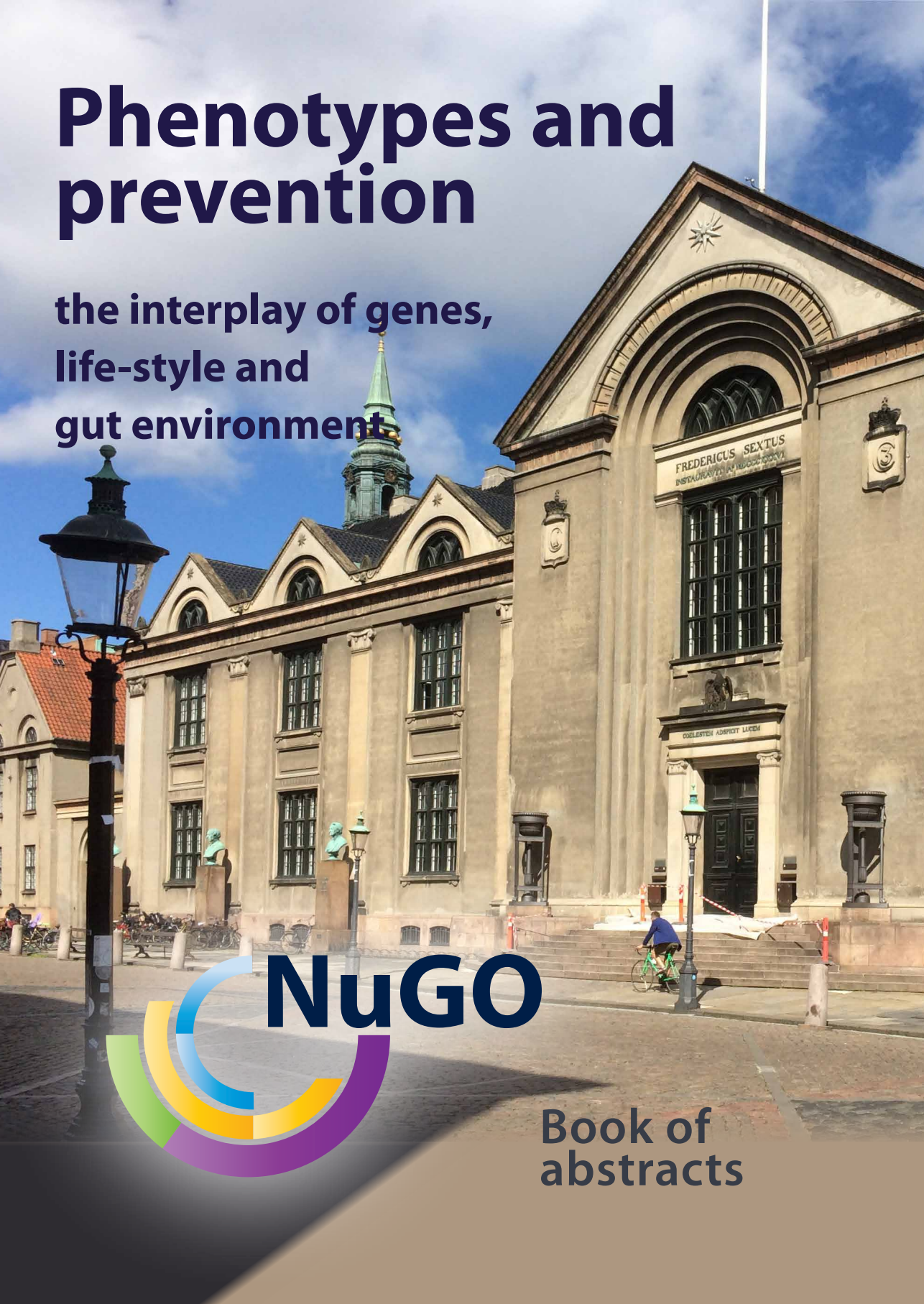
Phenotypes and prevention

the interplay of genes, life-style and gut environment



NuGO

Book of abstracts

Phenotypes and prevention

– NuGOweek 2016 –

Phenotypes and prevention

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Welcome

Welcome to NuGOweek 2016 in Copenhagen!

NuGOweek 2016 is number 13 in the series of NuGO's successful annual conferences and will take place in Copenhagen from Monday the 5th to Thursday the 8th of September 2016 with our pre-course and satellite meetings expanding this period to a full week.

The NuGOweeks have as their primary goal to stimulate new ideas and interactions between the attending researchers, strengthening networks and collaborations, and establishing new ones, not least among the early career investigators. Ideas for several of the 'frontier' projects in the EU and JPI funding programmes with conceptually new approaches have been conceived at NuGOweeks. We hope this year's version will also be the source of such new and important seeds for future progress.

Every NuGOweek has a theme and for the 13th NuGOweek we have chosen 'Phenotypes and prevention – the interplay of genes, life-style factors and gut environment'. This is to underline the importance of understanding the current phenotype as a consequence of the individual and her/his life while at the same time seeing it as a potential predictor of future health and disease risk. Of course nutrition, physical activity and other life style factors are at the heart of this vision as important modifiable factors for health. We have therefore sessions on genetics, various aspects of life style including diet, alcohol, and physical activity; sessions on infant health and the microbiota as determinants and sessions on biomarkers and on data integration for a systems view of health. In addition we have some hands-on sessions for tools in metabolomics and data integration as well as presentation of new tools for sharing and exploring data from nutritional studies.

For our early career investigators we offer a post-graduate course on 'Use of large scale data in dietary intervention studies' which will be held right before the official start of NuGOweek 2016. Younger investigators from several NuGO partners have again this year independently organized our 'Early career investigators session' but many investigators from this group have also been selected by our Scientific Committee for oral or poster presentations during other sessions.

On behalf of the Organizing Committee we would like to thank our sponsors, Scientific Committee, the young investigators group, and all people that helped to organise NuGOweek 2016; and we would like to welcome you here in Copenhagen, known as a wonderful and friendly city of the north. We are looking forward to enjoy excellent science and exciting discussions with you here in Copenhagen and we wish you all an enjoyable stay.

Lars Ove Dragsted

Scientific Committee

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Scientific programme

Session 1. Opening session

Session 2. Omics in disease prediction – integrating genes and phenotypes

Session 3. Early infant/childhood nutrition and later phenotypic response

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Session 4. Alcohol in prevention? – understanding the J-shaped response

Session 5. Hands-on software demo's

Session 6. Microbiota-host interplay as target in nutrition

Session 7. Early career investigators' session

Session 8. Exercise in disease prevention

Session 9. Biomarkers

Session 10. Genotypes, phenotypes and personalised responses

Session 11. Miscellaneous

Metabolic disease signatures translated to underlying mechanisms

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We seek to apply metabolomics and other 'omics' tools for understanding of mechanisms contributing to pandemic metabolic diseases of our era: diabetes, obesity, and cardiovascular disease. We have used these tools to define mechanisms underlying development of peripheral insulin resistance and glucose intolerance in animals and humans. For example, we have identified perturbations of branched chain amino acid (BCAA) catabolism in multiple cohorts of insulin resistant humans compared to normally insulin sensitive controls. Our studies and those of others have demonstrated the prognostic power of this signature to predict incident diabetes and intervention outcomes. These metabolites are also uniquely sensitive to the most efficacious interventions for obesity and diabetes. We have translated these findings to rodent models to demonstrate a contribution of BCAA to abnormalities in mitochondrial metabolism that contribute to the insulin resistant state, as well as to behavioral abnormalities associated with obesity. In hyperphagic Zucker obese rats, feeding of a standard chow diet partially restricted in BCAA content results in improved insulin sensitivity, with attendant changes in tissue metabolic profiles that suggest a relief of mitochondrial fuel overload as a contributing mechanism. Moreover, activation of BCAA catabolism by activation of the branched-chain ketoacid dehydrogenase complex by small molecule or genetic interventions improves glucose homeostasis. Finally, our studies provide evidence that the gut microbiome contributes to dysregulated BCAA homeostasis in obese humans. This work demonstrates the potential of metabolic profiling for defining novel metabolic disease mechanisms and new therapeutic strategies.

Forecasting chronic diseases using data fusion

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When the goal is to unravel the dynamics of a complex system such as the human metabolism, the complexity of the problem has brought out the necessity of data collection from multiple sources. Data fusion, i.e. extracting knowledge through the fusion of complementary data sets, is a topic of interest in many fields. For instance, in metabolomics, analytical platforms such as Liquid Chromatography - Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy are used for chemical profiling of biological samples. Measurements from different platforms are capable of detecting different types of chemical compounds with different levels of sensitivity. In this study, with a goal of forecasting chronic diseases, in particular, acute coronary syndrome (ACS), breast cancer and colon cancer, we jointly analyze LC-MS, NMR measurements of blood samples and the meta-data corresponding to the lifestyle of each subject from the Danish Diet, Cancer and Health (DCH) cohort study. In the DCH data set, subjects do not have the disease at the time of sample collection, but some will develop the disease years after the samples have been taken. Our preliminary results using supervised data fusion methods based on multiple kernel learning (MKL) demonstrate that (1) fusing LC-MS, NMR and meta data provides better separation of ACS cases and controls compared to individual data sets, (2) NMR data performs the best in terms of separating breast cancer cases and controls, and fusion degrades the performance significantly, and (3) neither the individual data sets nor their fusion performs well for colon cancer. Supervised data fusion, or multi-view learning, is a well-studied topic in machine learning; however, the use of multi-view learning in metabolomics is limited due to the lack of interpretability of the models. In our studies, the best performance has been achieved using rule-based MKL methods that train a classifier with the mean of linear kernels, and we have exploited the linearity of the models to identify the metabolites/features playing a significant role in the separation of cases and controls. Furthermore, we have validated our models and showed their limitations by discussing their performance in terms of capturing known biomarkers, e.g. biomarkers from smokers and coffee drinkers.

Epigenome- and transcriptome-wide response to vitamin D supplementation: *in vivo* human experiments

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The micronutrient vitamin D and its biologically most active metabolite 1,25-dihydroxyvitamin D_3 (1,25D) have direct effects on the human epigenome and modulate the response of the transcriptome *in vivo*. Monocytes, such as the THP-1 monocyte cell culture model, are a human cell type that is well responsive to 1,25D. Our RNA-seq (RNA expression), FAIRE-seq (accessible chromatin) and ChIP-seq (genomic association of the proteins VDR and CTCF, histone markers H3K27 and H3K4me3) datasets describe transcriptome and epigenome-wide the 1,25D response of THP-1 cells. Within 24 h the transcription of more than 1000 genes is significantly modulated by several thousand genomic VDR binding sites in concert with the 3D-chromatin organizer CTCF. Our triplicate datasets allow an accurate modeling of the transcriptome- and (epi)genome-wide effects of 1,25D *in vitro* and represent a reference for other human cell types. We extrapolate these models to the *in vivo* response of peripheral blood mononuclear cells (PBMCs) in human individuals receiving vitamin D supplementation, such in our long-term (VitDmet, NCT01479933, 71 elderly pre-diabetic subjects) and short-term (VitDbol, NCT02063334, 35 healthy young subjects) vitamin D intervention studies. One individual was investigated over a period of 2 years and multiple PBMC samples were taken, while from the remaining subjects up to 4 sample were available before and after vitamin D supplementation. RNA and chromatin was immediately prepared after PBMC isolation, i.e. the cells were not further cultured or manipulated *ex vivo*. RNA-seq analysis indicated several hundred genes significantly responding 24 h after vitamin D supplementation. Moreover, FAIRE-seq demonstrated that within the same time frame a comparable number of chromatin sites increased in their accessibility. Since monocytes are only a subset of PBMCs, there is only a partial overlap with THP-1 data. Nevertheless, computational tools, such as network analysis and self-organizing maps, indicated comparable principles of vitamin D signaling *in vivo* (PBMCs) and *in vitro* (THP-1) both on the level of target genes as well as on genomic VDR binding sites. However, *in vivo* there is a significant difference between short-term (24 h) and long-term (several months) responses to vitamin D supplementation. In conclusion, long-term and short-term vitamin D supplementation studies allow monitoring different aspects of the vitamin D responsiveness of human individuals and represent new types of human *in vivo* vitamin D investigations.

A multi-omics approach to identify biomarkers of fermented and non-fermented dairy product intake

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Food fermentation can be considered as a modification of nutrients by a microbial community that is external to the human organism and that is associated with health benefits. In this context, milk is an ideal matrix to investigate the impact of fermentation on nutrient bioavailability and the modulation of the gut microbiota. However, the identity of the bioactive components of these fermented products and their impact on physiological processes are not well-defined. To this end, we investigated the action of a fermented, probiotic dairy product compared to a non-fermented acidified milk on potential biomarkers of dietary intake. Fourteen healthy young men (age 24.6±4.7 years, BMI 21.8±1.8 kg/m^2) were enrolled in a twelve-week randomised cross-over study. Yoghurt containing the probiotic *Lactobacillus rhamnosus* GG and milk acidified with gluconic acid (control) were tested by acute, postprandial tests, completed over six hours (800 g unique dose) and by a two-week 'chronic' test phase (400 g/day). At the end of the chronic test phase fasting assessments were completed. Assessments included metabolomic analyses, completed using UHPLC-qToF and GC-MS platforms and transcriptomic assessments (RNA sequencing, Illumina HiSeq). Outcome measures were product and serum metabolomics and whole blood transcriptomics evaluated in acute and chronic conditions. Multivariate analysis (OPLS-DA) was used to compare the metabolomes of the products, as well as the metabolome profiles of the postprandial serum changes and of the fasting serum after two weeks product intake. Gene expression changes were evaluated by over-representation analysis (ORA) and Gene Set Enrichment Analysis (GSEA). Metabolomic analysis could differentiate the two dairy products as well as the two serum metabolite profiles associated with acute intake of each product (134 discriminative metabolites). In addition, 140 metabolites were found to be different between yoghurt and milk in the fasting state. Transcriptomic analyses also showed relative differences between the gene expression responses to the two acute meal tests. Using a multi-omic approach, we observe changes associated with the tested dairy products, ranging from product and circulating metabolome to gene expression changes. The integration of these analyses may provide insight into the physiological pathways that are regulated by the ingestion of fermented and non-fermented dairy products.

Associations between nut intake biomarkers assessed by metabolomics and cognitive decline

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Recent epidemiological studies suggest that healthy eating habits can help to prevent or delay the age-related cognitive decline. Nuts are considered a key component of healthy diets due to their composition and it has been suggested that they could hold an important role in the prevention of cognitive impairment and chronic diseases. However, most of the studies analysing the relationships between dietary nuts intake and human mental performance are based on data obtained from traditional methods of dietary assessment. Therefore, for the evaluation of the associations between nut consumption and cognitive decline, studies using nutritional biomarkers associated to dietary nuts intake are needed to confirm these protective effects. In this field, nutrimetabolomics is a valuable tool to better assess dietary exposure, allowing the discovery and validation of biomarkers of intake. Therefore, we applied an untargeted HPLC-Q-ToF-MS metabolomics strategy followed by multivariate analysis (OSC-PLS-DA) to characterize biomarkers of nuts intake among participants of the InCHIANTI cohort. After, we investigated their potential association with cognitive decline. Subjects were classified as non-consumers of nuts and weekly consumers of nuts (at least 2.9 g/day). Results showed that the dietary exposure to nuts was characterized by 13 urinary metabolites, including markers of polyphenol-derived microbial metabolites, compounds from fatty acid metabolism, and intermediate metabolites of tryptophan/serotonin pathway. In addition, four multi-metabolite biomarkers panels associated with habitual nut consumption were evaluated using tobit models. Furthermore, associations between dietary and urinary markers (individually and combined) of nuts intake and cognitive decline were analysed using linear and logistic regression models. The global performance of these associations was evaluated using receiver operating characteristic (ROC) curves and assessing the area under the ROC curves (AUC). These results provided a first overview of the association between biomarkers of nuts intake (assessed individually and combined in multi-metabolite models) and cognitive decline. These results will provide advances in epidemiological research with more objective and accurate data to evaluate the associations between dietary components and health outcomes.

Molecular phenotyping of sub-groups within a study population using next generation sequencing

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The promise of omics technologies to identify diet and lifestyle interventions that restore, maintain or improve health status has yet to deliver effective strategies. However, studies employing omics technologies to identify molecular phenotypes in study populations demonstrate unequivocally the inherent inter-individual variation in study participants and importantly their varied responsiveness to the interventions being tested. Previous studies in our lab identified distinct molecular sub-groups within a study population based on whole blood profiles measured by our custom multiplex assay of 28 cell defence gene markers. The molecular subgroups defined at baseline and characterised by differential SIRT1 were associated with markers of health status. SIRT1, associated with aging, oxidative and metabolic stress, has established roles in obesity and cancer and promotes metabolic efficiency *in vivo*. The molecular subgroup with lower levels of SIRT1 had reduced plasma HDL, increased TNFα and demonstrated a suppressed post-prandial response. This observed phenotype may reflect compromised SIRT1 stress responses and a key role in health status. More in-depth molecular phenotyping is required to gain further insight into molecular networks and biological processes associated with dysregulated SIRT1. The current study used next generation sequencing to explore differential gene expression across the transcriptome in healthy individuals stratified according to SIRT1 levels. Total RNA was extracted from fasted whole blood (males 21 to 60 years with no evidence of chronic illness) (n=16) collected using PAXgene® blood RNA tubes. Illumina dual indexed TruSeq mRNA libraries were prepared from 500 ng total RNA in duplicate samples from each participant collected on two separate occasions. Libraries were quantified by qPCR, equimolar pooled, sequenced at 32plex on a NextSeq500 with 75 bp single reads and 129 Gb total Q30 output. Reads were quality and adapter trimmed, aligned to reference, globin reads were removed and reads were normalised in DESeq2. Normalised reads were imported to Partek Genomics Suite v6.6 for differential gene expression and downstream Pathway and Gene Ontology enrichment analyses. Principal component analysis of the blood transcriptome clustered individuals by SIRT1 group. A total of 404 genes were differentially expressed between low and high SIRT1 groups (< vs ≥ median SIRT1; $P<0.05$ and Fold change ≥1.5). GO analysis identified cell senescence, aging, lipid metabolism, cellular homoeostasis and cellular response to nutrient levels as significantly enriched in this gene set. Pathway enrichment analysis identified cell cycle and wnt signalling as candidate SIRT1 regulated networks. These data further support SIRT1 as a candidate biomarker of health status and dysregulated SIRT1 molecular networks warrant further investigation. This work was supported by the Scottish Government's Rural and Environment Science and Analytical Services Division.

