slightly attenuated in analyses adjusted for established cardiovascular risk factors and potential confounders including cobalamin. Further, in patients with SAP, risk associations were predominately present in females and confined to patients with age and plasma neopterin or vitamin-A levels above the median (age and gender adjusted HRs [95% CI] per 1-SD increment of log-transformed MMA 1.34 [1.13-1.59], 1.22 [1.12-1.34], 1.21 [1.11-1.32] and 1.32 [1.18-1.48], respectively) (Pint<0.02 for all). CONCLUSIONS: Plasma MMA was positively associated with risk of AMI in patients with suspected or verified CHD. The associations were stronger in females, elderly and patients with higher vitamin A and neopterin levels. These results motivate further studies to elucidate the relationship between one-carbon metabolism, energy metabolism and atherosclerosis.

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Metabolomics Coupled with Pathway Analysis Reveal the Metabolic Fingerprint in Treatment- Naïve Ulcerative Colitis Patients

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Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory disorder in the gastrointestinal tract that affects up to 0.5% of the population of the Western world. The two major forms of IBD, ulcerative colitis (UC) and Crohn's disease (CD), are characterized by a dysregulated mucosal immune response triggered by several genetic and environmental factors in the context of host-microbe interaction. This overwhelming complexity of IBD makes it ideal for metabolomic studies to unravel the pathobiology of the disease and to improve patient stratification strategies toward personalized medicine. Therefore, we aimed to explore the mucosal metabolomic profile in UC patients, and to pinpoint the metabolic signature of IBD.

Colon mucosa biopsies were collected from 18 treatment-naive UC patients at the debut of the disease (inflamed mucosa), 10 UC patients in deep remission, and 14 healthy subjects. Metabolomic analysis of the colon biopsies was performed by combined gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF-MS) and ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). In total, 177 metabolites from 50 metabolic pathways were identified (Kyoto Encyclopedia of Genes and Genomes database (KEGG))

The relative abundance of 60 and 46 metabolites were altered in UC treatment-naïve patients compared with healthy controls and with UC remission patients respectively. The most prominent changes among the study groups were in lysophosphatidylcholine (LPC), acyl carnitine, and amino acid profiles. Several pathways were identified as the most perturbed according the integrated pathway analysis. These pathways ranged from amino acid metabolism (such as tryptophan metabolism, and alanine, aspartate and glutamate metabolism) to antioxidant defense pathway (glutathione pathway). Furthermore, the pathway analysis revealed a disruption in the long and short chain fatty acid (LCFA and SCFA) metabolism, namely the linoleic metabolism and butyrate metabolism.

In conclusion, the mucosal metabolomic profiling revealed metabolic signatures in active UC, and reflected the homeostatic disturbance in the gut. It seems that the microbiota is heavily involved in altering several metabolic pathways in the colon mucosa. This highlights the importance of integrating IBD-omes compartments by system biology approaches to identify key drivers of pathogenesis which prerequisite personalized treatment.

Title: Circulating endocannabinoids are dysregulated with respect to central CB1-receptor availability in male patients with first episode psychosis.

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Abstract text

There is an established link between psychosis and metabolic abnormalities such as altered glucose metabolism and dyslipidemia. However, the mechanism by which these metabolic changes occur remains unclear. Although antipsychotic drugs can contribute to the metabolic changes there is evidence that the alterations precede antipsychotic treatment. Metabolomics approaches have been used to quantify the metabolic changes occurring in first episode psychosis. Identifying dysregulated metabolism, could be used to predict the patients at risk of developing metabolic co-morbidities.

Using a quantitative liquid-chromatography triple-quadrupole mass spectrometry assay, nine endogenous endocannabinoids or related structures were measured from serum obtained from first episode psychosis patients (FEP, n=8) and healthy controls (HC, n=10). After serum sampling brain CB1R availability was quantified in the same individuals using positron emission tomography (PET) and specific cannabinoid-1 receptor (CB1R) tracer [18F]FMPEP-d2.

Circulating levels of arachidonic acid (p=0.02) and oleyl ethanolamide (p=0.04) were reduced in the FEP individuals. In order to compare the levels of circulating endocannabinoids to the brain CB1R availability PLS regression modelling was used. In HC there was strong association of arachindonoyl glycerol (1+2), stearoyl ethanolamide and palmitoyl ethanolamide with the CB1R availability in the grey matter of the hippocampus (R^2_{CV}=0.51) which was lost in the FEP patients (R^2_{CV}=0.10).

The dysregulation of circulating endocannabinoids in the circulation compared to CB1R following a FEP highlights a possible mechanism by which metabolic co-morbidities occur in psychosis. Despite the small number of patients in this study there is a clear dysregulation of the endocannabinoid system in patients with FEP.

Title: Proteo-metabolomic signature in people living with HIV on successful therapy

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Abstract text

Long-term HIV infection, even with successful combination antiretroviral therapy (cART), is associated with an enhanced and accentuated onset of premature-aging or age-related diseases in people living with HIV (PLHIV). The present study aimed to evaluate the levels of systemic inflammation and predict the risk of age-associated diseases by machine learning approach in PLHIV on long-term suppressive ART using a large number of biomarkers of the inflammation and immune activation and untargeted metabolomics profile. Blood samples were obtained from therapy naïve PLHIV (Pre-ART, n=43), PLHIV on ART for >5 years (ART, n=53), and HIV-negative healthy controls (HIVNC, n=41) after screening 258 individuals. Samples were analyzed for 92 markers of inflammation, sCD14, sCD163, and telomere length. In a subset of samples, untargeted metabolite profiling was carried out using Ultra-High-Performance Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (UHPLC/MS/MS). Despite a median duration of eight years of successful ART, sCD14, sCD163, 4E-BP1, ADA, CCL23, CD5, CD8A, CST5, MMP1, NT3, SLAMF1, TRAIL, and TRANCE levels continued to be significantly elevated in the ART group as compared to HC. Metabolic abnormalities in amino acid levels, energetics, phospholipids, and complex lipids, were observed, which may reflect known differences in lipoprotein levels in PLHIV that can resemble metabolic syndrome (MetS). Ingenuity Pathway Analysis of metabolites indicated a higher risk of inflammatory and neurological diseases in PLHIV, which was further supported by the plasma proteomics. Put together, these data suggest that HIV-1 infected individuals, even those on long term successful ART, may be at higher risk of developing inflammatory diseases leading to inflamm-aging. Metabolic abnormalities were observed in amino acid levels, energetics and phospholipids and complex lipids, which may reflect known differences in lipoprotein levels in PLHIV that can resemble metabolic syndrome (MetS).

Title: Intake of fatty fish according to dietary recommendations is associated with a beneficial lipoprotein subclass profile among healthy adults

Authors (presenting author underlined)

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Abstract text

Fish intake is associated with reduced risk of cardiovascular disease. Low density lipoprotein (LDL) cholesterol in the circulation is one of the most important modifiable risk factors for cardiovascular disease. High density lipoproteins (HDL) are responsible for reverse cholesterol transport, and are associated with reduced risk of cardiovascular disease. The aim of this study was to investigate the association between fish intake and lipoprotein subclass particle concentrations and composition. We performed a comprehensive plasma lipid profiling in 517 healthy adults, using a commercial high-throughput nuclear magnetic resonance spectroscopy platform. The participants were divided into tertiles for consumption of lean fish and fatty fish, reported through a validated self-reported food frequency questionnaire. The lipoprotein subclass particle concentrations and lipid composition was compared between the participants with lowest and highest tertiles of lean fish consumption and between lowest and highest tertiles of fatty fish consumption. We show that high (>223 g/week) versus low (<107 g/week) consumers of fatty fish have significantly higher particle concentrations of large and extra-large HDL particles, and significantly higher content of total lipids, phospholipids, total cholesterol and free cholesterol in large and extra-large HDL particles. No significant difference was found in particle concentration of VLDL and LDL between high and low consumers of fatty fish. We found no significant difference in the lipoprotein subclass profile between high (>180 g/week) versus low (<64 g/week) consumers of lean fish. High consumers of fatty fish seem to have a more beneficial lipoprotein profile compared to low consumers of fatty fish.

Effect of altered dietary lipid composition on the serum metabolome in the prevention of cardiovascular disease

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Background: Diets rich in unsaturated fatty acid have earlier been associated with beneficial effects in population groups at risk of cardiovascular disease. However, the changes in the metabolome after replacing saturated fat with unsaturated fat and the underlying potential health benefits are still unknown.

Aim: To identify metabolites that most strongly differentiate diets rich in saturated or unsaturated fats and to elucidate underlying mechanisms.

Methods: In a Norwegian 8-weeks, double-blinded, randomized, controlled trial 99 moderately hypercholesterolemic adults (25-70 years) were assigned to either a control diet (C-diet), rich in saturated fatty acids (SFA) or an experimental diet (Ex-diet), where products with SFA were replaced with closely matched products with polyunsaturated fatty acids (PUFA). Fasting serum samples were analyzed by untargeted ultra-performance liquid chromatography quadrupole-time-of-flight mass-spectrometry (LC-MS). Pre-processing by MZmine followed by PLS-DA modeling in Matlab^{R} was performed to detect features differentiating the two diet-groups based on baseline-corrected data. Identification of the metabolites predicting the diets was done by LC-MS/MS, comparison with online spectral libraries and authentic standards, and by plotting retention time-by-*m/z* curves for homologous series of phospholipids. Regression analysis was used to associate diet-specific metabolic profile scores with clinical outcomes.

Results: The metabolic profiles differentiated the metabolome of the Ex-diet and the C-diet groups with an area under the curve of 0.83. The Ex-diet group showed higher levels of unsaturated phosphatidylcholine plasmalogens, an unsaturated acyl-carnitine, and a secondary bile acid. The C-diet group was characterized by odd-numbered phospholipids and a saturated acyl-carnitine. The PCA scores of the serum metabolic profiles characterizing the diets were significantly associated with LDL-cholesterol, total-cholesterol and triglyceride levels.

Conclusion: The serum metabolic profiles confirmed compliance of the participants based on their altered diet-specific metabolome after replacing a diet rich in SFA with one rich in PUFA. The metabolic profiles of the diets were associated with CVD risk markers; however, the health outcomes were not significantly explained by the altered metabolome.