Signatures of acylcarnitine and acyl-CoA indicate changed mitochondrial pathways in diabetic mice

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Obesity, insulin resistance and diabetes are characterized by changes in plasma metabolite markers that are primarily derived from amino acid and fatty acid metabolism. We sought to determine changes in individual metabolic routes of mitochondrial amino acid and fatty acid oxidation by determining acylcarnitine profiles in plasma and tissues derived from mouse models representing different stages of obesity and diabetes. The ob/ob mouse with a leptin deficiency served is a model for insulin resistance with pancreas hypertrophy whereas the db/db mouse is a model with obesity and insulin resistance with pancreatic failure and consequently severe hyperglycemia. The streptozotocin-induced insulin-deficient mouse served as a model for type I diabetes or end-point stage type II diabetes with extreme hyperglycemia and ketoacidosis. A sensitive LC-MS/MS method was used to quantify around 45 acylcarnitines including isomers derived from individual pathways of fatty acid and amino acid oxidation. We furthermore determined concentrations of odd-numbered fatty acids. Since acylcarnitines are derived from the conversion of acyl-CoA we additionally quantified acyl-CoA species in liver and determined the correlation with the corresponding hepatic acylcarnitines. Particularly strong increases in acylcarnitines derived from branched-chain amino acid metabolism in plasma and tissues of all mouse models were found, but were most pronounced in STZ animals. All models also displayed increased levels in odd-numbered acylcarnitines in liver while dicarboxylic acylcarnitines derived from fatty acid omega-oxidation were strongly decreased in the obese ob/ob and db/db mice. Correlations between acylcarnitine and acyl-CoA concentrations in liver were strongest for monocarboxylic metabolites, while dicarboxylic acylcarnitines like malonylcarnitine and succinylcarnitine showed no or negative correlations with their respective acyl-CoA species. In summary, alterations of metabolic profiles in specific pathways of fatty acid and amino acid oxidation were found in the mouse models and could be associated with conditions of obesity, hyperinsulinemia, and diabetes.

DNA adductomics to reveal red meat and high fat intake related genotoxicity in rats

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DNA adducts are formed when endo- or exogenous genotoxic molecules attack and structurally alter DNA nucleobases, rendering DNA adducts to be potential biomarkers of exposure and/or effect. Hence, in-depth investigation of *in vivo* DNA adduct formation can be used to assess the possible genotoxic effects of the exposure to certain diet and lifestyle related chemicals. This can be of use in different fields of research; for example, to investigate the underlying cause of the plausible causal link between red meat consumption and colorectal cancer (CRC) in humans. The current red meat-CRC hypothesis states that the levels of highly reactive N-nitroso compounds (NOCs) and lipid peroxidation products (LPOs) in the gut increase due to the digestion of red (and processed) meat. Both NOCs and LPOs can exert genotoxic effects and are known to induce DNA adduct formation. In this study, 24 male Sprague-Dawley rats were either fed a low or high fat beef diet, or a low or high fat chicken diet during 14 consecutive days. After the feeding trial, targeted and untargeted DNA adduct detection in liver, duodenum and colon DNA was performed with high resolution mass spectrometry (HRMS(/MS); Q-Exactive). This enabled the targeted detection and quantification of 4 different NOC and LPO related (O^6-carboxymethylguanine, O^6-methylguanine (O^6-MeG), pyrimido[1,2-a]purin-10(1H)-one (M_1G) and α-methyl-γ-hydroxy-1,N^2-propanoguanine (CroG)) DNA adducts and other possibly relevant DNA adduct types by means of a complimentary top-down DNA adductomics approach. DNA adduct analysis was followed by extensive data processing (Xcalibur™ for targeted data processing, and ToxFinder™, Sieve™ and Simca™ for untargeted data processing), during which diet-related DNA adducts could be singled out by means of an in-house DNA adduct database. The O^6-MeG, CroG and M_1G DNA adducts could be detected in liver, duodenum and colon DNA although there was no clear distinction according to diet or tissue type. Untargeted full scan HRMS DNA adduct analysis revealed a different DNA adduct profile in each tissue type. Furthermore, several other DNA adduct types appeared to be significantly higher or lower in rats that were fed a different meat based diet; 24 putatively detected alkylation and oxidation induced DNA adducts could be selected. Since N-nitroso compounds and lipid peroxidation products are (1) hypothesized to rise upon red meat and high fat intake, and (2) prone to alkylate and oxidize DNA, the retrieved DNA adduct types could be highly relevant to the red meat-CRC hypothesis. In total, 16 DNA adduct types appeared to be significantly higher after consumption of beef (vs consumption of chicken), 5 DNA adduct types increased upon high fat consumption (vs low fat), whilst 3 demonstrated an increase after beef consumption (compared to consumption of chicken) as well as a high fat intake (compared to a low fat intake).

Expression of matrix-remodeling associated 5 (MXRA5) may be a marker of energy status

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Overweight and obesity lead to changes in adipose tissue such as inflammation, reduced insulin sensitivity and alterations in the production and secretion of many adipokines. The aim was to assess how altered energy balance by reduced food intake affect these processes. Methods: Healthy male and female Caucasians aged 50-65 years (overweight or obese; BMI 25-35 kg/m^2) were randomized to either energy restriction (~20% reduced intake of energy from food) or control (weight maintenance) for 12 weeks (n=11). Blood and adipose tissue sampling, blood cell count and markers of inflammation, magnetic resonance imaging (MRI) and spectrometry (MRS), and microarray analyses were performed before as well as after the intervention. The intervention was successful, reflected in a ~5% weight loss and reduced BMI. The amount of subcutaneous adipose tissue was reduced in the abdominal region as well as the rest of the body. Total internal adipose tissue was reduced, especially reflected in reduced amounts of liver fat. Gene expression changes in subcutaneous adipose tissue included increased immune-related pathways and reduced energy-related pathways. In addition, the adipose tissue secretome was substantially altered, revealing 30 interesting targets after validation in a second independent cohort and with RT-PCR. The most interesting target was MXRA5, which strongly correlated with the changes in adipose tissue depots. Interestingly, MXRA5 mRNA was linearly reduced in expression from 0-5-11-16% weight loss in a second cohort. Expression of MXRA5 mRNA correlated with TGFb mRNA and other transcripts related to extracellular matrix remodeling and cell-cell adhesion, indicating a role for MXRA5 in these processes. In addition, MXRA5 is equally expressed in viceral and subcutanous adipose tissue, over-expressed in subcutaneous adipose tissue in obese versus lean subjects and in cultured adipocytes from obese versus lean subjects. Furthermore, bariatric surgery reduced MXRA5 mRNA expression in subcutaneous adipose tissue. Finally, fragments of MXRA5 was detected in human blood serum, plasma and urine. MXRA5 mRNA expression is sensitive to weight loss and strongly correlates with alterations in the amount of adipose tissue. MXRA5 might serve as a marker of energy balance.

The maris study – a fully controlled 8 week dietary intervention study

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The importance of a healthy and balanced diet in the prevention of chronic diseases such as diabetes, cardiovascular disease (CVD), and cancer has been extensively studied. Most studies have focused on the relation of single dietary components with disease, whereas a diet is a mixture of many dietary components that can interact with each other. The Mediterranean (MED) diet has received considerable attention in this respect. It is characterized by a high intake of olive oil, fruits, vegetables, legumes, nuts, unrefined cereals, moderate intake of wine, and low to moderate intake of dairy products, meat, poultry and fish, and is considered as health promoting. The effect is often being ascribed to its high content of monounsaturated fatty acids (MUFA) and polyphenols. These bioactive compounds can affect gene expression directly, and might in this way regulate pathways and metabolites related to cardiovascular disease prevention. The objective of this study was to differentiate between the effect of a single food component versus the effect of a mixture of foods. To this end we examined the effects of replacement of saturated fat (SFA) by MUFA from refined olive oil in a western-type diet (MUFA diet), and the effects of a full MED diet high in MUFA from extra virgin olive oil (MED diet) on metabolomics in serum. Results of the study on plasma fatty acid profiles and transcriptomics have already been published elsewhere. In short, this study included 57 abdominally overweight males and females, whom were randomly allocated to either of the two diet groups (MUFA or MED diet), or a control group (SFA diet) for a period of 8 weeks. Main findings included that the MUFA diet significantly decreased serum LDL-cholesterol levels and total cholesterol levels, compared to the SFA diet. Moreover, consumption of the MUFA diet, compared to the SFA diet, decreased expression of oxidative phosphorylation (OXPHOS) genes. MUFA as replacement of SFA in a western-type diet had similar effects on lowering expression of OXPHOS genes as did the MED diet, leading to a more anti-inflammatory expression profile. Currently, we have additionally assessed the concentrations of 233 serum metabolites, including amongst others; different lipoproteins with varying particle sizes, and branched chain amino acids. Analyses of this metabolomics part are still ongoing. Since positive effects on cholesterol levels and gene expression profiles were found by replacement of SFA by MUFA, we now hypothesize that particle sizes of VLDL, LDL, and HDL are also beneficially changed by replacement of SFA by MUFA.

NMR metabolomics study of faecal extracts in patients with inflammatory bowel diseases

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Inflammatory bowel diseases (IBD) represent a chronic disorder, whose idiopathic aetiology is still not entirely known, affecting one or more parts of the intestine. Crohn's disease (CD) and ulcerative colitis (UC) are the two most thoroughly characterized forms of IBD. There is no specific diagnostic test for IBD, with diagnosis mostly based on endoscopic, radiologic, histologic and laboratory data, which are appropriately integrated with the evaluation of the medical case and the degree of disease activity. The combination of Nuclear Magnetic Resonance spectroscopy (NMR) and Multivariate Data Analysis was used to investigate the metabolic profile of the faeces hydrophilic extract. Samples derived from patients affected by IBD as well as from healthy individuals. Overall, 183 samples of faeces were collected from patients following a diagnosis of IBD, namely, 50 CD and 82 UC, together with 51 healthy controls. Orthogonal partial least squares-discriminate analysis (OPLS-DA) applied to NMR data showed the clear separation between patients affected by IBD from the healthy individuals, indicating significant differences among their metabolic profile. OPLS-DA was then applied in order to distinguish the two subtypes of IBD, CD and UC separately, from the healthy individuals. Also in this case, the analysis showed a good separation between the patients affected by CD and UC, respectively, and the healthy individuals. However, the comparison between UC and CD did not show a remarkable separation, which is indicative of a similar metabolic profile in the two major IBD conditions. The metabolites playing an important role in the separation of the samples belong to the classes of amino acids, sugars and decarboxylated microbial amino acids (i.e. biogenic amines). Finally, also metagenomics studies are ongoing on all the faeces samples included in the present study.

Versatile UHPLC-MSMS method for quantification of several alcohol intake related biomarkers

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Alcohol intake has been associated with preventive as well as negative effects on health. However, the intake estimates are often based on subjective reporting and therefore biased and the types of beverages consumed are often inaccurately reported. Accurate and specific quantification of alcohol related compounds in biological samples may help to understand dietary exposure and metabolic kinetics. The aim of this study was to develop a simple, rapid and versatile UHPLC–MSMS method able of quantifying several alcoholic beverage-related intake biomarkers as well as putative efficacy markers. The method was thoroughly validated for L–tartaric acid, ethyl sulfate, ethyl–β–D–glucuronide, indoxyl sulfate, p–cresol sulfate, resveratrol, estrone sulfate and dihydroepiandrosterone sulphate. Additional markers included for validation are some hops- and barley derived compounds and several ethyl esters of endogenous metabolites. All selected analytes were analyzed within 6 minutes in the negative ionization mode using multiple reaction monitoring. The method will be applied for the quantification of the compounds in human samples from dietary studies with beverages as well as for validating alternative sampling techniques such as dry urine or blood spots.

Early nutrition, metabolic effects and long-term health

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During the first 1000 days of life (pregnancy plus the first two years), environmental factors can influence the metabolism which exhibits a high plasticity in this period of life: a mechanism called early programming. A range of epidemiological studies have shown a close relation of early infant nutrition and later adverse physiological outcomes in child- and adulthood. Extremely rapid weight gain in the first days of life is associated with higher risk for later obesity and diabetes, while the risk for a rapid weight gain is increased in formula-fed infants. Moreover, the duration of breastfeeding was found to lower the risk of overweight in late childhood in a dose-dependent manner. Thus, breastfeeding is the most important nutritional influence during early life time. Apart from the very important socio-economic side-effect of breastfeeding, it seems to be rational that human breast milk is optimal designed to support healthy development of infants. A main focus of current nutrition research is to analyse the composition and function of human breast milk to facilitate the production of improved infant formula. In a randomised controlled clinical trial we showed that infant formula with a higher fat and lower protein content decreases the risk of later childhood obesity compared to a lower fat and higher protein formula. Thus, protein content is probably more important for growth than fat in the first year of life. This 'Early Protein Hypothesis' is one of the most important early programming hypotheses. A possible underlying mechanism is the insulinogenic ability of branched chain amino acids which are enriched in high protein formula fed infants and achieve such high concentration because they can escape their self-regulated degradation pathway. Branched chain amino acids can be used for energy provision by degradation to short chain organic acid in the skeletal muscle. The corresponding acyl-carnitines are also elevated in infants fed higher protein formula. To understand these metabolic processes, the determination of small molecules (metabolomics) has become an established research field which is currently conducted in large longitudinal studies. Among different technical approaches, targeted analysis based on liquid chromatography coupled to triple quadrupole mass spectrometry has been shown its ability to cover a broad range of relevant metabolites. Therefore, metabolomics is not only feasible to investigate underlying mechanism of present hypotheses, but also to raise new ideas and questions in the context of early programming. The questions, if unhealthy metabolic patterns in early life stay persistent over the period of infancy to childhood or even to adulthood, is investigated in the large (European) consortiums, like EarlyNutrition, DynaHealth and MetaGrowth, since only repetitive results in longitudinal human trials can create evidence for programming mechanisms and identify targets for early interventions. Meanwhile EarlyNutrition and MetaGrowth focuses on dietary influences from conception to childhood on physiological outcomes such as BMI and growth, the DynaHealth program covers the complex interplay between metabolism, psychological and socioeconomic factors throughout a life-course model.

The impact of breastmilk and delivery mode on the infant's gut microbiota and later health

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The first weeks after birth, the new parents are not the only ones fascinated by this emerging life. Birth and early life remain equally intriguing for microbiologists as the intestinal microbiota builds up, induces the immune system and impacts (long term) health. The babies' first encounter with a dense bacterial population happens during birth. Depending on delivery mode the new-born is first exposed to either the mother's vaginal and fecal flora or to the microbiota from the skin, medical personnel and hospital surroundings. Mode of delivery directly impacts the infant's gut microbiota composition after birth. But what happens in the long run? Due to its effect on the immune system, the bacterial colonization in early life could be the missing key to explain health outcome linked with caesarean birth. Could breastmilk provide some compensation for the bacterial contact that was lacking during a caesarean delivery? Here, we investigated the microbiome composition of stool and breastmilk samples from 30 mother-infant pairs after 3 months of exclusive breastfeeding. Mother infant pairs were stratified according to delivery mode. Both the fecal and breastmilk microbiota were analysed using DGGE and Illumina sequencing In comparison with the respective mother samples, the evenness of the fecal microbial ecosystem was clearly lower in the infants' gut microbiota, indicating a less mature community structure. In combination with the detected lower richness, domination by a few species in the infant gut was observed. Interestingly, the cluster analysis of the gut microbiota composition did not reveal differences according to delivery mode. Three months of exclusive breastfeeding had apparently erased the presumed microbiota differences between delivery mode shortly after birth. Taking a closer look at the microbial content of the milk, a lower evenness in the breastmilk from mothers undergoing caesarean section in comparison with those undergoing vaginal delivery. More research is needed to substantiate this difference in milk microbiota and identify the source of these bacteria. Microbiota being present in breastmilk suggests a possible microbiota transfer route between mother and child. Intriguingly two breastmilk samples from the c-section delivery group gave results similar to the vaginal group. Taking the meta data into account, these outliers were women who had an emergency c-section. C-section comprises two distinct biological processes namely the emergency and elective C-section. Preceding an emergency C-section, in contrast to a planned delivery, the mother undergoes labor with or without hormonal changes, rupture of the membranes and contractions. As the biological process prior to the surgical procedure is completely different, this could also affect the microbiota. A further subdivision of mode of delivery into 3 categories should be taken into account for further research and previous statements based on vaginal vs C-section comparisons because the delivery mode is an important determinant for the development of the infant gut microbiota.

LDL cholesterol in early pregnancy and offspring cardiovascular disease risk factors

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Cardiovascular disease (CVD) starts to develop in early life, potentially even in utero. A vast amount of data shows that maternal obesity, dysglycemia, diabetes and undernutrition prior to and during pregnancy associates with offspring CVD risk. However, associations between maternal gestational hypercholesterolemia and offspring CVD risk have scarcely been studied. The objective was to investigate the associations between elevated maternal gestational low-density lipoprotein cholesterol concentration (LDL-C) and CVD risk factors in 6-13 year old offspring. We recruited and examined 6-13 year old children whose mothers participated in the Stork pregnancy cohort, and who had either hypercholesterolemia or hypocholesterolemia during pregnancy, defined as LDL-C over the 90th percentile or below the 10th percentile within the Stork cohort, respectively. We measured CVD risk factors in the children, including body composition, blood pressure, plasma lipids, glucose concentration and total fatty acid composition, as well as dietary intake. Maternal plasma LDL-C at gestational week 14-16 was 4.0 and 1.4 mmol/l in the hypercholesterolemic (n=27) and hypocholesterolemic (n=34) groups, respectively (P<0.001). Interestingly, offspring plasma LDL-C was 0.4 mmol/l higher in children whose mothers had hypercholesterolemia during early pregnancy (P<0.01). There was no difference in birthweight in the two groups, and no other clinical or biochemical CVD risk factors or dietary intake whereas different between the groups at 6-13 years. Women with elevated cholesterol during early pregnancy have offspring with elevated LDL-C at the age of 6-13 years. Promoting a cholesterol-lowering healthy lifestyle among young fertile women with hypercholesterolemia may be particularly beneficial in offering CVD protection – not only for the individual woman, but also for children of the subsequent generation.

Antibiotics markedly alter the metabolome of preterm pigs susceptible to necrotising enterocolitis

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Necrotising enterocolitis (NEC) is a severe gut disorder involving the microbiota in preterm infants. Prophylactic oral and systemic antibiotics prevent NEC in premature pigs and change the proteomes of the small intestine and plasma of these pigs. It is hypothesised that prophylactic antibiotic treatment can also affect metabolism of preterm neonates, reflected as change in the plasma and urinary metabolomes. In the current study, plasma and urinary metabolomes of formula-fed preterm pigs that received antibiotics immediately after birth for four days were profiled by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) and were compared with those of pigs which were not given antibiotics. The antibiotic treatment comprehensively altered both the urinary and plasma metabolomes. Fourteen and 13 metabolites with significantly differential abundance were identified in plasma and urine, respectively. In the antibiotics-treated (AB) pigs, tryptophan metabolism is inclined towards the kynurenine pathway over the serotonin pathway shown by specific metabolites. Metabolites associated with the gut microbiome, including 3-phenyllactic acid, 4-hydroxyphenylacetic acid and phenylacetylglycine as well as three bile acids, were found at lower levels in AB pigs where the gut microbiome was extensively attenuated. Elevated levels of nucleoside metabolites including hypoxanthine and inosine, and N-acetyl-glutamic acid, pyroglutamic acid and N-acetyl-methionine in the untreated pigs can be attributed to NEC-associated infection and inflammation, and increased intestinal permeability. The appearance of microbial metabolites in body fluids may serve as early signs of NEC, which is important for timely clinical interventions. Besides the evident NEC-preventing effect, possible adverse effects of antibiotic treatment on nutrient utilisation and homeostasis in preterm neonates may be raised as a concern.

Novel plasma proteins in school-aged children are associated with a small head size at birth

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Undernutrition during fetal development may have long-term effects on health, mediated by permanent changes in cell size and number, and gene and protein expression. However, little is known about molecular signatures of early life nutritional insults that persist into childhood. We explored associations between birth anthropometry, as a proxy for fetal growth, and plasma proteins in a cohort of 500 6-8 year old children, born to mothers who had participated in an earlier prenatal micronutrient supplementation trial in Nepal, to assess plasma protein profiles in childhood associated with fetal growth restriction. A database of plasma protein relative abundance obtained from tandem mass spectrometry with isobaric mass tags was established for the 500 children in whom birth weight, length, and head circumference had previously been measured. Small birth size was defined as having a weight, length, weight-for-length or head circumference <-2 Z-scores of the WHO growth standards. We estimated differences in relative abundance of proteins between children born with small vs normal (≥-2 Z-scores) birth size. Pathway analysis using the Gene Ontology database was performed to identify functional protein clusters that are associated with birth size. Among 982 plasma proteins observed in >10% of children, 21 proteins were differentially abundant by head size at birth, while no proteins were differentially abundant by other anthropometric indicators ($q<0.10$). After adjusting for confounders including gestational age and child age, sex, and anthropometry, 19 proteins differed by head size at birth ($q<0.10$), 18 of which were 8-22% less abundant in plasma of children whose head circumference was small versus normal at birth. They are predominantly cytoskeletal proteins (α-actin, α-actinin, talin, filamin, etc.), proteins regulating actin-cytoskeleton interactions (tropomyosin 3/4), actin-binding motor proteins (myosin 6), and cytoskeleton-binding glycolytic enzymes. A correlation matrix revealed high, positive correlations among these proteins (average $r=0.68$). Relative abundance of one protein, angiopoietin-like 6, involved in vascularization was elevated in plasma of children with small head size at birth (vs children with normal head size). Pathway analysis confirmed that cytoskeleton-related protein sets were significantly negatively enriched in children with small head size ($q<0.10$). Plasma proteomics identified a novel cluster of proteins measured at 6-8 years of age specifically associated with a small head size at birth. As cytoskeletal metabolism and angiogenesis are fundamental processes in early development, identified proteins may be long-lasting markers of impaired cell and tissue growth and function during fetal life. Grant funding: Bill and Melinda Gates Foundation (OPP5241 and GH614).

Untargeted Urine Metabolome Profiling of Infants from the Danish SKOT I and SKOT II Cohorts

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There is a growing evidence that the mother`s nutritional status in pregnancy affects metabolism of the offspring. However, the link between mother´s nutritional status in pregnancy and infant´s metabolism is still not clear. The aim of this study is to explore whether the urine metabolome profile is different between infants born from obese mothers and mothers with body mass index (BMI) within the normal range. From the Danish SKOT 1 cohort of normal-weight mothers and children, a randomly selected set of 30 term infants were selected and compared with 40 term infants from SKOT 2 cohort of obese mothers. Urine samples collected with cotton inserts in the diapers at the age of 9 months and samples collected by normal spot voids at 36 months were included in the study. Urine samples were diluted and profiled using UPLC-Q-TOF/MS. Data were preprocessed with MZmine_2.19 followed by principal component analyses (PCA) and Partial Least Squares Discriminant Analysis (PLSDA) using PLS_Toolbox_8.1. There was a clear difference in urine metabolome profiles by PCA at the age of 9 and 36 months. At 9 months samples from SKOT 1 and 2 were clearly separated by PCA based on urine metabolome profile, but for the age of 36 months there was only a tendency for separation in PCA. Late eluting, fat soluble compounds with high VIP scores –possibly related to mother`s BMI- were detected in the PLSDA model at 9$^{th}$ and 36$^{th}$ months. Further analyses are needed to identify the compounds related to mother`s BMI and interpret the effect observed on offspring metabolism.

Precolos-Bovine colostrum as nutrition for preterm infants in the first days of life: a pilot study

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The optimal feeding regimen for preterm infants is not clear, especially when mother's own milk (MM) is not available. Infant formula (IF) and donor milk (DM) are potentially inferior to MM in promoting feeding tolerance, growth, and intestinal maturation. Bovine colostrum (BC) contains large amounts of protein, growth factors and immuno-regulatory components (IGFs, IgG, Lactoferrin) which may be beneficial. We wanted to investigate whether feeding BC to preterm infants during the first days of life would be safe, well tolerated and could promote nutrient uptake and gut maturation when MM is limited. We designed the PreColos study as a three-phased (A, B, C), dual-site (Rigshospitalet, RH and Foshan Women and Children's Hospital, FWCH), pilot feasibility study (ClinicalTrials.gov NCT02054091). The aim of this first part of the study (phases A and B) was to investigate the feasibility of bovine colostrum given as a supplement to maternal milk in the first days of life for preterm infants. The results of phases A and B are summarized here. In phases A and B, 12 infants delivered with gestational age (GA) between 27+0 and 32+6 weeks (RH) or birth weights (BW) 1000-1,800 g (FWCH) were recruited before the first feeding. BC was administered as a supplement to MM for up to 10 d with the maximum total protein intake of 4.5 g/kg/d. Outcomes were feeding intolerance (FI), time to enteral feeding at 120 ml/kg/d (TTF120), growth, combined incidence of serious infections/NEC, plasma amino acids, and plasma bovine IgG. Data are presented as median (interquartile range). The BW and gestational age (GA) for the 12 infants (7/5, female/male) were 1499 (1231-1650) g and 30.4 (29.8-31.9) weeks. Infants received BC for 7.5 (5.8-9.0) d at a dose of 17.7 (12.2-25.3) ml/kg/d and 1.4 (1.0-2.0) g/kg/d protein from BC. At 37 weeks or discharge, body weight reached 2280 (2112-2510) g and average growth velocity was 12.3 (10.3-13.8) g/kg/d. TTF120 and days on PN were 10.0 (6.0-14.5) and 11.0 (0.0-15.5) d. Seven infants showed FI in the first week and in 1 infant in the second. The total volume of gastric residual was 39 (6-79) ml in the first week and 4 (1-12) ml in the second. On day 7, five infants showed a transient hypertyrosinemia, which disappeared on day 14 for all infants. Plasma bovine IgG was below the detection limit (5 μg/ml, n=7). No adverse clinical reactions to BC were observed. BC supplementation was tolerated and raised enteral protein intake markedly in very premature infants during the first week of life. The results were used to initiate phase C, a randomized, controlled pilot trial with 40 infants in total comparing bovine colostrum with currently used standard diets to further examine the safety of bovine colostrum supplementation and clarify relevant endpoints and reasonable sample size for a larger randomized, controlled trial, which will allow us to study the effects of early bovine colostrum feeding on long-term growth and cognitive development in preterm infants.

Dairy and plant based food intakes are associated with altered faecal microbiota in children

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The first 1000 days (conception to 24 months) is when gut microbiota composition and eating patterns are established, and a critical period influencing lifelong health. The aim of this study is examine the association between food intake and microbiota composition at the end of this period. Diet was quantified for 37 well-nourished Australian children aged between 2 to 3 years by using a food frequency questionnaire and 24 h recalls. Both dairy and plant-based (fruit, vegetables, soy, pulses and nuts) food intakes were associated with distinct microbiota profiles. Dairy intake was positively associated with the Firmicutes:Bacteroidetes ratio, and in particular *Erysipelatoclostridium* spp., but negatively associated with species richness and diversity. Vegetable intake was positively associated with the relative abundance of bacteria assigned to the *Lachnospira* genus, while soy, pulse and nut intake was positively associated with the relative abundance of bacteria related to *Bacteroides xylanisolvens*. Fruit intake, especially apples and pears, was negatively associated with the relative abundance of bacteria most closely related to *Ruminococcus gnavus*. In this cohort of 2 to 3 year old Australian children dairy and plant based food intakes were found to be associated with altered microbiota composition. Further exploration is needed to elucidate the effect of these dietary and microbial differences on host phenotype.

Neonatal gut microbiota perturbation by peripatum antibiotics in rats lead to decreased weight gain

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Cross talk between a mammalian host and its intestinal microbiota plays a role in immune mediated diseases such as allergies, asthma, type 1 diabetes, as well as in obesity and auto immune diseases. Over the past decades, a significant increase of these diseases in young children in the developed world has been documented. In Western countries the pattern of initial colonization of the gut during the first days of life has changed dramatically. Among factors potentially modulating initial colonization, the use of antibiotics is particularly important. Antibiotics are frequently administered orally to either mothers or young children to treat or prevent bacterial infections not necessarily related to the gastrointestinal system. This has adverse effects on the commensal gut microbial community, as it disrupts the intricate balance between specific bacterial groups within this ecosystem, potentially leading to dysbiosis. We hypothesized that modulation of community composition and function induced by peripartum antibiotics affects intestinal microbial composition and general health of the offspring. To address this, 33 pregnant Wistar rats were dosed by oral gavage with either amoxicillin (AMX), vancomycin (VAN) or water (CON) daily from 8 days before delivery until weaning of the offspring. Significant lower weightgain of the offspring of antibiotic treated dams compared to the control were observed. The antibiotic treated dams had significantly larger caecum size and higher caecal pH as well as spleen size than control animals. Offspring were dissected at different time points and significant changes in liver, spleen and epididymal fat were measured between groups. Composition of the gut microbiota, alpha diversity, caecum short chain fatty acid levels, caloric contents of faeces, bile salt levels, acute phase protein haptoglobin in blood, social and locomotive behavior as well as gene expression of tight junction proteins are currently being analyzed.

Possible mechanisms of prevention by moderate alcohol intake

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Moderate alcohol consumption has been associated with lower risk of cardiovascular disease and diabetes in observational cohorts for over four decades, forming the basis for a 'J-shaped' curve of alcohol with chronic disease. Possible mechanisms for the associations of moderate alcohol intake with cardiometabolic disease and other chronic diseases, like breast cancer and osteoporosis, have been extensively studied in short- and intermediate-term feeding studies and confirmed in large epidemiological studies. These mechanistic studies help to provide a plausible foundation for the classic J-shaped curve and focus the effects of alcohol on several metabolic pathways, including reverse cholesterol transport, insulin resistance, inflammation, platelet function, and sex steroid hormones. The most widely studied effect of alcohol is to increase levels of HDL-cholesterol. Although the effect is nearly a simple dose-response, it appears to be modified by baseline levels and possibly by genetic variation in the HDL metabolic pathway and extends to *ex vivo* cholesterol efflux. Statistical modeling suggests that half of the benefit of alcohol observed in cohort studies may be attributable to increased levels of HDL-cholesterol. Of greatest importance for diabetes, alcohol consumption reduces insulin resistance, particularly in women, and lowers markers of glycemia in feeding studies. The best studied mechanism to date involves adiponectin, whose expression in adipocytes is directly increased by alcohol consumption. A third potential pathway involved in several cardiometabolic disorders is inflammation. Randomized trials confirm that alcohol consumption lowers levels of fibrinogen, an acute-phase reactant, although effects on other inflammatory markers have been observed if less consistently. The observed effects of alcohol consumption to inhibit platelet function and raise levels of estrone and DHEA sulfate in short-term trials may explain its competing associations with coronary heart disease and other chronic diseases. For example, alcohol consumption lowers risk of myocardial infarction and ischemic stroke, but increases risk of hemorrhagic stroke and possibly gastrointestinal bleeding, which cumulatively point to an anti-thrombotic effect. Likewise, alcohol consumption increases HDL-cholesterol and bone density while increasing risk of hormone-sensitive breast cancers, suggesting effects mediated by endogenous estrogens. Other issues complicate a simple categorization of the effects of alcohol. Genetic differences in alcohol metabolism appear to modify its effects on cardiovascular disease, perhaps by modifying the HDL-raising response. Further, the acute effects of alcohol often demonstrate biphasic responses, with differential changes based upon timing since last dose. Finally, the upper limb of the J-shaped curve may be dictated by a number of pathways activated primarily with heavy alcohol consumption, including depletion of endogenous antioxidants, acute increases in hemodynamic parameters, and adverse nutritional and behavioral responses.

Biomarker model for detecting beer intake

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Alcohol intake is a strong confounder in many nutrition studies but research is hampered by lack of accurate tools (biomarkers) characterizing the exact origin of the consumed alcoholic beverages. Therefore, we applied untargeted metabolomics approach to identify plasma and urine metabolites associated with recent beer intake and used a characteristic metabolite pattern representing raw materials and beer production as a sensitive qualitative biomarker of beer intake. The first meal study (MSt1) was a randomized, crossover, single-blinded meal study with 18 participants who were given one at a time of four different test beverages: (1) strong lager, (2) regular lager, (3) non-alcoholic beer and (4) a soft drink together with a standardized meal. In an additional meal study (MSt2), four participants were assigned to have lager either with high hops or low hops. For MSt1, plasma and urine samples were collected up to 3 h and 24 h, respectively and up to 40 h (only urine) for MSt2. In addition to the samples, test beverages, wort and hops extract used in the production of the beer were analyzed with liquid chromatography mass spectrometry. ASCA was used to isolate the effect of beer from that of alcohol and the discriminant metabolites (beers vs soft drink) were selected by PLSDA. Urine and plasma metabolites associated with beer intake were characterized according to their origin: (1) hops, (2) wort (barley or malting), (3) fermentation, and (4) human biotransformation and from each of the three groups, one metabolite was selected for establishment of a specific biomarker model for beer. Metabolites originating from hops were iso-alpha-acids (IAAs) and their degradation products which were further rearranging to tricyclohumols and/or conjugated in the body. Maltose, pyro-glutamyl proline, hordenine, N-methyl tyramine (NMT), isoleucine/leucine and tyrosine were characteristic of the wort component. 2-ethyl malate was identified as a metabolite originating from fermentation. The biomarker model was based on four selected metabolites, i.e. pGlu-Pro (malting), NMT sulfate (barley), 3-ethyl malate (fermentation) and the sum of IAAs and tricyclohumols (hops) in 24 h pooled urine, for MSt1. The model was validated using MSt2, which predicted all the samples collected before and up to 12 h after beer intake 100% correctly (AUC=1). Beer intake affected a number of metabolites, particularly in urine. Although none of the identified beer intake markers were specific or stable biomarkers individually, a biomarker model including four metabolites representing both beer raw materials and production steps provided a specific and accurate tool for measurement of beer consumption. The use of aggregated biomarkers to assess intake of complex processed foods is a novel concept and may be a powerful tool for compliance assessment and for exposure assessment in observational studies.

Metabolomics pre-processing and compound annotation

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Mass spectrometry coupled with liquid chromatography (LC-MS) has become a widely used analytical tool for analysis of biological samples in metabolomics and proteomics research. Due to its high sensitivity, LC-MS experiments produce large amounts of data with complex chemical information which makes it unsuitable for application of any data analysis tool in its raw form. The next step is therefore to pre-process the data which will arrange and reduce the data in such a way that relevant information is kept. Before any pre-processing can commence, however, the LC-MS data need to be converted from proprietary vendor-specific formats to open formats. We will show you how to use these tools and how to ensure that you preserve the integrity of your data. Data pre-processing is crucial for the quality of the quantitative information as well as for the final compound identification, and therefore the resulting biological interpretation. Many software packages for pre-processing of LC-MS bas ed metabolomics data are freely available such as XCMS and MZmine. Some performs only specific steps in the pre-processing pipeline, whereas others cover many steps. These tools typically include specific algorithms for the two key steps in data pre-processing, (1) peak detection and (2) alignment. Each software tool creates a list of peaks denoted by a specific mass and retention time and each peak has a signal intensity denoting peak area. Correct and robust data pre-processing requires understanding of the raw data structure so that parameter settings of the data pre-processing tool can be optimized. This could be a difficult task unless the selected data pre-processing tool provides visual tools allowing inspection of raw data characteristics. MZmine offers simple visual tools for each step of data pre-processing which allows users without programming skills to pre-process the data with optimized settings. Therefore, we will provide an illustration of data pre-processing with MZ mine using a set of samples from a metabolomics study. Once the raw data has been pre-processed statistics are applied to select the features that are of interest in the context of the specific study. We will not cover this step in this workshop. One of the major bottlenecks in metabolomics is that the molecular structure of the metabolites are initially unknown until their identity has been confirmed by standard compounds. In addition each metabolite can lead to several peaks and it is therefore important to recognise peaks associated with the same compound and what the relationship between these peaks are, which can provide useful information for identification. In this workshop we will show you how to apply the tool CAMERA that will group the peaks and annotate their relationship. Finally we will briefly summarise how you can proceed with compound identification. In summary this workshop will provide a brief walkthrough of: (1) conversion of vendor-specific raw data to open format s such as mzData, mzXML or mzML; (2) illustration of basic steps in data pre-processing using MZmine; (3) illustration of peak grouping and annotation using CAMERA; and (4) a brief overview of the tools you can employ for compound identification.