Keywords: cardiovascular risk markers, Nordic diet, fatty acids composition, phospholipids, metabolomics

Title: Lipidome associates with metabolic co-morbidities in first-episode psychosis

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Abstract text

Patients with schizophrenia have lower life expectancy, mostly due to the increased prevalence of cardiovascular disease, type 2 diabetes and metabolic syndrome. Identification of psychotic individuals who are at highest risk of rapid weight gain and the associated development of metabolic co-morbidities is therefore a major challenge for public health.

Here we applied lipidomics in a prospective study comprising 48 controls, 44 FEP patients and 22 individuals at clinical-high-risk (CHR) for psychosis, from two study centers (Turku/Finland and London/UK). Lipidomics (UHPLC-MS) was applied in baseline serum samples, while body mass index (BMI) was assessed at baseline and after 12 months. We found that baseline triacylglycerols with low double bond count and carbon number positively associated change in one year BMI. The result shows that the overall pool of plasma phospholipids remained inversely associated with the weight gain in FEP patients, but not in CTR. In addition, a signature comprising two triglycerides lipids were predictive of the weight gain in psychotic individuals, with an area under the receiver operating characteristic curve (AUROC) of 0.77.

Our study thus suggests that lipidomic signature may serve as a predictor of weight gain and may thus provide a useful marker for identifying patients who are most vulnerable to the development of metabolic co-morbidities in psychosis.

Title: The Effect of the Dairy Matrix on Postprandial Plasma Phospholipids: A Randomized Crossover Intervention Study

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Abstract

Postprandial dyslipidemia contributes to cardiovascular disease (CVD) risk through metabolic comorbidities such as obesity, insulin resistance and diabetes mellitus type 2. Dietary lipids are emulsified in lipoproteins, consisting largely of triglycerides, protein and phospholipids. Decreased circulating ether-phospholipids, increased lysophosphatidylcholine and perturbations in the ratio of phosphatidylcholine and phosphatidylethanolamine have been positively associated with obesity and insulin resistance; conversely, sphingomyelin have shown to reduce CVD risk through decreased bioavailability of cholesterol in the intestinal lumen. However, while circulating phospholipids appear to be related to CVD risk, little is known on their postprandial kinetics and how this affects CVD risk.

Therefore, elucidation of the role of the dairy matrix on postprandial phospholipid absorption kinetics is of great interest. The present study investigates the effect of textural and structural alterations made to the matrix of four dairy products with similar nutrient composition, on phospholipid absorption kinetics.

The study is a randomized crossover trial, consisting of four different interventions with a wash out period of minimum 2 weeks in-between. The following dairy products were included in the study; cheddar cheese (i.e. intact protein network and milk fat globules); homogenized cheddar cheese (i.e. loss of protein network and potential alterations to the milk fat globules); MCI drink (micellar casein isolate (MCI) with added cream); MCI gel (gel product made from the MCI drink).

Twenty-five healthy, normal weight male subjects between the ages of 19-40 years were recruited for the study. The subjects received four test meals, each consisting of one of the dairy products, bread and water, matched to obtain similar macronutrient and isocaloric composition. Blood samples were drawn during fasting (time 0 min) and 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after intake of the test meals.

A targeted liquid chromatography tandem mass spectrometry method is currently employed to quantify approximately 50 glycerophospholipids and sphingolipids in the blood samples. Using univariate and multivariate data analysis strategies, the plasma lipid profiles from the present study will be evaluated to examine the effects of the applied textural and structural alterations of the dairy matrices.

Urine metabolite profiles and nutrient intake evaluated from 4-day weighed food diary in habitual vegans, vegetarians and omnivores

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Increasing interest for diets excluding meat and other products of animal origin increase the importance of objective and reliable methods to measure dietary exposure, prove associations and causation between diet and health, and to evaluate nutrient intake in vegetarian diets.

This study aimed to investigate if nuclear magnetic resonance (1H-NMR) analysis of urine samples may serve as an objective method to discriminate vegan, vegetarian ± fish and omnivore diets. A secondary aim was to assess the influence of dietary nutrient intake on the metabolomics results.

Healthy volunteers (43 men and 75 women) complying with habitual vegan (n=42), vegetarian (n=25), vegetarian adding fish (n=13) or omnivore (n=38) diets were enrolled. Data were collected on clinical phenotype and lifestyle including a 4-day weighed food diary. Urine was analyzed for metabolites by 1H-NMR spectroscopy and data normalized using Probabilistic Quotient Normalization and pareto-scaled before multivariate analysis. Before Orthogonal Projections to Latent Structures with Discriminant Analysis (OPLS-DA), volunteers were assigned as meat eaters or nonmeat eaters, vegans or nonvegans.

It was possible to separate meat- and nonmeat consumers and vegans and non-vegans in multivariate models. However, the correct classification of vegan individuals was only 58%. Reported intake of protein was higher in omnivores and saturated fat lower and fiber higher in vegans, when compared to the other groups. Micronutrients such as riboflavin, selenium and vitamin D were highest among omnivores and lowest among vegans. Discriminating metabolites were related to differences in protein intake.

1H-NMR urine metabolomics appears suitable to objectively identify and predict habitual intake of meat in healthy subjects, but results should be interpreted with caution since specific foods rather than food groups contribute to the patterns. Vegans had a better macronutrient composition, but omnivores a better micronutrient intake, demonstrating the different benefits of these diets.