Insights in working with complex datasets: benefits and limitations using MetaCore

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Advances in data acquisition, such as whole genome microarray analysis, have changed how we address and investigate basic and applied scientific questions. Today, we are able to generate complex datasets to tackle the challenges of the 21st century, including a growing and ageing population, metabolic diseases such as type 2 diabetes and obesity as well as human nutrition underlying health and disease. The ability to analyse datasets effectively is of critical importance for a deep understanding of modern biology and physiology. This ultimately requires skills in the field of quantitative research in order to uncover and understand the underlying molecular mechanism of complex biological processes. This tutorial will focus on introducing and discussing the bioinformatics tool MetaCore and its ability to underpin modern bioscience and nutrigenomics in the context of physiological and pathophysiological interpretations. We aim to demonstrate key analytical tools for microarray analysis using MetaCore in order to extract valuable knowledge from complex transcriptomic datasets generated in your research projects. The session will be designed for participants to acquire essential skills for the pathway analysis and interpretation of differential gene expression data of human and animal studies. This will be combined with the presentation and discussion of successful published studies regarding a metabolic remodelling and endocrine regulatory function of skeletal muscle. Finally, participants will be made familiar with benefits and limitations of using MetaCore to accelerate their scientific research as well as future perspectives to gain extensive understanding of biological systems.

Hands on session for the ENPADASI DASH-IN software

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The objective of ENPADASI is 'project is to deliver an open access research infrastructure that will contain data from a wide variety of nutritional studies, ranging from mechanistic/interventions to epidemiological studies including a multitude of phenotypic outcomes that will facilitate combined analyses in the future'. From this the Data Sharing In Nutrition (DASH-IN) infrastructure has been developed. The DASH-IN infrastructure has been designed to meet the FAIR Principles. It provides a means to upload data in a fashion that ensures that data has a precise and measurable set of qualities; qualities that ensure that the data is Findable, Accessible, Interoperable, and Reusable. This training session will proved an overview of the DASH-IN infrastructure and will guide users through uploading of data. The training work package of ENPADASI has developed on-line resources including a step-by step video on how to upload data. All information can be found at http://www.enpadasi.eu/wp6.html.

Role of the gut microbiota in over- and under-nutrition

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Gut microbiota collectively represents 40 trillion microorganisms living in the gut and is nowadays considered as a crucial regulator of host immunity and metabolism. Gut microbiota has been proposed to be involved in the occurrence and/or evolution of several metabolic and inflammatory diseases, such as obesity, diabetes, inflammatory bowel diseases, asthma and allergies. Therapeutic benefits from the gut microbiota can be harvested through the use of probiotics and prebiotics. During this presentation, I will discuss our recent studies aiming to evaluate the role of the gut microbiota in over- and undernutrition. The first part of the talk will be dedicated to the systematic evaluation of the role of the gut microbiota in the health benefits conferred by resistant starches in metabolic syndrome. Resistant starches (RS) improve insulin resistance in human nutritional trials and animal experiments, and these benefits are often hypothesized to be mediated through modulation of the gut microbiota. Using comparative studies performed in conventionalized and germ-free mice fed a Western diet, we demonstrate that some metabolic benefits exerted by dietary RS, especially improvements in insulin levels, can occur independently of the microbiota. This work also sets a precedent for future mechanistic studies regarding the role of the gut microbiota in mediating the benefits of other functional foods. In the second part of the talk, I will present experimental results suggesting that nutritional modulation of the gut microbiota could constitute an interesting therapeutic approach for cancer cachexia. Up to 50% of cancer patients suffer from cachexia, defined as a progressive loss of adipose tissue and skeletal muscle atrophy. Inflammation and anorexia are often associated with cachexia and participate to its progression. A community-wide analysis of the caecal microbiota from two mouse models of cancer cachexia identified common microbial signatures, including decreased *Lactobacillus* spp. and increased Enterobacteriaceae and *Parabacteroides goldsteinii*/ASF519. Building on this information, we administered a synbiotic containing inulin-type fructans and live *Lactobacillus reuteri* 100-23 to leukemic mice. The treatment improved many of the features associated with cancer and cachexia, such as muscle atrophy, hepatic cancer cell proliferation and lifespan. Cancer cachexia was also associated with alterations of the gut barrier function. Administration of the synbiotic treatment restores the expression of antimicrobial proteins controlling intestinal barrier function and gut immunity markers. Altogether, this set of experiments provides evidence that the development of cancer outside the gut can impact intestinal homeostasis and the gut microbial ecosystem and that a symbiotic intervention, by targeting some alterations of the gut microbiota, confers benefits to the host, improving lifespan and reducing cancer cell proliferation and cachexia. Finally, the host-microbiota crosstalk in alcohol-dependent populations will be evoked.

Populating the preterm infant gut with probiotics

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Initial colonisation of the gut by pioneer bacterial species is the first key step for host well-being including immune defence development. The process of initial gut microbiota colonisation in preterm babies (1:10 live births defined as <37 weeks gestation) is radically interrupted due to a variety of factors including mode of delivery and antibiotics. This aberrant colonisation of premature infants appears pivotal to the development of a number of diseases, including necrotising enterocolitis (NEC). Importantly microbiota modulation via probiotic supplementation may aid in prevention of NEC in preterm infants. I will discuss how probiotics, including Bifidobacterium represent a powerful opportunity for strategically manipulating the wider early life microbiota (from birth up to 1 year) when bacterial assembly is disturbed within the context of preterm birth and correlations to health outcomes.

Colonic transit time is related to bacterial metabolism and mucosal turnover in the human gut

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Little is known about how colonic transit time relates to human colonic metabolism, and its importance for host health, although stool consistency, a proxy for colonic transit time, has recently been negatively associated with gut microbial richness. To address the relationships between colonic transit time and the gut microbial composition and metabolism, we assessed the colonic transit time of 98 subjects using radiopaque markers, and profiled their gut microbiota by 16S rRNA gene sequencing and their urine metabolome by ultra performance liquid chromatography mass spectrometry. Based on correlation analyses, we show that colonic transit time is associated with overall gut microbial composition, diversity and metabolism. A relatively prolonged colonic transit time associates with high microbial species richness and a shift in colonic metabolism from carbohydrate fermentation to protein catabolism as reflected by higher urinary levels of potentially deleterious protein-derived metabolites. Additionally, shorter colonic transit time correlates with metabolites likely reflecting increased renewal of the colonic mucosa. Together, this suggests that a high gut microbial richness does not per se imply a healthy gut microbial ecosystem and points at colonic transit time as a highly important factor to consider in microbiome and metabolomics studies.

Mitochondrial function controls intestinal epithelial stemness and proliferation

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The intestinal epithelial cell (IEC) layer constitutes a rapidly self-renewing interface in intimate contact with the enteral environment and the immune system of the host, enabling intestinal homeostasis. Disturbances of this homeostasis can give rise to chronic degenerative diseases of the gastrointestinal tract such as colorectal cancer (CRC) or inflammatory bowel diseases (IBD), which are often associated with metabolic disturbances. In line, epithelial mitochondrial dysfunction and failure in cellular oxidative metabolism have been implicated in the pathogenesis of IBD and CRC. We used tissue-specific mouse models deficient in heat shock protein 60 (HSP60), a mitochondrial chaperone, to investigate the impact of mitochondrial function on IEC homeostasis. Induction of HSP60 deficiency activated the mitochondrial unfolded protein response (MT-UPR) and was accompanied by mitochondrial dysfunction including reduced respiratory capacity and a drop in cellular ATP levels. An observed loss of stemness and cell proliferation in HSP60-deficient crypts was associated with epithelial release of WNT signaling-associated factors WNT10A and RSPO1. Sporadic failure of Cre-mediated Hsp60 deletion gave rise to hyperproliferative crypt foci originating from $OLFM4^+$ stem cells. The importance of WNT10A and RSPO1 for epithelial proliferation and regeneration was confirmed *ex vivo* by intestinal organoid culture. Our data show that HSP60 deficiency leads to mitochondrial dysfunction and antagonizes epithelial stemness. At the same time, mitochondrial dysfunction is associated with induction of WNT-related signals in IEC. The observed compensatory hyperproliferation of $HSP60^+$ escaper stem cells suggests the paracrine release of WNT-related factors from HSP60-deficient, functionally impaired IEC to be pivotal in the control of the proliferative capacity of the stem cell niche. Conclusively, these data indicate a crucial role for mitochondrial function in maintaining intestinal stemness and homeostasis.

Infant gut microbiota development is driven by transition to family foods

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The first years of life are paramount in establishing our endogenous gut microbiota, which is strongly affected by diet and has repeatedly been linked with obesity. However, very few studies have addressed the influence of maternal obesity on infant gut microbiota, which may occur either through vertically transmitted microbes or through the dietary habits of the family. Additionally, very little is known about the effect of diet during the complementary feeding period, which is potentially important for gut microbiota development. Here, the gut microbiotas of two different cohorts of infants, born either of a random sample of healthy mothers (n=114), or of obese mothers (n=113), were profiled by 16S rRNA amplicon sequencing. Gut microbiota data were compared to breastfeeding patterns and detailed individual dietary recordings to assess effects of the complementary diet. We found that maternal obesity did not influence microbial diversity or specific taxon abundances during the complementary feeding period. Across cohorts, breastfeeding duration and composition of the complementary diet were found to be the major determinants of gut microbiota development. In both cohorts, gut microbial composition and alpha diversity were thus strongly affected by introduction of family foods with high protein and fiber contents. Specifically, intake of meats, cheeses and Danish rye bread, rich in protein and fiber, were associated with increased alpha diversity. Our results reveal that the transition from early infant feeding to family foods is a major determinant for gut microbiota development.

Dietary intakes and gut microbiota composition in a Swedish population

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Several studies have shown that diet affects the constitution of the human gut microbiota. However, many of these studies have been conducted within controlled settings with limited generalizability. We aim at investigating if intakes of fiber, whole grain, fruits, vegetables and meat associate with the gut microbiota in the ongoing Malmö Offspring Study (MOS) using dietary records. The present study included 184 individuals (mean age 41y, 51% women and no use of antibiotics in previous 6 months) from MOS. DNA was extracted from fecal samples and the 16S rRNA V1-V3 region was sequenced. High-quality sequence reads were binned into operational taxonomic units (OTU:s) using QIIME software. Singletons and low abundance (<0.001%) OUT:s were removed. Dietary data on fiber, whole grain, vegetables, fruit and meat was obtained from a validated 4-day dietary record, and then energy adjusted (g/kJ). We applied a linear regression model adjusted for age and sex, to examine associations between tertiles of dietary intakes and relative abundance of the bacteria (logarithmically transformed). False discovery rate was applied to correct for multiple testing. After quality control we observed 8 OUT:s at phylum level and 62 at genus. We did not observe any significant associations between the bacteria and the food groups at phylum level. However at genus level we observed significant association between specific bacteria and the different food groups. The strongest associations were seen for wholegrain and decreased abundance of *Alistipes* (p for trend 0.003), for fiber and decreased abundance of *Acidaminococcus* (p for trend 0.008), for fruit and decreased abundance of *Blautia* (p for trend 0.017) and for meat and increased abundance of *Sutterella* (p for trend 0.014). However, the associations did not remain significant after correction for multiple testing. Intakes of fiber, whole grain, fruits and meat showed nominally significant associations with relative abundance of several gut bacterial genera. Currently we are sequencing 500 feces samples to confirm and extend these findings.

Dairy probiotic or prebiotic intake reduces postprandial inflammation and modulates gut microbiota

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Intestinal dysbiosis has been implicated in the pathogenesis of metabolic diseases including type 2 diabetes mellitus and obesity. Several dietary strategies have been explored to address this issue including probiotic and prebiotic supplementation for their potential action on the gut microbiota: probiotics deliver of live microorganisms to the microbiota while prebiotics selectively promote growth (or alter activity) of microorganisms. However, the role of these interventions on the microbiota and their benefits on metabolic disease risk remain controversial. The current study aimed to assess the impact of probiotic compared to prebiotic dairy interventions on the gut microbiota and metabolic health. A twelve week randomized cross-over design was used to evaluate the impact of daily intake of a yogurt containing *Lactobacillus rhamnosus* GG compared to a milk acidified with the prebiotic δ-gluconolactone (400 g/day for two weeks) in fourteen healthy young men (age 24.6±4.7 years, BMI 21.8±1.8 kg/m^2). The intervention phases were separated by a three-week wash-out phase during which normal milk was consumed. In addition to fasting assessments, the effect of the dairy products on the postprandial inflammatory response was assessed with a standardised high fat meal test. Temporal changes of the faecal microbiota were evaluated by sampling at eight different stages of the study (16S rRNA sequencing, Illumina MiSeq). Neither probiotic yogurt nor acidified milk affected fasting markers of metabolic health or inflammation. However, after both dairy interventions, the postprandial response of interleukin 6, tumour necrosis factor-α and chemokine ligand 5 after a high fat test meal was significantly reduced (iAUC, $P<0.001$). Significant changes were also observed after the chronic interventions on certain microbiota taxa including the detection of yoghurt strains *Lactobacillus delbrueckii* and *Streptococcus salivarius* (padj. <0.0001). The sensitive detection of the strains used to ferment the yoghurt was a useful marker of compliance with the intervention. Nine operational taxonomic units (OTUs) were altered after acidified milk intake, notably including *Bifidobacterium* strains, which is consistent with the prebiotic action of δ-gluconolactone. Probiotic or prebiotic dairy interventions appear to reduce the postprandial inflammatory responses associated with a high fat meal. These changes are accompanied by alterations of the microbiota, and multivariate analysis of the clinical markers and faecal microbiota are in progress. These data suggest that dietary modulation of the gut microbiota may modulate the metabolic changes occurring in the early stages of metabolic disease.

Dissecting the female gut microbiota – 106 participants, 115 measurements and millions of bugs

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Individual gut microbial composition is closely related to environmental factors, lifestyle habits and nutrition. The gut microbiome is essential for conversion of food to energy and might therefore have an influence on digestion, satiety and body weight. Additionally, it has different communication pathways with the central nervous system, called the gut-brain-axis. Gut dysbiosis, representing a gut-brain-axis disruption could thus be a key factor for understanding health and disease in women. We have investigated the composition of the gut microbiome in a large cohort of 106 female participants: anorexia nervosa patients (n=18), athletes (n=20), normal weight (n=26), overweight (n=22) and obese women (n=20). DNA was extracted from stool samples and subjected to 16s rRNA gene analysis by Ion Torrent based deep sequencing. A comprehensive bioinformatics pipeline based on QIIME (www.qiime.org) was applied to all data. In addition 115 anthropometric and clinical parameters were determined from all participants. Microbiome analysis revealed diverse microbiota in all participants with substantial differences between participants. Alpha diversity is reduced in anorexic (BMI<17,5) and obese participants (BMI>30). Statistical analysis by ADONIS test reveals a correlation of BMI, subcutaneous fat measurements on chest, neck and hip, Carotin counts and Fagerström test with weighted Unifrac distance of microbiota. Kruskal Wallis test identified the bacterial class of Clostridia, the order of *Rickettsia* and the family of Lactobacillales as contributing factors for these differences. The fecal concentration of Clostridia was significantly higher in athletes compared to overweight (P=0.037) and obese controls (P=0.009). In the order of *Rickettsia*, significant abundance differences could be found between AN and obese controls (P=0.009) as well as between AN and obese controls (P=0.013), normal weight and overweight (P=0.03) and normal weight and obese participants (P=0.04). Concerning the family of Lactobacillales significant abundance differences appeared between AN, normal weight (P=0,003) and adipose controls (P=0.03) as well as between athletes and adipose controls (P=0.019). More extensive bioinformatics analysis of this dataset is ongoing. This study generated data for the analysis of the impact of diverse antropomorphic parameters on the female gut microbiome. We identified several antropomorphic measurements correlated to the microbiome. In-dephth bioinformatics analysis of the dataset is ongoing. Keywords: microbiome, gut microbiota, anorexia nervosa, athletes, exercise, gut-brain-axis

Probiotics do not protect against high concentrations of bacteria in nasogastric feeding tubes

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Preterm infants are vulnerable to pathogens and at risk of developing NEC or sepsis. Nasogastric feeding tubes (NG-tubes) might contaminate feeds given through them due to biofilm formation. We wanted to determine if NG-tubes yielded larger amounts of bacteria the longer they had been in use and if probiotics given through the NG-tube protected against this. We performed an observational study where we collected used NG-tubes of all admitted infants in a tertiary neonatal department for a period of two months. Preterm infants born before 30 weeks of gestation received probiotics in the form 2 capsules of 10^9 cfu *Lactobacillus rhamnosus* GG (LGG) + 10^8 cfu *Bifidobacterium animalis* ssp. *lactis* (BB12) from day 3 of life through the NG-tube. After removal, the NG-tube was flushed with a 1-ml saline solution, the density of bacteria was determined by culture and the isolates identified by VITEK 2 system and/or MALDI-TOF. Concentration and types of bacteria were related to the time the feeding tube had been in use and any probiotic administration through the tube. Out of the 94 NG-tubes, 89% yielded more than 1000 cfu/ml bacteria, and 55% yielded the potentially pathogenic Enterobacteriaceae and/or *Staphylococcus aureus*. The mean concentration in the yield was 5.3 (SD 2.1, maximum 9.4) log10cfu/ml. Neither the presence of contamination nor the density was associated with the time the NG-tube had been in use. Probiotics had been given through 78% of the NG-tubes. We isolated probiotic bacteria from 15 (21%) of these and from none that had not been used for probiotic administration (P= 0.02 Fishers exact test). The administration of probiotics through the NG-tubes was not associated with the risk of contamination (88 vs 90% of probiotic and non-probiotic NG-tubes, respectively), nor contamination with potential pathogens (56 vs 52% of probiotic and non-probiotic NG-tubes, respectively). NG-tubes yielded high densities of bacteria even within the first day of use and probiotics did not protect against this. Further studies are needed to determine if changing the NG-tubes between meals or once a day will make a positive impact on tube contamination and clinical parameters.