Influence of blue mussel intake on fatty acid composition and serum metabolites

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Intake of blue mussels decreased disease activity in women with rheumatoid arthritis (RA) in the randomized cross-over MIRA (Mussels, inflammation and RA) trial(1). This study investigates potential causes of the decreased disease activity by analysing fatty acid composition in erythrocytes and plasma phospholipids and serum metabolites in the participants of the MIRA trial.

Twenty-three women completed the randomized 2 × 11-week cross-over dietary intervention, exchanging one cooked meal per day, five days a week, with a meal including 75 g blue mussels or 75 g meat. Fatty acid composition in erythrocytes and plasma and ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR) metabolomics data were analysed with multivariate data analysis. NMR-data were non-normalized and UV or UVN scaled. Principal component analysis (PCA) models and Orthogonal Projections to Latent Structures (OPLS) were used to explore clustering patterns of observations, trends in the data in relation to known factors and outliers. OPLS with Discriminant Analysis (OPLS-DA) and OPLS with effect projections (OPLS-EP) were performed to compare the two diets.

The fatty acid profile in erythrocytes was different after intake of blue mussels compared to the control diet, and all samples were correctly classified to either the blue mussel diet or control diet in OPLS-DA. Changes following blue mussel intake included significant increases in omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at the group level but not for all individuals. The fatty acid profile in plasma phospholipids and ^{1}H-NMR serum metabolites did not differ significantly between the diets.

To conclude, modelling fatty acids in erythrocytes may be a better biomarker for seafood intake than only EPA and DHA content. The change in fatty acid pattern in erythrocytes could be related to reduction in disease activity, although it cannot be excluded that other factors than omega-3 fatty acids potentiate the effect.

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Title: Clustering molecular features originating from the same metabolite in LC-MS metabolomics

Authors (presenting author underlined)

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Abstract text

Non-targeted metabolite profiling approaches necessitate complex data-analytical procedures that continue to be developed within the metabolomics community. One of the major problems in LC-MS based metabolomics is that a single metabolite is not typically represented by a single molecular feature, but merely as a varying composition of signals resulting from isotopes, in-source fragments, dimers, and adducts, which all change the mass of the compound entering the mass spectrometer and are thus interpreted as separate features. While peak extraction software have algorithms to spot the most common adducts, the peak tables extracted from raw LC-MS data files still commonly contain multiple peaks that originate from the same metabolite. These extra peaks can cause problems in the data analysis phase, especially in metabolite identification, where a large amount of manual work is required to identify features originating from the same metabolite. (1) In addition, the redundant peaks aggravate the multiple testing problem in statistical analysis, where p-values of hypothesis tests are corrected using methods such as the Benjamini-Hochberg false discovery rate procedure. This in turn lowers statistical power, resulting in an inflated type II error rate.

We present a novel method for clustering features that possibly originate from the same metabolite. The method links molecular features with similar retention time and high Pearson correlation coefficient, forms an undirected graph of the features and finds densely connected clusters of features that probably originate from the same metabolite. Each feature in a cluster is then assigned a cluster label. The algorithm can be used to compress the data matrix before statistical analysis, reducing collinearity, thus potentially increasing the performance of multivariate statistical models. The cluster labels can also be used to decrease the workload in metabolite identification. We have tested the method on several datasets at University of Eastern Finland and the preliminary results are promising. The algorithm is implemented in R (2) (version 3.5.0 or above is recommended) and licensed under the MIT license, thus free to download and use.

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Title: Intake of beta-glucan and changes in plasma metabolic profile. A short-term intervention in healthy subjects

Authors

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Abstract text

Epidemiological studies have linked a diet high in fiber to reduced risk of coronary vascular disease (CVD) and type 2 diabetes Mellitus (T2DM) [1-3]. Consumption of the soluble fiber beta-glucan has especially been shown to reduce total and LDL-cholesterol and post-prandial glycaemic responses [4, 5]. The beneficial effect of beta-glucan on metabolic regulation has been attributed to viscous gel forming in the small intestine, interfering with absorption in addition to effects via fermentation by microbiota primarily in the large intestine. We have previously conducted a short-term cross-over intervention in order to investigate the effect on glycemic regulation after intake of beta-glucan in fourteen normal weight, healthy individuals. Different amounts of beta-glucan (low, medium and high) was provided as evening meals for three consecutive days. The results demonstrate that intake of beta-glucan reduced the postprandial glycemic response and changed the gut microbial composition. However, no effect on serum total cholesterol was observed. In order to further investigate the effect of beta-glucan on metabolic regulation, plasma metabolic profile was analyzed using an NMR-based platform (nightingale Health Ltd). The metabolic platform included data on lipoprotein subclasses and lipids as well as low-molecular-weight metabolites. In particular, the results suggest that a short-term intake of beta-glucan alters the concentration and lipid content of the HDL subclasses. Whereas no effect on the LDL and LDL subclasses was observed. Data on plasma metabolic profile will be presented.