Effects of whole grain wheat compared to refined wheat consumption on liver and metabolic health

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Whole grain wheat (WGW) products contain a large proportion of dietary fiber and bioactive compounds and are therefore described as nutritionally superior over refined (RW) products. Consumption of WGW has repeatedly been linked to a reduced risk of type 2 diabetes and cardiovascular diseases. Intervention studies on WGW products mainly focused on cholesterol and a few other outcome parameters related to vascular health or glucose metabolism. However, metabolic health is the result of a complex interplay between different metabolic organs, such as adipose tissue and liver. In the current study, we investigate the effects of WGW compared to RW on metabolic health, by determining organ-specific health markers as well as investigating the individual's capacity to respond to a mixed meal challenge. The study was a randomized controlled, double blind, parallel trial with 50 male and female participants aged 45-70. Participants had mildly elevated levels of cholesterol and a BMI between 25 and 35 kg/m^2. After a 4wk run-in period with RW products, they were randomly assigned to a 12wk intervention of either WGW (98 g/day) products or colored RW products. Intervention products consisted of bread and cereals. Before and after the intervention period, participants were examined by ^{1}H-MRS to assess intrahepatic triglyceride accumulation (IHTG), donated a subcutaneous adipose tissue sample and consumed the mixed meal. Prior to consumption of the meal and up to 4 hours after consumption, blood was sampled and vascular function was assessed. Execution of the study was successful, none of the participants dropped out and all remained weight stable. Compliance was very high as determined by recall of product packages and plasma alkylresorcinol levels. IHTG increased significantly in the RW group and remained stable in the WGW group. Fasting levels of the liver enzymes ALT, AST and GGT and marker for hepatic β-oxidation beta-hydroxybutyrate did not show any changes. There were no changes observed in fasting and postprandial levels of plasma glucose, insulin and free fatty acids between the WGW and RW group. Fasting plasma triglycerides levels did not differ between the two groups, but 4 h postprandial levels in the WGW group were significantly higher after 12 wks of intervention. With regard to effects on adipose tissue, no differences were observed in fat-cell size, gene expression analyses are pending. The protective effect of consumption of WGW on IHTG points towards a beneficial effect of consumption of WGW compared to RW on liver health. Additional analyses, including adipose tissue gene expression analysis, might reveal other interesting early organ adaptations to WGW.

Mother's secretor status affects development of children's microbiota composition and function

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One mechanism by which early life environment may influence long term health is through modulation of the gut microbiota. It is widely accepted that the optimal source of nutrition in early life is breast milk, with human milk oligosaccharides (HMOs) thought to play an important role in nourishing the developing microbiota. However, mothers with inactive secretor genes have altered HMO composition and quantities in their breast milk. In this pilot study we examine the influence of secretor status and breast-feeding on microbiota composition at 2 to 3 years of age. 37 children and 17 eligible mothers were recruited. Secretor status was determined from blood and saliva samples using hemagglutination inhibition technique and faecal microbiota composition was examined by 16S rRNA gene sequencing. Secretor status was determined for 28 eligible children with 20 being secretors (S, 71.4%). Eleven of the 17 mothers were secretors (S, 64.7%). Mothers' secretor status had a strong association with the children's stool microbiota composition and explained 16.9% of the variation in the weighted UniFrac distances. The children of non-secretor (NS) mothers were found to have increased abundance of *Prevotella* compared to children with S-mothers. Our results also suggest that a mother's secretor status has an even more pronounced effect if the children were exclusively breast-fed for at least 4 months of life, with the abundance of *Bifidobacterium* being higher in the breast-fed children of S-mothers. In contrast, the abundance of OTU related to *Bacteroides plebeius* and *Bacteroides uniformis* were decreased in these children. Mothers' secretor status has an impact on children's microbiota composition at 2 to 3 years of age. This effect was even stronger in children who were exclusively breast-fed for at least 4 months.

Interactions of exercise and diet in health prevention

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A large and consistent body of epidemiological evidence indicates that a low levels of physical activity, low levels of cardiorespiratory fitness and high levels of sedentary behaviour are associated with increased risk of cardio-metabolic diseases. In patients with impaired glucose regulation, lifestyle interventions, including physical activity as a component (often in combination with weight loss), are effective at reducing risk of incident diabetes. There is however, emerging evidence that the beneficial effects of physical activity may not be the same for everybody, and the optimal levels of physical activity for prevention of diabetes and cardiovascular disease may differ between individuals. In particular, individuals of South Asian ethnicity, those with a family history of diabetes, and those who are obese or have low levels of fitness and low levels of muscular strength may particularly benefit from engaging in high levels of physical activity. Dietary intake and physical activity both contribute to energy balance and body weight regulation. There is evidence that individuals with high levels of fat oxidation may be protected from future weight gain. Exercise increases fat oxidation both during exercise and over the hours following the exercise session. The timing of exercise relative to food ingestion influences the magnitude of this effect, with exercise performed before meal ingestion having a greater effect on total fat oxidation over the day, than exercise performed after eating. We have also shown that exercise-induced increases in fat oxidation vary between individuals, with individuals exhibiting the largest increases in fat oxidation at rest in response to an exercise training intervention having the greatest exercise-induced reductions in fat mass. Physical activity influences cardio-metabolic disease risk by a number of mechanisms. One of these is by improving the handling of postprandial lipids. There is a large body of evidence showing that exercise lowers postprandial triglyceride concentrations. This appears to be an acute effect of recent exercise, rather than a long-term training adaptation. The effect cannot be replicated by a dietary-induced energy deficit of similar magnitude, but removal of the exercise-induced energy deficit markedly attenuates the extent of triglyceride lowering. Our recent data suggests that this effect is mediated, at least in part, by exercise increasing the affinity of very-low-density lipoproteins for clearance by the enzyme lipoprotein lipase.

Creatine supplementation during knee immobilization attenuates changes in muscle transcriptome

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Knee immobilization can induce rapid loss of muscle mass and strength in humans. We investigated whether creatine supplementation during immobilization can attenuate muscle and strength losses. In addition, we assessed the effect of 7 days of knee immobilization with or without creatine supplementation on the skeletal muscle transcriptome and metabolome. Sixteen healthy young men underwent knee immobilization for 7 days using a full-leg cast. Subjects received 20 g creatine or a placebo supplement per day for 5 days prior to immobilization, followed by 5 g creatine or placebo per day during immobilization. Muscle biopsies were obtained from the vastus lateralis prior to immobilization and immediately after cast removal. Transcriptomics were performed on the biopsies using Affymetrix Human Gene 1.1ST arrays. Metabolomics were performed on a subset of the muscle biopsies (12 subjects in total, 6 subjects per group). Measured metabolites included amines, acyl carnitines, nucleotides and organic acids. Knee immobilization caused muscle mass (-506±115 mm^2 whole leg cross-sectional area, $P<0.01$) and strength loss (-7.5±1.8 kg leg extension 1RM, $P<0.01$) in all participants, with no differences between creatine and placebo supplemented groups. Knee immobilization significantly induced expression of ubiquitin mediated proteolysis genes, whereas glucose metabolism, fat metabolism and mitochondrial genes were downregulated. We observed no or mild changes in the canonical muscle atrophy genes MuRF1 (TRIM63) and MAFbx (FBXO32). Genes belonging to the HDAC4-Myogenin axis were significantly upregulated, which was attenuated by creatine supplementation. Overall, gene expression changes due to immobilization were smaller in the creatine group. Knee immobilization induced a significant decrease in several metabolites. These metabolites included fatty acid derived acyl carnitines, putrescine, proline and lactic acid. Creatine supplementation did not significantly alter the muscle tissue metabolome response to immobilization. Knee immobilization appears to induce the HDAC4-Myogenin axis, which is primarily associated with denervation and motor neuron diseases. Transcriptional changes in skeletal muscle due to knee immobilization were attenuated by creatine supplementation, although this was not associated with attenuation of muscle mass and strength losses. We hypothesize that the attenuated transcriptional response is due to antagonization of the HDAC4-Myogenin axis by creatine-induced activation of the p38-MAPK pathway. The metabolome showed changes consistent with the decreased expression of oxidative phosphorylation and energy metabolism genes.

Genetics and athletic performance – the genome era

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Complex traits, such as diabetes, obesity and cancer on one hand, and mental and athletic performance, on the other, are recognized as multifactorial, with both environmental and genetic components. Genetic factors influence several phenotypic traits related to exercise and elite athletic status, with reported heritability of roughly >50% for elite athletic status, with the rest being attributed to environmental factors. Mapping the genetic composition of athletic performance has been massively dealt with in the last two decades. Our research identified several associations between genetic polymorphisms and elite performance, including; FTO, NRF2, ACTN3 and NOS3. To date, approximately 25 SNPs have been found to be associated specifically with elite athletic status by others and us, and their number is increasing every year. Studies of the genetics of elite athletic performance are complex by definition, as elite athletics are a rare phenotype and thus limit the number of samples available for research. Inter-study differences in the competition levels of athletes, sex, and ethnic groups further complicate this issue. In the future, these limitations could be partly overcome through larger collaborative scientific efforts and utilization of new 'omics' technologies.

Inter-individual responses to high intensity interval training: interactions with the sirtuin system

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Type 2 diabetes (characterised by impaired glucose control) is increasing in the UK (~3.7 million type 2 diabetics and ~7 million pre-diabetics). This is attributed to lack of physical activity, excess weight and genetic factors. High intensity interval training (HIIT) is a time-efficient exercise regime that improves blood glucose control and may be a useful public health tool. The sirtuins and associated genes involved in producing the chemical, nicotinamide adenine dinucleotide (NAD), required for sirtuin function in the body, are emerging as key players in blood glucose control. This study investigated the interplay between the sirtuin/NAD system and individual variation in insulin sensitivity responses after HIIT. Recreationally active individuals (n=20), not training in a specific sport, 18-35 years, non-smokers, free of cardiovascular, metabolic or haematological disorders were recruited. Body mass, height and fat percentage were measured and oral glucose tolerance tests (OGTT) performed. Each HIIT session consisted of a warm up (3 min at a workload of 50 Watts) followed by 4-6 (building over the first 6 sessions) maximal 30 s sprints at a resistance equivalent to 7.5% of body mass with 3 min unloaded cycling between sets, followed by a cool down (3 min at 50 Watts). Participants performed HIIT three times/week for four weeks. A second OGTT was carried out at least two days after the final training session. Plasma glucose, insulin, lipid profiles and vitamin B3 were measured and insulin sensitivity calculated (Cederholm index). RNA extracted from whole blood collected at baselines (pre and post HIIT) and 60 min post OGTT in PAXgene® blood RNA tubes was assayed for sirtuin and NAD biosynthetic enzyme gene targets using an in-house custom designed assay, the hSIRTNADPlex. The hSIRTNADPlex incorporates 25 gene targets (all 7 mammalian sirtuin genes and 15 enzymes involved in conversion of tryptophan, bioavailable vitamin B3 and metabolic precursors to NAD). NAD/NADP was measured in whole blood. Significant reductions in body weight and body fat post-HIIT were associated with altered lipid profiles, NAD/NADP and regulation of components of the sirtuin/NAD system (NAMPT, CD38 and ABCA1). Improvements in measured metabolic health parameters were achieved in some, but not all, of the young adults tested. Notably, metabolic improvements in response to HIIT were achieved in individuals with poor health parameters measured pre-HIIT. Further investigation of the causes of the marked inter-individual variation in observed responses to HIIT will be critical in formulating optimal public health messages for HIIT and potentially other forms of exercise. This work was supported by the Scottish Government's Rural and Environment Science and Analytical Services Division and NHS Grampian Endowment.

Exercise and diet influences the interplay between adipose tissue immune cells and adipocytes

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Both physical exercise and dieting are major strategies in the treatment and prevention of obesity, and may counteract adipose tissue inflammation and insulin resistance. The aim was to compare the effect of energy restriction and physical exercise on adipose tissue response. We studied overweight/obese sedentary subjects in two intervention studies, each lasting 12 weeks affecting energy balance either by energy restriction (~20% reduced intake of energy from food; NutriTech), or by enhanced energy expenditure due to physical exercise (combined endurance- and strength-training; MyoGlu). We monitored mRNA expression by microarray and mRNA sequencing from adipose tissue biopsies, measured several plasma parameters and monitored adipose tissue depots with magnetic resonance imaging (MRI) and spectroscopy (MRS). We thoroughly compared microarray, RNA sequencing and RT-PCR as different methods of mRNA analyses, revealing strong correlations between the three methods. Physical exercise as well as energy restriction reduced visceral, subcutaneous and liver fat markedly, but the effects on adipose tissue gene expression were strikingly different in the two interventions. Whereas physical exercise increased energy-related pathways and reduced inflammatory pathways, energy restriction decreased energy-related pathways and increased inflammatory pathways. In the energy-restricted subjects (body weight reduced by 5% during the intervention) there were clear signs of enhanced lipolysis as monitored by mRNA in adipose tissue and plasma concentration of free fatty acids. After energy restriction there was increased expression of markers for pro-inflammatory M1-like macrophages in adipose tissue, possibly as a result of buffering local increase in free fatty acids. In the exercising subjects (insulin sensitivity increased by 29% during the intervention) there was a marked reduction in anti-inflammatory M2-like macrophages and Th1-cells. Furthermore, there were strong correlations between T-cells and macrophages, suggesting that physical exercise affects a complex network of immune cell regulation, which ultimately reduced inflammation in adipose tissue without reduction in body weight. Energy restriction and physical exercise affect energy-related pathways as well as inflammatory processes in adipose tissue differently, especially related to the interplay between adipocytes and adipose tissue immune cells.

Fetuin-A and free fatty acids interacts in determining changes in insulin sensitivity after exercise

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The hepatokine fetuin-A may together with free fatty acids (FFAs) stimulate adipose tissue inflammation and insulin resistance in adipose tissue via toll-like receptor (TLR) 4 both in mouse and humans. Some of the health benefits of exercise might be explained by altered release of myokines from skeletal muscle, although it is not well documented if some of the beneficial effects may be explained by altered secretion of hepatokines from the liver. Study the relation between exercise, fetuin-A and insulin resistance. Methods: Twenty-six sedentary men underwent 12 weeks of combined endurance and strength training. Blood samples and biopsies from m. vastus lateralis and adipose tissue were collected. Plasma fetuin-A concentration was reduced 10.5+2.4% after long-term exercise and interacted with circulating FFAs to predict some of the change in insulin sensitivity after exercise. Interestingly, the interaction between fetuin-A and FFA negatively influenced the adipose tissue by reducing insulin receptor signaling and increasing the number of pro-inflammatory M1-macrophages. However, these effects of fetuin-A and FFA were not observed in skeletal muscle. Our data strongly suggests that the reduction in plasma fetuin-A in response to exercise contributes to improved adipose insulin sensitivity.

Integrative analysis of gene expression network for atherosclerosis-related disease and excercise

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Analyze the genes associated with atherosclerosis disease and hypercholesterolemia susceptible to modify their level of expression in a profile of microarray data by effect of the interaction with some bioactive components present in the lycopene. Atherosclerosis is a complex disease requiring improvements in diagnostic techniques and therapeutic treatments. Atherosclerosis include factors such as lifestyle, exercise, diet and metabolic and gene expression processes. The gene expression data modeling is a current bioinformatics tool in the field of nutrigenomics for researching the complex gene networks and the relationship with nutraceuticals topics. We have formulated the clustering of hypercholesterolemia gene networks (APOB, LDLR, LDLRAP1, and PCSK9) by applying the mathematical procedure Principal Components Analysis (PCA) to the gene expression data point by introducing an interaction between neighboring points. We reviewed the responsible genes of hypercholesterolemia and their expression levels using international databases and we assessed the posible interaction role of lycopene and excercise in specific genes to evaluate the interaction patterns between them. Also we identify gene networks expression of cardiovascular disease (PPARs, FADS1, LTA4H, FABP4, APOA, APOB and APO E) and the possible therapeutic nutraceutical effect. Conclusions: The identification and comparison of gene expression levels associated with atherosclerosis-related disease will help to guide future research work focus on effective treatments to prevent health problems like hypercholesterolemia. The integrative analysis based on gene networks modeling offers a conceptual framework that could be important to understand human medical pathologies. Keywords: Atherosclerosis, hypercholesterolemia, lycopene, gene network, nutraceuticals

Dual specificity phosphatase 5/6 are oppositely regulated in human skeletal muscle by acute exercise

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Dual specificity phosphatase (DUSP) 5 and 6 specifically dephosphorylate and deactivate ERK1/2; therefore, they are important regulators of ERK1/2 signaling. DUSP5 is located in the nucleus, whereas DUSP6 is found in the cytoplasm. Both DUSPs also bind to inactive ERK1/2 to sequester it in nucleus and cytoplasm, respectively. Depending on the stimulus DUSP5/6 expression is promoted or reduced. Thereby, a stimulus-dependent pattern of DUSP5/6 determines the ERK1/2 activity in a compartment-specific way. This might be an important regulatory mechanism in determining physiological consequences of ERK1/2 signaling. In human skeletal muscle (SkM), ERK1/2 activity increased by acute exercise and rapidly reduced upon cessation. However, the regulation of DUSP5/6 by exercise and its implication has not been studied so far. Sedentary men categorized either as control (BMI=23.5±2.0 kg/m^2; normal fasting and 2 h serum glucose levels; n=13) or dysglycemic (BMI=28.9±2.5 kg/m^2; fasting glucose ≥5.6 mmol/l and/or 2 h serum glucose ≥7.8 mmol/l; n=11) were subjected to 45 min cycling (70% VO_{2max}). SkM biopsies were taken before, after, and 2 h after exercise. RNA was isolated followed by mRNA sequencing and differential gene expression analysis. Primary human myotubes (SkMC) were acutely exposed to dexamethasone (dex) mimicking the cortisol increase during exercise, and ionomycin (iono) to increase intracellular Ca^{2+} concentrations. Using Western blot and qRT-PCR, ERK1/2 activity and DUSP5/6 expression were analyzed. Acute exercise promoted DUSP5 expression 9.1-fold (P<0.001) directly after exercise while DUSP6 was reduced by 43%, with no difference between the groups. After 2 h rest DUSP5 returned to basal values whereas DUSP6 was still 30% lower compared to baseline. Basal DUSP5/6 levels were not different between the groups. During *in vitro* differentiation of SkMC, DUSP5 expression decreased by 50% from day 0 to day 1 and remained stable thereafter. DUSP6 expression increased during differentiation (day 6 vs day 0: 3.5-fold, P<0.005, n=3). Incubation with dex increased ERK1/2 phosphorylation after 15 min (1.6-fold, P<0.001, n=3), which returned to basal level after 30 min. Moreover, dex stimulation promoted DUSP5 expression 2.6-fold (P<0.05, n=4-6) after 1 h, which returned to basal level after 4 h. DUSP6 expression was significantly reduced by 42% after 2 h and 4 h, and returned to basal level after 6 h. A similar pattern but with different kinetics was observed for iono, which increased DUSP5 expression 2-fold after 6 h, whereas DUSP6 was reduced by 40% after 3 h and returned to basal level after 6 h. The prominent regulation of DUSP5 and 6 expression by acute exercise in human skeletal muscle indicates an important role in generating a specific spatio-temporal pattern of ERK1/2 activity during exercise. Stimulation of human myotubes with dex and iono mimicked this pattern of DUSP5/6 *in vitro*, and enables future studies aiming to decipher the physiological consequences of a proper regulation of DUSP5 and DUSP6.