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Title: Systems genetics analysis of hepatic lipid metabolism in mouse models of insulin resistance, obesity, and nonalcoholic fatty liver disease

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Abstract text

Overconsumption of a diet rich in refined carbohydrates and saturated fat is an important contributing factor to the development of obesity, insulin resistance and non-alcoholic fatty liver disease (NAFLD). Variation between individuals in ectopic accumulation of lipids in non-adipose organs such as the liver might be an important contributor for the observed difference in susceptibility to metabolic diseases. Here we generated and report a new population resource for analysis of hepatic lipid metabolism in mice fed a high fat, high sucrose (HF/HS) diet. The Hybrid Mouse Diversity Panel (HMDP), consisting of 102 inbred strains, were quantified for 256 hepatic lipid species and results were integrated with genetic variation, phenotypic traits, and global gene expression. Genome-wide association studies identified 133 loci for 98 lipid species (lQTL). Upon integration with hepatic transcriptome data, we identified novel mechanisms for hepatic lipid regulation in response to the HF/HS diet. These include *Pex16, Ifi203* and *Map2k6* as high-confidence candidate genes, which control liver LPC content, PC homeostasis and TAG accumulation, respectively. In addition, we provide *in vivo* validation for *Ifi203* and *Map2k6* regulating lipid metabolism and suggest specific mechanistic links for how these genes influence metabolism. Our findings provide an overall framework for data integration to investigate genetic regulation of individual hepatic lipid species. These data also provide a novel resource for researchers studying metabolic traits, including NAFLD and type 2 diabetes.

Title: Novel MALDI imaging solution empowered by a MALDI-QTOF and dedicated bioinformatics pipeline for identification of metabolites and lipids from tissue

Authors

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Abstract text

MALDI Mass Spectrometry Imaging (MALDI-MSI) has emerged as a technique to spatially resolve different metabolic processes in tissue sections. We present a novel workflow solution consisting of a high spatial resolution MALDI source and stage mounted on a commercially available QTOF. A range of metabolomics imaging applications will be highlighted. This includes measurements of endogenous metabolites; typically these measurements were previously performed using extremely high mass resolving instruments. Measurements of mouse intestine sections revealed differential abundance of multiple endogenous metabolites localized to different anatomical regions.

Kidneys from rats treated with substance Factor Xa antagonist and untreated rats were analyzed for the distribution of the compound, compound metabolites and lipid signals. The compound (m/z 432.15) and associated metabolites were detected in the renal medulla; neither were detected in the non-treated samples. A number of lipid signals were increased or decreased in both the renal cortex and medulla of compound-treated animals when compared to those not receiving treatment.

Glycoconjugates can be measured as metabolic endpoints of glucose metabolism. N-glycan imaging was conducted on human liver carcinoma sections and results compared against a list of glycans generated using a high-mass-resolving MRMS system. 61 out of 61 N-linked glycans signals were detected in the sample measured on the MALDI-QTOF, with clear differences in tumor versus non-tumor regions.

These results demonstrate the capability of our MALDI-QTOF instrument for the MALDI-MSI of different metabolomics applications.

Title Diet quality in European children and associations with the plasma and urinary metabolome and markers of beta cell function.

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Abstract text Background: The role of diet quality in glucose homeostasis and diabetes development in children remains unknown. Metabolomic profiling may help to understand metabolic alterations in response to diet quality and determine underlying mechanistic pathways. Objective: We aimed to examine associations of ultra-processed food (UPF) consumption and Mediterranean diet (MD) adherence with serum and urinary metabolites and markers of β-cell function in children. Methods: We studied 1100 children from 6 European birth cohorts (mean (SD) age, 7.9 [1.5] years). We assessed consumption of UPF based on the NOVA classification using a validated food frequency questionnaire. We also estimated MD adherence with an a priori defined score (KIDMED). Urine and serum metabolomic profiles were measured using 1H nuclear magnetic resonance spectroscopy and targeted LCMS/MS metabolomic assay (Biocrates AbsoluteIDQ p180 kit), respectively. C-peptide and insulin levels were assessed in child plasma with the multiplex Luminex system. Results: After adjusting for child BMI and other covariates, a 5% increment in UPF consumption was associated with a 10.7% increase in C-peptide levels (95% CI: 4.2, 17.7). Each one-point increment in KIDMED was associated with a 9.6% decrease in C-peptide (95% CI: -15.1, -3.9). No associations were observed with insulin. Higher UPF consumption was associated with lower serum levels of 2 monounsaturated, 10 polyunsaturated and 6 saturated glycerophospholipids, 5 sphingolipids, 2 acylcarnitines, and the branchedchain (isoleucine, leucine, valine) and aromatic (phenylalanine, tryptophan, tyrosine) amino-acids, as well as higher serum glycine and citrulline. Higher KIDMED score was associated with higher serum levels of 7 saturated and 9 polyunsaturated glycerophospholipids, lower serum levels of leucine, valine, alanine, glycine and glutamine, and lower urinary sugars (sucrose, fructose), An integrated analysis identified distinct metabolite patterns that characterized the associations of diet quality with C-peptide. Conclusion: Plasma metabolomics profiles can reflect diet quality in childhood. A high adherence to Mediterranean diet with low ultra-processed food intake may be protective for β-cell function in children.

The impact of orange juice consumption in the serum metabolome of overweight and obese subjects from the BIONAOS study: a semi-targeted metabolomics approach

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Background: The increasing rates of obesity are considered a public health problem. A large body of evidence clearly suggests that obesity is associated with a chronic proinflammatory state, which could lead to insulin resistance, metabolic syndrome, and atherosclerosis. Previously, in the BIONAOS study, we have shown that orange juice (OJ) consumption might help to reduce BP and regulate some oxidative stress and inflammatory related metabolites (1, 2)

Aim: To characterize the effects of orange juice with OJ containing different doses of flavanones on endogenous metabolites in a human intervention study using semi-targeted metabolomics in serum.