The FoodBAll online resources to support discovery of novel dietary biomarkers with metabolomics

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One of the aims of the FoodBAll (Food Biomarkers Alliance) project (http://foodmetabolome.org) is to develop the missing tools and resources to facilitate the identification of food intake biomarkers using metabolomics. A compound database with extensive coverage of the food metabolome and a food intake biomarkers database were identified as the priority needs. The most comprehensive database on food constituents and their chemical and biological data, FooDB (www.foodb.ca), as well as the expert-curated database PhytoHub (www.phytohub.eu) focused on dietary phytochemicals are being enriched to include new contents on food non-nutrients and their human metabolites, including the known metabolites described in the literature and in silico predicted metabolites. In parallel, a new database, Exposome-Explorer, is being developed to include all known dietary biomarkers (currently n=142) and rich information on their measurement in various populations. Beyond databases, the lack of commercial standards for food-derived metabolites is another major limitation in nutritional metabolomics. FoodComEx (Food Compound Exchange, http://foodcomex.org) is a new chemical library initiated to facilitate the sharing of not easily accessible standards for diet-related compounds. FoodComEx is an online catalog of pure compounds made available by academic laboratories. Compounds are stored in the laboratory where they have been isolated or synthetized. Anyone interested in one compound can contact the provider and a bilateral negotiation will define the terms of collaboration, within the rules defined in a charter of good practices. FoodComEx is a collaborative initiative widely open to new contributors and users. Another resource developed in the FoodBAll project is a web portal (http://foodmetabolome.org/wpkg4) which presents and links to the most useful tools, databases, libraries of spectra, softwares for nutritional metabolomics and dietary biomarker discovery, and will propose tutorials, webinars, and news related to the Food metabolome. Funding: JPI HDHL FoodBAll project (2014-2017)

Food history and micronutrients profile and their relation to DNA damage in children in Brazil

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High energy density food and reduction in fruits and vegetables consumption is common in the usual dietary pattern of children, resulting in insufficient intake of vitamins and minerals. These nutrients are distributed in several functions in human body, including cellular protection as antioxidants. These antioxidant properties work by inhibiting oxidation reactions or through the mediation of DNA repair damaged reactions. Unrepaired cellular DNA damage is associated with the development of cancerous tumors. In obese, oxidative stress is high, therefore these individuals are more susceptible to the emergence of malignancies. DNA damage has been widely studied using electrophoresis in single cell gel (comet assay), considered a fast, simple and economic method. The aim of this study is to investigate the association between DNA damage and micronutrient intake and plasma levels in children and adolescents in Ribeirão Preto (São Paulo, Brazil). This is a cross-sectional clinical sub study of the project: 'New Strategy to analyze gene-nutrient interaction.' 9 to 13 year old students were selected from three schools in the city of Ribeirão Preto (São Paulo, Brazil), totalizing a sample of 120 subjects. Data collection included anthropometry, body composition, assessment of food intake using a food frequency questionnaire, and blood sampling for vitamins dosage and determination of DNA damage with comet assay. The subjects were divided into two opposing groups according to DNA damage using the classification proposed by Wollowski *et al*. One group with less DNA damage (n=108) and group 2 with increased DNA damage (n=12). Nutrient intake patterns were also generated through cluster analysis and associated with DNA damage. Two clusters were generated based on intake of amino acids (valine, phenylalanine, leucine, tryptophan, isoleucine, niacin, aromatic amino acids, branched amino acids) and some micronutrients and metabolites (phosphorus, pantothenic acid, cyanocobalamin, purines, chrome, manganese, zinc, copper, magnesium, inositol, choline): cluster 1 (n=27) with a higher consumption of these nutrients and cluster 2 (n=58) with a lower consumption. The cluster with lower consumption of amino acids and micronutrients had a higher energy intake and DNA damage (tail intensity: 11.54 ±4,9 versus 9.41 ±4,17; P=0.05). After applying ANCOVA with adjustment for BMI, mean values of serum retinol, beta-carotene and riboflavin, were significantly lower in the group with higher DNA damage. These findings corroborate the literature, asserting protective role of micronutrients against DNA damage. This study has been sponsored by Nestlé Institute of Health Science.

The association between Brazilian healthy eating index revised and biomarkers

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Validation of the Brazilian Healthy Eating Index Revised (BHEI-R) adapted from the Healthy Eating Index 2005 using biomarkers hasn't been done. The objectives was to correlate the Brazilian Healthy Eating Index Revised (BHEIR) with biomarkers in children and adolescents in Brazil. Methods The study was performed in two consecutive years (2013-2014) in the same community with participants selected from schools in Ribeirão Preto (São Paulo, Brazil). Three 24-hour recalls on nonconsecutive days were used in subjects from 9 to 13 years old to calculate the scores of the Revised Brazilian Healthy Eating Index (BHEI-R). The metabolites were analyzed by the method of HPLC (High Performance Liquid Cromatography) or through the Mass Spectrometer. The data of nutrient intake was calculated using the program Diet Win®. Due to outliers related to under-reporters and over-reporters, the participants who ate lower than 0.79× basal metabolic rate and higher than 2.4× basal metabolic rate were excluded from these analyses. 2013, n=89 and 2014, n=79. Correlations analyses were performed through Spearman test (non-parametric data). The Partial correlation with adjustment for confounding variables (age, sex and body mass index) was used. P values lower than 0.05 were considered statistically significant. BHEI-R scores and biomarkers vales were split into 4 groups on the basis of quartile analyses. There were statistical associations through correlation tests, agreement in the same quartile and misclassification in opposite quartile, respectively, between the following variables: Total vegetable vs Linolenic fatty acid r=0.36 (37.27 and 5.6%); Total vegetable vs Docosahexaenoic fatty acid (DHA) r=0.34 (31.05 and 4.97%); Total vegetable vs Beta carotene r=0.22 (35.67 and 9.55%); Dark green and Orange vegetable vs Retinol r=0.18 (34.38 and 10%); Dark green and Orange vegetable vs Beta carotene r=0.20 (27.39 and 11.46%); Dark green and Orange vegetable vs Nudi r=0.17 (28.8 and 9.38%); Dark green and Orange vegetable vs Linolenic fatty acid r=0.25 (32.92 and 6.83%); Total Fruit vs Beta carotene r=0.23 (33.12 and 8.92); Total Fruit vs Linolenic fatty acid r=0.30 (27.95 and 6.21%); Total Fruit vs Aracdonic r=0.30 (34.16 and 6.83%); Total Fruit vs Eicosapentaenoic fatty acid r=0.26 (36.02 and 6.21%); Total Fruit vs Docosahexaenoic fatty acid r=0.29 (33.54 and 6.21%); Whole Fruit vs Beta carotene r=0.26 (38.21 and 7.01%); Milk vs Retinol r=0.20 (26.9 and 10%); Milk vs Piridoxal r=0.21 (27.85 and 10.13%); Meat vs Creatine r=0.34 (P=0.001); Meat vs Nudi r=0.20 (P=0.012); Oil vs Alpha Tocopherol r=- 0.21 (p =0.007); Saturated Fat vs Alpha Tocopherol r=- 0.22 (32.03 and 17.5%). The Brazilian Healthy Eating Index Revised is validated for the above components in this population. This study has been sponsored by Nestlé Institute of Health Science.

A dynamic scheme for biomarker classification

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Biomarkers are potentially efficient tools to measure intakes of food components, their biological effects and host susceptibility. The search for biomarkers has accelerated due to the flourishing of the 'omics era. The biomarker area is therefore under fast development and new putative biomarkers are continuously emerging in the scientific literature. In order to better understand the potentials of biomarkers and to communicate their use and application it is helpful to have a theoretical framework for biomarker classification that may provide ontologies for the area. We provide here a dynamic scheme for biomarker classification for the nutrition area based on the intended use of a biomarker rather than the applied technology or nature of the outcome. The scheme recognizes that single biomarkers may serve several purposes depending on study design and intended use and that they may also be part of more complex metabolite patterns associated with exposure, efficacy or susceptibility. Many biomarkers have been proposed but not further validated. Biomarker validation goes beyond analytical validation and a number of studies need to be performed in order to provide a well-validated marker to the research community. In order to further support the biomarker development we also propose a validation scoring system for the subclass of food intake biomarkers as well as a framework for evaluating the usefulness of such a validation score by reviewing biomarkers for all major food groups within the transnational FoodBAll collaboration.

NMR-based metabolomic analysis of the impact of chlorogenic acid enriched coffee on urine metabolome

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Many studies have highlighted that coffee could provide some benefits for human health. However, little is known about the role of chlorogenic acids (CGAs), the major polyphenol compounds of coffee. Therefore, through a ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR)-driven untargeted metabolomics approach, we aimed to assess the effect of CGAs from coffee in the urinary metabolome of ten male volunteers that participated in a crossover randomized and placebo-controlled intervention study with a rich CGAs coffee extract beverage (CEB: 223 mg/100 ml of CGAs). For the study, a daily dose of CEB or a placebo beverage with equal caffeine dose was supplied during 28 days. For long-term analysis of the CEB impact, fasting urines were collected at the first and last days of each period of the study. Additionally, 4-hour postpandrial urines after the first intake of each beverage were taken for studying the effects of the acute CEB intake. ^{1}H-NMR profiling of CEB used in the study was also performed in order to confirm the dietary origin of those biomarkers related with food metabolome. Multilevel partial least squares discriminant analysis (ML-PLS-DA), as well as a univariate analysis model for crossover studies were performed to assess the significant changes. A higher excretion of 2-furoylglycine was observed in the acute consumption of CEB, likewise endogenous compounds related to citric acid cycle, such as succinic, citric, 3-methyl-2-oxovaleric and isobutyric acids. On the other hand, an influence on the gut microbiota was shown in the sustained consumption through the increase of microbiota-derived compounds hippuric, 3-(3-hydroxyphenyl)-3-hydroxypropionic and 3-hydroxyhippuric acids in urine. Moreover, trigonelline was excreted after both acute and sustained intakes, and it was also identified in the composition of the beverage, exhibiting a direct excretion with the absence of any biotransformation and non-interindividual variation.

GC-MS analysis of organic acids in human urine using ethyl chloroformate as a derivatization agent

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There is an increasing interest in measuring organic acids in biological samples such as urine. The aim of this work was to develop a fast and reliable method for profiling organic acids in human urine. The method developed is based on rapid esterification of carboxylic acids in aqueous pyridine-containing media with ethyl chloroformate used as derivatization agent. The reaction conditions were standardized for various analytical parameters and optimal results were obtained at a pH range of 8-9 and inlet temperature of 260 °C. Initial GC oven temperature was held at 80 °C for 4 min, ramped to 110 °C at a rate of 4 °C/min, to 140 °C at a rate of 10 °C/min, to 240 °C at a rate of 10 °C/min, to 280 °C at a rate of 4 °C/min, and then held at 280 °C for 3 min. For development of the method, a total of ten standard organic acid compounds were used. All 10 standards had an acceptable linearity at concentration range of 1-50 mg/l. The injections were performed in triplicate for each concentration. The correlation coefficient (r^2) was >0.994 for all the analysed standards. The instrument repeatability was evaluated at 25 mg/l for all the standards. The average RSD% for these measurements was 1.83 with a range of 0.42-3.16%. Application of the method to human urine led to the identification of 31 metabolites. While the current protocol serves the purpose for the screening of organic acids in human urine, our future line of study will focus on other analytical method validation parameters such as method repeatability, stability, recovery and quantification of urinary metabolites to achieve better method evaluation.

Current food intake markers and introduction of a new validation scoring system – a FoodBAll project

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The measurement of dietary exposure is one of the main issues in nutritional science, as it is of crucial importance for the discovery of true associations between dietary intake and effects on health. By far, the most commonly applied tools for estimating dietary exposure are based on self-reporting (e.g. food frequency questionnaires) that may often be biased. In this context, biomarkers of food exposure, measured in biological samples, may provide an objective estimate of actual intake, representing a promising supplement to the actual self-reporting tools. Anyway, only a limited number of foods are covered by validated exposure markers and a general validation system is still missing. Therefore, common criteria for biomarker validation are required. One of the main goals of the FoodBAll (Food Biomarkers Alliance) project (http://www.foodmetabolome.org) is to provide a new biomarker classification system and an overview of current food intake markers, as well as a new biomarker scoring system. For this purpose, a list of food groups has been held in order to get a good coverage of the food intake in different population groups within Europe. Nine main food groups have been identified: (1) alcoholic beverages; (2) food of animal origin; (3) fruit and vegetables; (4) cereals and wholegrain; (5) fats and oils; (6) legumes; (7) non-alcoholic beverages; (8) confectionary; (9) spices and herbs. For each group a systematic review on the status of current intake markers has been carried out and each marker identified has been scored for analytical validity, biokinetics/metabolism, robustness and consistency. Based on the results of the validation scoring system, the quality of existing markers has been evaluate and eventual missing investigations required to fully validate each putative marker have been pointed out. Funding: JPI HDHL FoodBAll project (2014-2017).

Identifying new targets and biomarkers for nutritional interventions with global metabolomics

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In order to devise and develop the nutritional interventions of the future preclinical research aims to approximate an expected effect in humans to define biomarkers, targets, or the efficacy of an intervention. Clinical research aims to elucidate the same type of information in the more relevant, but less controlled, scenario. The information converges around translational science. The use of metabolomics for translational science is increasing; particularly in nutrition research. In nutrition research metabolomics has even greater relevance since, in addition to accounting for genetics, it can account for microbiota and diet/lifestyle/environmental influences. Hence, it is valuable for understanding how exogenous treatments (dietary, prebiotic, probiotic) influence the host in the context of all of these inputs. Further, due to the conservation of metabolism, the biomarkers are highly translatable, further increasing the utility of metabolomics in translational studies. Illustrating this will be recent results in which new targets (microbiota-related, endogenous metabolic targets) and intervention strategies (probiotics, supplements) have been elucidated in preclinical studies using metabolomics. In the preclinical realm the metabolic biomarkers are often used to hone-in on a target. In clinical studies, metabolomic biomarkers are also used to monitor efficacy, compliance and pharmacokinetics. Highlighting the inception of the use of these types of clinical biomarkers will be recent biomarker discoveries in clinical cohorts.

Two complementary metabolomics studies to identify biomarkers of banana intake

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The intake of fruits and vegetables has been associated to a lower risk of developing metabolic diseases and cancer. Banana is ranked amongst the most eaten fruit in the world. However, in spite of its popularity, its specific health effects have been poorly studied and there are no biomarkers of banana intake reported in the literature. Therefore, the identification of novel biomarkers for banana is of great value to provide more reliable consumption data than dietary questionnaires for clinical and observational studies. Untargeted metabolomics allows a holistic analysis of the food metabolome allowing a deeper inquiry in the metabolism of different compounds and the recognition of patterns and individual differences that may lead to the identification of specific bioactives or biomarkers of consumption. As part of The FoodBall initiative (http://foodmetabolome.org), we aim to identify biomarkers of intake of banana and tomato using an untargeted approach on urine and serum of 12 volunteers that participated in a crossover, randomized, controlled study (BioBanaTom study). Volunteers consumed three different test foods: (1) 240 g of banana; (2) 300 g of tomato; and (3) Fresubin 2 kcal as control. Serum and urine samples were collected in kinetics over 24 h and processed to be analyzed using LC-QTof analysis. The metabolomics profiles are compared using univariate and multivariate statistical methods. The identification of discriminant compounds is performed by tandem mass fragmentation with a high resolution LTQ-Orbitrab Mass spectrometer and by an extensive inquiry of different online databases. First results obtained for 24 h urines of the BioBanaTom study will be presented. In parallel 20 low and 20 high consumers of banana have been selected in the French Cohort SU.VI.MAX2 on the basis of questionnaire data collected over 15 years. An untargeted LC-QTof analysis of one morning spot urine samples has been conducted and the discriminant ions associated to habitual banana intake will be identified and compared to the discriminant ions observed in the BioBanaTom study after acute consumption of banana. Funding: JPI HDHL FoodBAll project (2014-2017), ANR PhenoMeNEp.

Host genome and gut microbiome in health and disease

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Monogenic diseases offer clear insight into the relation between genome and diseases, but the importance of the host genome in relation to more complex multifactorial diseases such as obesity and diabetes has proven more difficult to establish. During the last decade it has become well established that the gut microbiota plays an important in relation to metabolism and immune functions, and evidence has been presented that the gut microbiota may even affect behavior. However, the exact molecular mechanisms by which bacteria in the gut exert their actions still remain elusive. We have been involved in large-scale genomics and metagenomics projects using high throughput Illumina-based sequencing. In this lecture I will briefly summarize our data on the mouse, the pig, and the human gut microbiomes, pointing to differences and similarities. In relation to studies on humans, the projects have in particular focused on the genetics of obesity and type 2 diabetes, and on characterizing changes the gut microbiota associated with these metabolic disorders, cancers and autoimmune diseases. Whereas whole genome sequencing has provided limited insight into the genetics of obesity and diabetes, studies on human cohorts have discovered changes in the gut microbiome that characterize obese individuals, individuals with type 2 diabetes, and patients suffering from rheumatoid arthritis and colorectal cancer revealing characteristic changes in the diversity and functional competences of the gut microbiota. I will summarize our recent results illustrating how mouse model experiments can be used to demonstrate the role of specific gut bacteria, how metagenomics analyses can be used for early non-invasive diagnosis of colorectal cancer, describe examples of how such analyses can be used to predict efficacy of treatment in relation to rheumatoid arthritis, and even stratify patients prior to start of treatments. I will conclude the lecture by discussing possible functional consequences and perspectives of these findings.

Hepatic estrogen receptor alpha and sex-differences in lipid homeostasis

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Among obesity-associated diseases, non-alcoholic fatty liver disease (NAFLD), a syndrome characterized by excess triglyceride (TG) accumulation within hepatocytes, represents an increasing public health issue due to its emerging association with atherogenesis and cardiovascular diseases. Although the higher prevalence of obesity among female population, women result to be somewhat protected from NAFLD, at least until menopause, by suggesting that the sex-related differences in NAFLD susceptibility could be mainly rely on estrogen signalling. We postulated that these sex-differences may be due to the activity of hepatic Estrogen Receptor alpha (ERa), which, in females, might have acquired a regulatory activity superior to the others nuclear receptors (NRs) to adapt lipid metabolism to the needs of reproduction. To date, the mechanisms underpinning the liver- and sex- specific effects of estrogens remain to be fully elucidated. Taking advantage of the Cre-lox technology, we generated a liver conditional ERa KO mouse (LERKO) and investigated the extent to which ERa was able to maintain the lipid homeostasis in the liver of female and male mice fed with a diet low in fat (10% kcal from fat, LF) or enriched in fat (60% kcal from fat, HF). The study shows that HF leads to fat deposition and morphological alterations more in the liver of male than female control mice, by reproducing the sex-dependent incidence of NAFLD seen in humans. These sex-differences are ascribable to the specific action of hepatic ERa, as the lack of this NR in the liver of LERKO females leads to metabolic impairment and morphological defects similar to those seen in the liver of male control mice. Furthermore, hepatic ERa play a crucial role in counteracting the development of a pro-atherogenic profile, as demonstrated by the increased LDL-cholesterol (low-density lipoprotein) fraction in the plasma of LERKO females, even when fed with a diet low in fat. Surprisingly, the lack of ERa reverses the effects of HF in the liver of LERKO males that show reduced fat deposition. However, the decreased lipid accumulation in the liver of LERKO males occurs at the expense of circulating lipids. In fact, LERKO males have altered plasma cholesterol levels, with a significant increase in the plasma LDL-cholesterol fraction, by suggesting that in males the lack of hepatic ERa leads to a reduced lipid uptake by the liver. According to this view, HF fails to induce the expression of LDLR (LDL receptor) in the liver of LERKO in comparison to control male mice. The reduced lipid uptake by the liver and the concomitant excess of dietary lipids might further contribute to the development of a pro-atherogenic profile in LERKO males fed with HF. These data demonstrate the relevance of hepatic ERa in maintaining lipid homeostasis in a sex-specific fashion and suggest hepatic ERa as a target for developing new, appropriate therapies able to counteract the development of liver-biased cardio-metabolic diseases.

Personalized nutrition using clinical and gut microbiome data

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Elevated postprandial blood glucose levels constitute a global epidemic and a major risk factor for prediabetes, type II diabetes and obesity, yet existing dietary methods for controlling them may have limited efficacy, as a food that induces a high postprandial (post-meal) response in one person may induce a low response in another. Indeed, using continuous monitoring of week-long glucose levels in 800 people, we measured responses to ~50,000 meals and found high variability in the response of different people to the same meals and in how macronutrient content, time of day, and exercise affect the response, altogether suggesting that universal dietary recommendations may have limited utility. To unravel the determinants of this variability, we measured gut microbiota, blood parameters, anthropometrics, physical activity, and self-reported lifestyle behaviors in this cohort, and integrated this multi-dimensional data into a machine learning algorithm that successfully predicted individualized postprandial responses to any real-life complex meal, as was additionally validated in an independent 100-person cohort. Finally, a blinded randomized controlled dietary intervention based on this algorithm resulted in significantly lower postprandial responses and consistent alterations to gut microbiota composition. Together, our results suggest that personalized diets may successfully modify elevated postprandial blood glucose and its metabolic consequences, and thus provide direct treatment for the pre-diabetic stage and assisting in the worldwide battle against the obesity and diabetes epidemic.

Genetic variations as modifying factors to dietary zinc requirements – a systematic review

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A drop in costs of genetic testing and the increasingly accessibility of these tests to health practitioners and consumers make it appealing to use the acquired knowledge for developing tailored nutrition advice. However, what we currently know is that interaction between genes and environment (such as nutrition) is very complex, with most likely several gene variants within a number of biochemical pathways contributing to recommended nutrient requirements in pathologic and healthy contexts. Scientific literature describing links between specific polymorphisms, dietary requirements and the risk of developing nutrition related pathologies is not clear and presents with significant gaps, making difficult for health professionals to translate the new information into dietary and nutrition practice. Zinc is a micronutrient with a multitude of diverse functions, its homeostasis is maintained through the activity of numerous proteins with corresponding genes present in the population in alternative polymorphic variants. Zinc status has been associated with chronic conditions including diabetes, Alzheimer's disease and cardiovascular conditions, and Single Nucleotide Polymorphisms of genes coding for zinc transporters have been reported to be associated with these conditions. With the present study we sought to systematically review the literature available to assess the relationship between genetics, zinc and health, and assess the current research to see whether specific guidelines on zinc intake could be recommended according to genotype. The present systematic search of the literature databases resulted in the extraction of 18 studies (10 cross sectional, 7 case-control and 1 a meta-analysis); 31 Single Nucleotide Polymorphisms were investigated for their association with dietary Zn or Zn status. In 15 studies the association was measured in relation to disease risk biomarkers. 5 studies looked at the effect of Zn intake and/or status in modulating type 2 diabetes risk; Zn intake was found to interact independently with 2 polymorphisms in the Zn transporter gene SLC30A8 to affect glucose metabolism indicators. While the outcomes were statistically significant the small size of the effect and lack of replication in larger and diverse populations raises the issues regarding translation into nutrition and dietetic practice. 2 studies looked at the relationship of SNPs and cognitive performance; 7 at the association between a range of outcomes linked to chronic conditions in the aging population; 2 papers described the analysis of the genetic contribution in determining Zn concentration in human milk; and 2 determining Zn concentration in plasma. These studies took into consideration different polymorphisms and biomarkers each with a small size effect and different population. This systematic review summarises the available evidence regarding modification of zinc requirements on the basis of genetic makeup, highlighting the need to coherently summarise nutrigenetics' results to allow health professionals to translate the evidence into dietary recommendations and guidelines.

BodyKey by NutriliteTM: a modern weight-management program

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In 2007, the UK government arranged a study involving over 200 experts from various science fields and asked them to identify the factors influencing weight gain. The aim of the research project was to identify and understand the complex systematic progression of weight gain until the stage of obesity. The result was a map showing the relationships between different factors contributing to obesity. The complexity of weight management is reflected in the fact that many who attempt weight loss are unsuccessful. In 2015, Amway conducted a survey in eight European countries. The primary outcome was that the majority of people (63-80%) have attempted to lose weight. According the foresight study, the following factors affect weight management: social environment, food production, food consumption, individual psychology, individual activity, physical activity environment and biology. Consequently, a weight-management program is more likely to be successful if most of the above factors are identified and addressed. Although some factors such as the physical activity environment cannot be influenced by a commercial weight-management program, most other factors identified can be. BodyKey by NutriliteTM addresses the individual biology by providing a personalized diet and exercise recommendation based on the individual genetic profiles and personal preferences. In addition to this, recipes following the personalized diet are offered along with online coaching for motivation. Furthermore, the details of calorie, macronutrient, and micronutrient intake are analyzed. Since 2013, more than 30.000 individuals have enrolled in the program. Not surprisingly, weight-loss results are better if the duration of participation is long.

Association between 5-HTTLPR L/S polymorphism and ADHD in childs: implications in diet and obesity

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It has been postulated that the presence of functional polymorphism in the promoter region HTTLPR gene serotonin transporter (SLC6A4) may be related to certain compulsive eating behaviors and increased obesity. The main objective was to assess the presence of this polymorphism in children and adolescents with attention deficit disorder and hyperactivity disorder (ADHD) and its potential association with the presence of overweight / obesity and eating habits in these patients. A total of 120 children and adolescents (60 with ADHD newly diagnosed and 60 controls) were studied in a sex- and age-matched case-control study. ADHD diagnosis was performed according to the DSM-IV-TR by trained psychiatrists. Energy, dietary intake and adherence to Mediterranean diet were objectively measured. DNA was obtained for serotonin transporter gene-linked polymorphism (5-5-HTTLPR) genotyping. You have identified statistically significant differences ($P<0.05$) between cases and controls regarding their BMI and BMI Z - score. In addition, children and adolescents with ADHD have lower adherence to the Mediterranean diet ($P<0.001$). The Long variant of the 5-HTTLPR polymorphism is more prevalent in controls, whereas in cases predominates over the Short allele ($P<0.05$). It has also been identified: the Short allele carriers are inattentive and have a higher rate of ADHD, compared to subjects with ADHD but Long allele carriers. Regarding hyperactivity no statistically significant difference between the two alleles was observed. No associations between carriers of different alleles, adherence to the Mediterranean diet or obesity were observed. In the studied population, children with ADHD have a higher incidence to present the Short variant of the control subjects HTTLPR polymorphism. This could lead to an increase in the presence of own symptomatology of ADHD such as inattention. Conversely, the presence of this polymorphism could not relate with less adherence to Mediterranean diet pattern or with more obesity.

Fruit consumption in different photoperiods influences biomarkers of seasonal rhythmicity in rats

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Circannual cycles are dominant features of the seasonal biology of mammals. They involve different genetic and physiological changes that synchronize the animals to environmental variations. In accordance with the Xenohormesis theory, which considers plant metabolites as an interspecific chemical signals, we hypothesize that fruit consumption can modulate the animals metabolism in a season-dependent manner. The aim of this study was to investigate the genetic and physiological effects caused by the fruit consumption in two different seasonal times, spring and autumn. Three groups (n=6) of Fisher 344 rats fed standard diet were housed in two different light schedules of 6 hours of light per day (L6, n=18) or 18 hours of light per day (L18, n=18) in order to emulate season's day length. After 1 month under these conditions, animals in each photoperiod were orally supplemented with 2 different kinds of lyophilized fruit (100 mg per kg of body weight/day): grape (RC, n=12) as an autumn fruit and cherry (CI, n=12) as a spring fruit for 10 weeks. Another group used as control received the vehicle (VH, n=12). Hypothalamus and hypophysis were used to study the expression profile of photoperiod-marker genes by real-time RT-PCR. Furthermore, multivariate statistical analyses, Principal Component Analysis (PCA) and Random Forest (RF), were performed using 45 variables related to hormone plasma levels, biometrical, body composition and gene expression data using the software Metaboanalyst in order to identify global changes induced by each treatment respect the vehicle in the two different photoperiods. Gene expression analyses showed that the consumption out of season of each fruit produces changes in hypothalamic Dio2/Dio3 and pituitary Eya3/Chga ratios, key biomarkers of the animal seasonal rhythmicity. According to PCA and RF analyses, grape, an autumn fruit, induced clear changes at the hypothalamus-pituitary axis level in the L18 photoperiod, while cherry, a spring fruit, induced alterations at a peripheral level when the animals were treated in the L6 schedule, as suggested the clear separation between groups. Growth hormone, thyroid stimulating hormone, Chga expression, total fat, food intake and retroperitoneal white adipose tissue depot had the most important influence in these differential effects. These findings indicate that specific fruit consumption induces genetic and physiological alterations depending on the seasonal consumption. Moreover, our results suggest that the most important changes are induced when both fruits are consumed out of their natural season. These results might be relevant to understand the role of chrononutrition in the development of metabolic alterations that lead to highly prevalent diseases such as obesity, cardiovascular disease or the metabolic syndrome. This work has been supported by the Spanish Ministry of Economy and Competitiveness (MINECO), (AGL2013-49500-EXP, FRUITOBES project).

A systematic review for the association between SNPs and energy and/or macronutrient intake

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Several studies have shown that dietary intake differs among individuals and is also associated with genetic variants. Data about these associations are limited and inconsistent. The aim of this systematic literature search was to study the association between single nucleotide polymorphisms (SNPs) and energy and/or macronutrient intake. Four different databases (PubMed, Embase, Cochrane Library, Web of Science) have been searched for specific terms on genetic variants and energy and/or macronutrient intake. Studies in humans, in English and German language and published between 1994 and September 2015 were included. Exclusion criteria referred to basic research and special collectives (e.g. children, pregnant/breastfeeding women or persons with cancer). Two reviewers screened independently titles, abstracts, and full text for eligibility criteria. In case of any discrepancies, a third researcher has been consulted. All identified publications have been assessed for quality check. In total 12,552 articles have been found. After removing duplicates and screening of articles, 113 publications with a total of 93 different gene loci have been identified as relevant. Detailed study information has been exported for the five most frequently published gene loci representing 44 publications: fat mass and obesity associated (FTO) gene, melanocortin 4 receptor (MC4R) gene, peroxisome proliferator activated receptor gamma (PPARG) gene, apolipoprotein A2 and A5 (APOA5, APOA2) genes. Over 50% of the articles including 15 different SNPs dealt with the association between the FTO gene locus and energy and/or macronutrient intake. The FTO SNP rs9939609 was the most common SNP which has been described in 17 articles. Significant as well as non-significant associations were found for this SNP. Similar results were observed for the MC4R gene locus, whereas three out of ten studies showed significant results. One study has shown a significant association between PPARG rs1801282 and total fat intake (P=0.03). There have been no significant associations for the APOA5 gene locus compared to the APOA2 gene locus for which a significant association with energy and/or macronutrient intake has been observed. The literature identified through this systematic search is heterogeneous, complicating the comparison and conclusion of results. Studies investigating the FTO gene locus were over-represented. The analyses of the five most frequently published gene loci showed no clear evidence for significant associations between these SNPs and energy and/or macronutrient intake. The review was partly funded by Amway GmbH, Puchheim and is registered in PROSPERO (CRD42015025738).

Stability of metabotypes revealed by dietary challenges

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Many phenotypic traits are associated with dynamic responses to environmental cues such as a meal. The concentrations of many plasma metabolites respond in a timely manner to the meal and the inter-individual variability of this response is much higher than the variability in the fasting state. To assess to which extend these responses are a stable 'metabotype' we profile around 400 metabolites in plasma during different dietery challenges. Within the Nutritech project, 72 healthy volunteers (men and women, age=60±5 yrs., BMI=30±2 kg/m^2) underwent an oral glucose tolerance test (OGTT), a mixed meal tolerance test (MMTT) and a mixed meal tolerance test combined with physical activity (MMTT+PA). The challenges were repeated after 13 weeks, during which 40 volunteers abide to a 20% energy restriction and 32 others followed a supervised diet without energy restriction. Metabolite profiling was performed in plasma samples using LC-MS/MS, GC-MS, NMR and clinical chemistry methods. Despite an average weight loss of 5.62±2.95 kg, the caloric restriction did not induce major changes in plasma metabolite concentrations during the dietary challenges or in the fasting state (based on mean values). The inter-individual differences accounted for more than 50% of the observed variability. Assessed by ANOVA, the overall contribution of the caloric restriction to the variation of metabolite concentrations was negligible, ranging from 0.25% for lyso PC C18:2, to about 2.3% for Alanine. This indicates the stability of individual responses (or phenotypes) to the challenges. To assess this stability in metabolite responses to the repeated challenges correlation analyses based on the average concentration of each metabolite during the challenges was performed. Most metabolites displayed stable responses (butyrylcarnitne r=0.9, e.g.) but some had very different kinetics between the two challenges (non-conjugated bile acids r=0.5, e.g.). When assessing the 'stability' of the response for all metabolites in each individual, an r>0.95 for most of the individuals was obtained. Understanding the reasons for this high stability in response of metabolites and individuals should help to identify markers of health and disease.