Methods: An LC-MS based semi targeted analysis was developed in samples from one hundred thirty subjects (18-65 years) included in the BIONAOS study. The BIONAOS study was a randomized, crossover, double-blind, controlled study performed in overweight and obese subjects following a dietary intervention that entailed the consumption of 500 ml of OJ containing normal (NPJ) or high concentrations of flavanones (HPJ) for 12 weeks (1). Raw data were pre-processed using TargetLynx and the identification was prepared using an internal database. Normalization and imputation of missing values was done using MetaboAnalyst. Multivariate modeling was performed using SIMCA 15 including orthogonal partial less square-effect projection (OPLS-EP) models. The selection of the most relevant compounds was based on both, a VIP > 1 and using the jack-knifing technique.

Results: In total, 484 compounds were detected including glycerolipids, glycerophospholipids, sphingolipids, non-esterified fatty acids, sterols, fatty acid amides, oxidized fatty acids, bile acids, peptides, amino acids, and derivatives and acyl carnitines. The visual inspection of the OPLS-EP model including samples from both groups revealed a more remarkable metabolic response related to the HPJ intervention (CV-ANOVA = 1.109e-07). We identified a metabolic signature related to the response comprised of 131 compounds being predominant the presence of glycerophospholipids and sphingolipids among others.

Conclusions: The consumption of an OJ with a higher content of flavanones during 12 weeks shows to have a larger modulation on the serum metabolome of overweight and obese subjects than a regular OJ. Further work is needed to identify the metabolic pathways involved and their relevance.

Keywords: Orange juice, flavanones, obesity, overweight, metabolomics

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Title: Significant impact of a lifestyle educational program on children with genetically driven hypercholesterolemia

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Abstract text

Background and Aims:

The first line of therapy in hypercholesterolemic children is therapeutic lifestyle changes (TLSC). The efficacy of lifestyle intervention in children with familial hypercholesterolemia (FH), where LDL-C levels are genetically driven, deserves focused studies.

Aims:

1)Appraise the diet and physical activity (PA) in FH and non-FH hypercholesterolemic children. 2)Prospectively, evaluate the impact of an intensive lifestyle educational program on food patterns, PA and lipid profile in FH and non-FH children.

Methods: 274 children (4 to 18 y/o) were recruited but 238 were selected because available full basal information. A quantitative food frequency questionnaire (FFQ) including 137 items was performed. Physical activity was assessed by Minnesota questionnaire. The lipid profile was assessed by 2D-1H-NMR (Liposcale test). 127 children participated in the prospective phase and were reevaluated after 1 year TLSC intervention. 97 of these, have NMR at baseline time and one year follow-up.

Results: At basal time, we did not find differences between groups in the composition of the diet and in the amount of PA. The only differences were in the lipid profile and in age. FH children were younger. FH had more cholesterol and arrived before at the Lipid Unit.

After one year of intervention of TLSC, the percentage of fats and saturated fatty acid was reduced and the amount of fiber increased significantly in both groups. The percentage of protein increased slightly. The percentage of children who performed 1hour/day of PA increased significantly by 56% in the FH and by 53% in the non-FH. The global lipid profile was also positively modified. Total LDL-C and the small LDL particles (the most atherogenic particles), were reduced in both groups. The magnitude of change was bigger in FH group. This reduction could be explained because patients with FH were more aware. The small LDL particles were reduced in both cases but the percentage did not change.

Conclusions: Educational strategies to implement TLSC in children leads to empowerment, increased adherence and overall metabolic improvement in hypercholesterolemic children including those with FH.

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Title: Biomarker validation and identification of metabotypes for personalized nutrition in the Diet, Cancer and Health – Next Generations cohort

Authors

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Abstract text

Diet is a major modifiable lifestyle determinant for prevention of non-communicable diseases. Current dietary recommendations are population-based; however, a recent study has shown that individuals following a personalized diet are more optimal for health and wellbeing (1). Foods tailored for different types of metabolism, i.e. metabotypes could be one effective strategy to better motivate consumers to healthy eating and combat non-communicable diseases. Factors such as genetic heritage, lifestyle, diet and microbiota determine the metabotypes (2). In order to investigate this hypothesis a sub-sample of 720 subjects from the Diet, Cancer and Health – Next Generations cohort (DCH-NG) were re-invited to join a validation study and participate in two additional examinations at 6 and 12 months after baseline, stratified by gender, age and fasting status. At each visit subjects donated blood, urine, saliva and faeces samples. BMI, waist/hip circumference, blood pressure, fat and muscle mass was measured and comprehensive questionnaires about diet and lifestyle were completed as well multiple 24-hour dietary recalls. By collecting repeated samples over a 12-month period we will 1) assess the validity and reproducibility of the semi-quantitative food frequency questionnaire, 2) assess long-term reproducibility of plasma and urine metabolites, 3), assess changes in the metabolome related to reported changes in major dietary food groups and from analysed changes in gut microbiota and 4) identify general metabotypes for personalized nutrition.

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Title: Associations of perfluoroalkyl substances with proteomic biomarkers of inflammation

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Abstract text

Perfluoroalkyl substances (PFAS) have been linked to immunotoxicity in experimental studies (1). Although PFAS exposure is associated with altered immune function in epidemiological studies of children, it is less known whether this is observed also in adults. We evaluated a panel of 86 proteins in plasma from 965 elderly individuals from Sweden (all aged 70, 50% women) using a multiplex proximity extension assay (PEA) including a number of inflammatory markers, such as monocyte chemoattractant proteins, tumor necrosis factors, and interleukins. Plasma PFASs concentrations were determined using isotope-dilution ultra-pressure liquid chromatography coupled to tandem mass spectrometry. We examined associations using adjusted multivariable linear regression. Nineteen proteins were negatively associated with levels of five PFAS following adjustment for sex, sample storage time in freezer, and correction for multiple testing. Associations of PFAS and hepatocyte growth factor (HGF) and macrophage colony-stimulating factor 1 (CSF-1) remained significant for all following adjustment for smoking, exercise habits, education, energy, and alcohol intake, body mass index (BMI) and glomular filtration rate (GFR). CSF-1 was inversely associated with perfluorohexane sulfonic acid (PFHxS) β: -0.08: 95% confidence interval (CI); -0.13, -0.02), perfluorooctanoic acid (PFOA) β: -0.04: 95% CI; -0.07, -0.006, perfluorononanoic acid (PFNA) β: -0.04: 95% CI; -0.08, -0.003, perfluorodecanoic acid (PFDA) β: -0.03: 95% CI; -0.06, -0.003, and perfluoroundecanoic acid (PFUnDA) β: -0.05: 95% CI; -0.08, -0.02. Comparable relationships were also observed for HGF. Our findings implicate PFAS exposure with decreased levels of plasma inflammatory markers, findings supporting immunotoxicological actions of PFASs also in elderly humans.

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Title: Using Parallel Accumulation Serial Fragmentation (PASEF) to speed up untargeted lipidomics and metabolomics LC-MS/MS workflows

Authors

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Abstract text

A crucial step in lipidomics and metabolomics studies is the acquisition of MS/MS data. To reliably identify analytes, the MS/MS fragmentation has to be fast enough to generate a reasonable number of high quality spectra over a chromatographic peak. This becomes even more complicated if the LC run times are very short.

While there is an in-depth oriented approach to ID as many lipids as possible, clinically-oriented projects often demand a high-throughput for large sample cohorts. Therefore, a short cycle time per sample is needed to realize studies with hundreds or even more samples in a reasonable time frame. In order to keep up with this, the analytical instrumentation needs to deliver a high data quality at high acquisition speeds. This is realized by the PASEF (Parallel Accumulation Serial Fragmentation) acquisition mode on the timsTOF Pro instrument.

In this work, extracts of the NIST reference SRM 1950 plasma were investigated for the number of identified lipids using different LC run times. The benefit of the additional ion mobility separation is to get clean MS/MS spectra of co-eluting compounds, e.g. isobaric lipids. This is demonstrated on a pair of PC / PE lipids. Furthermore, the ability to reliably identify differences between sample groups, even at short LC run times will be presented.

The acquisition of MS/MS data from the lipid extract was supported by the TIMS technology as it adds a complementary ion mobility dimension to the prior LC separation. This allowed for a very fast MS/MS acquisition using a serial fragmentation of co-eluting precursors. The so-called Parallel Accumulation Serial Fragmentation (PASEF) acquisition allowed measuring MS/MS spectra at speeds up to 100 Hz.

In order to evaluate the potential of the PASEF MS/MS performance to do faster chromatographic separations, different gradient run times were applied to separate SRM 1950 lipid extracts. Features for precursor ions and corresponding MS/MS spectra were extracted automatically and searched against the LipidBlast MS/MS library.

First results showed that for more than 100 target lipids evaluated in detail (including phosphatidylcholines, lysophosphatidylcholines, sphingolipids, and triglycerides) the number of identified lipids remained very similar even when using a four times shorter LC gradient length. This identification rate also highlighted that the PASEF MS/MS spectral quality was compatible with 5 minute LC separations allowing to measure more samples per day compared to typical separations requiring 20 or more minutes. Another effect is the reduction of solvent usage: 300 ml were saved when the gradient time for a measuring time of 24 h was reduced from 20 to 5 min.

Genome-scale metabolic modelling of gut microbiota in progression to type 1 diabetes

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Abstract Taxonomic and functional profiling of gut microbiome in a cohort of 33 infants from Finland and Estonia, genetically predisposed to Type 1 Diabetes (T1D), has shown remarkable decrease in alpha-diversity of the microbes, and elevation of microbial markers involved in inflammation1. Furthermore, significant changes in faecal metabolites have been reported between non-converters, seroconverters and T1D cases. Herein, we developed mechanistic genome-scale models (GEMs) to explore and identify any metabolic dysregulation associated to T1D, at 0 (cord blood), 6, 12, 24, 36 months of age. The shotgun metagenomics data was processed and abundance of individual microbe in a community was estimated. Persistently, higher abundances of Faecalibacterium prausnitzii, Bacteroides dorei, Eubacterium rectale and Ruminococcus bromii, Bifidobacterium longum, was exclusively marked in children who progressed to T1D along the age, while Bacteroides ovatus was abundant at 36 months. Moreover, Bacteroides uniformis had higher abundance, both in seroconverters and T1D cases. Multiple comparision using MaANOVA showed differential abundances of gut enzymes involved in pan-metabolism. A total of 201 abundant microbe species that corresponds to 236 strains or taxa with known genomes were identified. 199 microbial genome-scale models (GEMs) were retrieve from public domains2 and 37 microbial GEMs were reconstructed. A community model of gut microbiota was developed. Compartments simulating lumen and epithelial cells that enabled exchange of diets and pool metabolites were added. The community model for each child was personalized by their normalized metagenomic abundance. The gut microbiota model is currently used to study carbohydrate, amino acid, short-chain fatty acids (SCFAs) production, vitamin biosynthesis and bile acid biotransformation in the gut of non-converters (healthy/controls), seroconverters and T1D cases. The GEM predictions will be validated by faecal metabolomics data measured in our lab. The secreted microbial metabolites (predicted) will be correlated with the circulating plasma metabolome, that might elucidate the metabolic role of microbes in modulating host machinery during seroconversion and in progression to T1D.

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Title: Exposure to PFOA alters lipid and polar metabolites profile in PPARα humanized mice

Authors

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Abstract text

Per- and polyfluoroalkyl substances (PFASs) have structural similarities with fatty acids and may interfere with fatty acid metabolism and lipid synthesis in the liver. These compounds are widely used as surface treatment agents in papers, textiles, firefighting foams and many other materials. However, due to the toxicity, bioaccumulation potential and persistency in the environment, perfluorooctanoic acid (PFOA) and its salts were added to the Annex A of Stockholm convention on persistent organic pollutants in May 2019.

In this project, we explored the lipid and metabolomic changes in peroxisome proliferator-activated receptor alpha (PPARα) humanized transgenic mice and corresponding knockout mice. Following the exposure to PFOA, the lipidomic profiling of mice serum and liver samples, was conducted on UPLC-Q-TOF/MS and polar metabolite profiling was carried out on GC-Q-TOF/MS instrument.

Statistically significant changes in lipid and polar metabolite profiles were observed between control and treatment groups of both PPARα humanized and knockout mice. Several lipids and polar metabolites, including upregulated ceramides, phosphatidylcholines and indole-3-propionic acid and downregulated sphingomyelins were showing similar regulation patterns in humanized and knockout mice models. However, triglycerides containing polyunsaturated fatty acids were significantly upregulated in PPARα humanized mice but downregulated in knockout mice. Our finding suggests that exposure to PFOA in mice have a significant impact on lipidomic and metabolic profiles in PPARα humanized and knockout mice, but not always in a similar manner. Moreover, the lipid changes showed distinct similarities to lipid alterations observed in non-alcoholic fatty liver disease, with upregulation of several lipotoxic lipids.

Title: Development and validation of a dietary biomarker panel reflecting multiple foods

Authors

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Abstract text

Diet is a major risk factor for chronic disease, yet efforts to measure and monitor diet are hampered by a reliance on self-reporting methods suffering from large measurement errors. Dietary biomarkers, molecules derived from food measured in blood or urine, can be used as objective measures of specific food intakes and may reduce measurement error for diet. Over the past 10 years, a large number of molecules that are specific to different foods have been discovered. However, little work has been undertaken to validate these new markers across different populations. Moreover, high-throughput methods that allow determination of multiple dietary biomarkers, reflecting several food items in a single analysis are urgently needed but lacking. Applications of such methods using simple and novel sampling techniques applicable outside a clinical setting are desirable to widen the application of dietary biomarkers. In this poster, we will present a project that focuses on developing a rapid method to detect biomarkers covering a range of common foods relevant to health. The method will be applied in two human studies to assess dietary biomarker response in several matrices under controlled and free-living conditions. We will also test if measuring dietary biomarkers in cheek cells, which are easily collected, may better reflect longer term intake. The studies outlined here will constitute a major step towards enabling dietary biomarkers to be applied widely in precision nutrition to guide individuals towards healthy diet and improve research on food and health.

Title: Novel and comprehensive metabolite profiling in cord blood reveal sex differences: A sub-study from the Norwegian Mother, Father and Child Cohort study

Authors

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Abstract text

Elevated cholesterol concentrations may track from early childhood to adult life, and increase the lifelong cholesterol burden and the risk of developing cardiovascular disease. Higher cholesterol concentration has been shown in girls than in boys below 25 years of age however, the cholesterol- and lipoprotein subclass concentrations among newborn boys and girls have been scarcely studied. We included 361 girls and 352 boys who participated in the Norwegian Mother, Father and Child Cohort Study from 2002-2008. Cord blood metabolites were measured by nuclear magnetic resonance spectroscopy. Sex differences were found among several of the 85 detectable metabolites in the present study. Girls compared to boys have higher cord blood concentrations of total-, low-density lipoprotein-, high-density lipoprotein-, esterified-, and free cholesterol, apolipoprotein A1, polyunsaturated fatty acids, histidine and particle concentrations of intermediate-density lipoprotein, large-, medium-, and small low-density lipoprotein and extra-large, medium-, and small high density lipoprotein, while lower concentrations of monounsaturated fatty acids and ratio of triglycerides to phosphoglycerides ($0.001 \leq P \leq 0.04$). Girls have significantly higher cholesterol concentrations than boys at birth suggesting that the sex-related differences in lipid metabolism has evolved already during the pregnancy.