Adiponectin and tumor necrosis factor gene polymorphism in Egyptian obese children

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Childhood obesity is associated with many comorbidities e.g. type 2 diabetes, hypertension, hyperlipidemia, accelerated growth and bone maturation, ovarian hyperandrogenism and gynecomastia, cholecystitis, pancreatitis, and pseudotumor cerebri. It has been increasingly recognized that adipose tissue is an endocrine organ that secretes a number of biologically active 'adipokines' like tumor necrosis factor-α, plasminogen activiator inhibitor-1, leptin, resistin, angiotensinogen, and adiponectin. Plasma concentration of adiponectin is reduced in human obesity, particularly visceral, and negatively correlated with insulin resistance (IR). Tumor necrosis factor (TNF), an important proinflammatory cytokine, plays a role in the regulation of cell differentiation, proliferation and death as well as in inflammation, innate and adaptive immune responses, and also implicated in a wide variety of human diseases.. The aim of this work is to study adiponectin and TNF gene polymorphism in a group of Egyptian obese children and to determine the possible association between this polymorphism and complications of obesity. The present study was a case-control comparative study that included 200 children: 100 of them were obese as defined by WHO, 2011 and the remaining 100 were apparently healthy non-obese children, age and sex matched served as a control group. Informed consent will be taken from parents of all studied cases. Molecular studies: Adiponictin and TNF gene polymorphism by PCR amplification and restriction enzymes PCR (RE-PCR) was done. According to the result we conclude that: (1) No statistically significant difference was found between the two studied groups as regard variants of adiponectin gene polymorphism. 10% of controls as well as 6%of cases fail to show any variant of adiponectin gene polymorphism. (2) The two studied groups as regard variants of TNF gene polymorphism. 10% of controls as well as 6%of cases fail to show any variant of TNF gene polymorphism. (3) Variants of adiponectin` gene polymorphism as regard laboratory investigation in patient group. 4-Variants of adiponectin gene polymorphism as regard sex in patient groups. (4) Variants of TNF gene polymorphism as regard sex in patient group. (5) TNF gene polymorphism and each of age, age of onset of obesity and anthropometric measurements in patient group. (6) Variants of TNF gene polymorphism as regard laboratory investigation in patient group. There was statistically significant increase in the mean levels of TG, LDL and FBS and significant decrease in the mean HDL level in the patient group when compared to controls. A Statistically significant difference was found between GA,AA genotype variant of adiponectin gene polymorphism and each of Waist circumference & BMI while no significant relation was found with other parameters in patient group.

Consumption of protein-enriched milk has minimal effects on inflammation in elderly subjects

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Aging is associated with increased levels of inflammatory markers which may contribute to the development of inflammatory related diseases. Growing evidence also indicate that age-related inflammation is associated with loss of muscle mass and muscle strength. Dietary strategies to prevent the onset of inflammation are therefore suggested. The aim was to investigate the effects of daily intake of low-fat, protein-enriched milk on markers of inflammation among community-dwelling elderly men and women ≥70 years of age with reduced physical strength. In a randomized placebo controlled intervention study, subjects were randomly allocated into two groups, one protein (n=14, age 76.9±4.9 yrs) and one control group (n=17, age 77.7±4.8 yrs), receiving either protein-enriched milk (2×0.4 l/d; 2×20 g protein/d) or an isocaloric control drink (2×0.4 l/d) for 12 weeks. We measured the mRNA levels of 44 genes in peripheral mononuclear blood cells (PBMC) before and after the intervention. Serum levels of IL6, TNFα, sTNFRSF1A and hs-CRP were also analyzed. To determine body composition and asses muscle strength, we used dual-energy X-ray absorptiometry and chest press and leg press, respectively. After the intervention, changes in mRNA levels of NR1H3 and INFG were significantly different between the two groups (P=0.02). We observed a decreased mRNA level of TNFRSF1A (P=0.03) and an increased mRNA level of DPP4 (P=0.01) in the control group. In contrast, no significant changes were observed in the protein group. The circulating level of TNFα was significantly increased in the control group (P=0.03) only, while sTNFRSF1A was significantly increased in both groups (P=0.02 in the protein group and P=0.01 in the control group). We observed no significant differences in serum levels of IL6, hs-CRP or in the TNFα/TNFRSF1A ratio, and there was no correlation between changes in body mass or muscle strength and circulating inflammatory markers. The results indicate that the consumption of a low-fat, protein-enriched milk for 12 weeks had minimal effects on inflammatory related markers, compared to an isocaloric intake of carbohydrate.

Effect of rs174546 on the expression of FADS1 and its application to genotype-based functional food

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The rs174546 occurs in 3' untranslated region (3'-UTR) of fatty acid desaturase 1 (FADS1) gene. Several Genome-wide association studies (GWASs) have proposed that single nucleotide polymorphism (SNP) rs174546 would be associated with plasma triglyceride and total cholesterol levels. Here, we investigated the effect of existence/nonexistence of rs1801282 (C/T) on FADS1 gene expression. Constructs containing SNP C/T variants were made by cloning of 3'-UTR of FADS1 into the end of luciferase gene and point mutation (C/T), and luciferase activity assay was performed after transient transfection. As a result, rs174546 reduced luciferase activity by approximately 15-20%, implying that the SNP decreases FADS1 transcription. This result also means that the person having the SNP would be helpful to eat something can increase FADS1 expression to maintain normal plasma lipid level. Therefore we next constructed the luciferase based assay system that can evaluate the effect on the FADS1 expression through assessing its promoter activity. This assay system was functionally validated by applying a positive control material and, using this system, we found KFS002, a bioactive food component which increase FADS1 expression. Then we confirmed the effect of KFS002 on FADS1 expression by Western blot analysis. In conclusion, we experimentally validated the impact of SNP rs174546 on FADS1 gene expression. Also, we tried to applicate this result to develop the SNP-based functional food that could be helpful to maintain blood lipid level by regulating FADS1 expression.

Replacement of dietary SFAs with PUFAs upregulates the mRNA expression levels of the LDLR and LXRA

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Solid evidence indicates that replacing saturated fatty acids (SFAs) with polyunsaturated fatty acids (PUFAs) reduces total cholesterol and LDL cholesterol (LDL-C) and thereby coronary heart disease (CHD) events. The molecular mechanisms of the LDL-C lowering effects are however not completely elucidated. To further understand the molecular mechanisms behind the cholesterol lowering effect, we examined the gene expression level of lipid related genes in peripheral blood mononuclear cells (PBMCs) in a human randomized controlled dietary intervention study where we previously have shown that total cholesterol and LDL-C was reduced when SFAs were replaced with PUFAs. In an 8-week double blinded study, healthy adults (n=95) aged 25-70 years with moderate hypercholesterolemia were randomly assigned to an experimental diet group low in SFAs but high in n-6 PUFAs (Ex-group) or a control diet group high in SFAs but low in n-6 PUFAs (C-group). PBMCs were isolated and the mRNA gene expression analysis was measured by quantitative real-time polymerase chain reaction. We analyzed the mRNA expression changes of target genes at the beginning and the end of the intervention. Exchanging SFAs with PUFAs increased LDLR mRNA expression as well as LXRA, FASN and ABCG1 mRNA expression in PBMCs. The mRNA expression of UCP2, a gene involved in lipid oxidation, was significantly down-regulated in the Ex-group compared to the C-group. The LDLR mRNA expression was positively correlated with the LXRA mRNA expression and was negatively correlated with percentage change in serum LDL-C. The novel and important finding of the present study is that the effect on plasma cholesterol obtained by replacing SFAs with PUFAs seems to be mediated through an upregulation of LDLR mRNA expression, potentially leading to increased intracellular cholesterol concentration, subsequently increasing the mRNA expression of LXRA and LXRA target genes.

Effects of short photoperiod exposure on glucose and lipid metabolism in liver and skeletal muscle

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Nearly all organisms use sunlight to adjust their periods of activity and resting to optimize survival, being able to respond to altered day length with changes in behaviour and physiologic parameters in order to anticipate relatively predictable annual variations. It is known that photoperiod exposure alters some biometric, physiologic and behaviour parameters. However, its effects on glucose and lipid metabolism and the molecular mechanisms that can mediate these effects have not been fully elucidated. The aim of this study was to determine whether the exposure to a short photoperiod can alter glucose and lipid metabolism in liver and skeletal muscle of normoweight rats. During 14 weeks, 12 male Fischer rats were fed with a standard diet and distributed in two groups corresponding to different day lengths (photoperiods): L12, submitted to a normal length day (12h light from 9.00 am to 9.00 pm and 12h darkness from 9.00 pm to 09.00 am) and L6, submitted to a short length day (6h light from 9.00 am to 3.00 pm and 18h darkness from 3.00 pm to 09.00 am). The animals were sacrificed one hour after the beginning of the light cycle (10.00 am) and blood, liver and soleus muscle samples were collected for further analyses. Compared with L12 rats, an increase of serum glucose and non-esterified fatty acids (NEFAs) was observed in rats exposed to the short photoperiod. These metabolic changes were accompanied by a significant down-regulation of the hepatic mRNA levels of glut2 and CD36, two key genes involved in glucose and fatty acid uptake, respectively. Furthermore, lower mRNA levels of CD36 were also observed in the soleus muscle of L6 animals, which also showed decreased gene expression levels of had and cpt1β, two genes related with the β-oxidation process. The exposure to a short photoperiod increases the circulating levels of glucose and NEFAs, two effects that could be mediated, at least in part, by the down-regulation of key genes involved in glucose uptake and fatty acid transport and β-oxidation in liver and skeletal muscle. The research described here received funding from the Spanish Ministry of Economy and Competitiveness (MINECO), AGL2013-49500-EXP, FRUITOBES project.

Blood cell models to analyze antioxidant and anti-inflammatory power of nutrients

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The biomarkers used to detect changes in nutritional interventions are of a great range and often fail to measure minor metabolic responses induced by food. Stress challenge tests are applied to disrupt body's homeostasis and the rate of recovery and biological changes are indicative for the nutrient effects. The present study aims to compare the application of challenge tests in different blood cells based models: using peripheral blood mononuclear cells (PBMCs) (own results) and platelets-based model (literature data). PBMCs are representative for a complex tissue, which metabolism is dynamic and dependent on body's metabolic state and the environmental input. Platelets play a key role in hemostasis and their impaired function is associated to cardio-vascular diseases. These blood cells are easily accessible and sample material is often used for investigation of biological responses to different compounds. Bacterial lipopolysaccharides (LPS) have been widely used in models studying inflammation response both *in vitro* and *in vivo*. LPS are a strong activator of TNF-α, IL-1 and IL-6 synthesis, and of production of reactive oxygen species (ROS). Oxidative stress is additionally inducing the inflammatory responses. Individuals with inflammatory diseases often exhibit modified platelet function. LPS can also play a role of platelet agonist and the LPS-induced hyperactivity of platelets leads to overproduction of ROS. Anthocyanins (ATH) display powerful antioxidant properties and protect blood platelets against oxidative damage induced by ROS. *Agrimonia eupatoria* L. (agrimony) is a valuable source of polyphenols and has antioxidant and anti-inflammatory properties. Our study established that PBMCs transcriptomics is affected by an agrimony tea intake in both normal weight (NW) and overweight (OW) healthy subjects. Tea intake suppressed LPS stimulated inflammatory response in PBMCs in NW subjects by suppressing the IL-6 and IL-1β gene expression levels. SOD1 expression in NW and GCLc expression in OW subjects were suppressed presumably due to the antioxidant properties of the herb. Saluk *et al*. demonstrate that pretreatment with ATH protects the platelets against LPS-induced oxidative stress. The immunomodulatory roles of platelets are controversial. Ando *et al*. demonstrate the immunosuppressive effects of platelets by applying a model of macrophage cell culture incubated with platelet-conditioned medium. The suppression of macrophage inflammatory responses to LPS are presented as decreased concentrations of TNF-α and IL-6 in the culture supernatants and suppressed gene expression levels. At the same time studies of Scull *et al*. and Waehre *et al*. report that activated platelets upregulate the production of inflammatory cytokines in monocyte-derived macrophages and PBMCs models. These models are representative for complex responses of blood cells to different stimuli. A comparative study could possibly evaluate the applicability of these blood cells based stress test models in nutrition studies. A model that would provide a measure for both inflammatory and oxidative stress responses, e.g. the one that uses LPS stimulation, would be informative enough to assess the capacity of a nutrient to improve cell response to stress.

Identification of metabotypes and development of tailored dietary advice for a European population

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Traditionally, personalised nutrition was delivered at an individual level. However, the concept of delivering tailored dietary advice at a group level through the identification of metabotypes or groups of metabolically similar individuals has emerged. Whilst this approach to personalised nutrition looks promising, further work is needed to examine this concept across a wider population group. Therefore, the objectives of this study are to: (1) identify metabotypes in a European population; and (2) develop targeted dietary advice solutions for these metabotypes. Using data from the Food4Me study (n=1,607), k-means cluster analysis revealed the presence of three metabolically distinct clusters based on twenty-seven metabolic markers including cholesterol, individual fatty acids and carotenoids. Cluster 2 was identified as a metabolically healthy metabotype as these individuals had the highest omega 3 (6.56±1.29%), carotenoids (2.15±0.71 μM) and lowest total saturated fat levels. Based on its fatty acid profile, cluster 1 was characterised as a metabolically unhealthy cluster. Tailored dietary advice solutions were developed per cluster using a decision tree approach. Testing of the approach was performed by comparison with the personalised dietary advice, delivered by nutritionists, to Food4Me study participants (n=180). Excellent agreement was observed between the targeted and individualised approaches with an average match of 82% at the level of delivery of the same dietary message. Future work should ascertain whether this proposed method could be utilised in a healthcare setting for the rapid and efficient delivery of tailored dietary advice solutions.

Replicable LDL decrease following a 6-week micronutrient intervention in a healthy pediatric cohort

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Insufficiency of micronutrients not only affects undernourished populations but can also be observed in develop or developing countries. Optimizing micronutrient intake through dietary interventions in healthy populations could potentially fine tune metabolic processes and prevent the advent of pathophysiological processes. We conducted a short-term intervention study to evaluate the metabolic benefits of optimizing micronutrient status. The intervention consisted of a 6-week multi-micronutrient supplementation followed by a 6-week washout in a Brazilian cohort of healthy children aged 9 and 13 years. The intervention was done twice, a year apart, in cohorts of 141 and 139 participants. Participants consumed a multi-micronutrient bar prior to their classes 5 day a week. Fasting blood biochemistry, proteomics, metabolomics and circulating micronutrients, and dietary habits were assessed at baseline, after a 6-week intervention period, and following a 6-week washout period. Genomics data was also assessed at study initiation. The intervention increased circulating levels of vitamins contained in Nestrovit® reproducibly in both years. Furthermore, decreases in glucose, total cholesterol and LDL-C levels (after correction for regression to the mean effect) were observed in both years. Interestingly, LDL-C levels of 45 and 35% of participants from both years exceeded the normal intra-individual variation (i.e. observed to be 10%). No baseline cardiometabolic parameters or global dietary patterns other than baseline level of LDL-C could further explain the LDL-C response to intervention. However, we have observed strong correlation between the variation of some vitamins and LDL-C level. Results from this trial reveal that optimizing micronutrient status, even in asymptomatic children, could improve metabolic health (for example lipid profiles). Further analyses on proteomics, metabolomics and genomics are ongoing to better understand inter-individual variability in response to intervention.

Variation in Zbtb16 modulates metabolic response of pregnant rats to high sucrose diet feeding

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Over-nutrition in pregnancy and lactation affects development of the fetus/infant, which can result in metabolic disorders in adulthood. Previous studies have shown the effects of improper maternal diet and associated them with altered fetal development, however little is known about involvement of genetic factors influencing this process. We suggested that as a significant energy metabolism regulator, Zbtb16 gene could be important for pathogenesis of metabolic syndrome and tested a hypothesis that variation of Zbtb16 gene modulates the effect of high-sucrose diet (HSD) induced metabolic and fetal developmental changes. We established a new congenic rat strain containing Zbtb16 gene from a metabolic syndrome model, the polydactylous rat (PD/Cub) strain, within the spontaneously hypertensive rat (SHR) strain genomic background. Using 16-week-old female rats of both strains we tested whether this gene is involved in nutrigenetic interactions with HSD in pregnancy. SHR and SHR-Zbtb16 female rats were fed standard diet during pregnancy and 4 weeks of lactation (control groups) or a high-sucrose diet (HSD, 70% calories as sucrose) in the same period. We assessed comprehensively the metabolic profiles of the four groups including glucose tolerance tests, levels of insulin and interleukins and also concentrations of triglycerides and cholesterol in 20 lipoprotein fractions. Two-way ANOVA with STRAIN and DIET as major factors was used. SHR dams displayed overall worse glucose tolerance when fed HSD compared to SHR-Zbtb16. We identified significant STRAIN*DIET interactions for levels of M-CSF, IL-13, VEGF, PYY and insulin. In both strains we observed an increase of cholesterol and triglyceride concentrations in large particles (chylomicrons, VLDL) and decrease of cholesterol and triglyceride concentrations in medium to very small LDL particles when fed HSD. Our results show that Zbtb16 gene is involved in metabolic changes in pregnant rat dams exposed to HSD. The nutrigenomic aspects of HSD programming effect on offspring are currently under investigation.

Association between sugar intake and coronary event risk in the Malmö Diet and Cancer cohort

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Previous studies have suggested that a high intake of sugar-sweetened beverages is positively associated with the risk of a coronary events. However, few studies have examined the association between sucrose (i.e. sugar) and incident coronary events. The aim of the present study was, therefore, to examine the associations between sucrose intake and coronary event risk in a large cohort. We performed a prospective analysis on 26,190 individuals (62% women) free from diabetes and without a history of cardiovascular diseases from the Swedish population-based Malmö Diet and Cancer cohort. Over an average of 17 years of follow-up, 2,493 incident cases of coronary events (fatal or non-fatal myocardial infarction or death attributable to ischemic heart disease) were identified from registers. Sucrose intake was obtained from an interview-based diet history method, including 7-day records of prepared meals and cold beverages and a 168-item diet questionnaire covering other foods. Cox proportional hazards regression was used to model the association between sucrose and coronary event risk adjusted for gender, age, energy intake, dietary method, season, smoking, waist, alcohol consumption, physical activity, educational level, intake of fruit and vegetables, whole grains, coffee, fermented milk, meat, and fish. A restricted cubic spline was computed to examine the shape of the association between sucrose intake and coronary event risk. Participants who consumed more than 15% of their energy intake (E%) from sucrose (5% of this population) had a 37% (95% CI=13-66%) increased risk of a coronary event compared with the lowest sucrose consumers (<5 E%). In addition, we observed a non-linear association indicating that the risk increased above the median intake (8.2 E%), and no benefit of having a lower sucrose intake was observed. The results indicated that high sucrose consumption is associated with an increased risk of a coronary events.

Obesogenic effects of TCDD dioxin in mice after a low dose and during chronic exposure

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There is considerable public, scientific and regulatory concern over the possible adverse health effects of chronic exposure to trace levels of persistent organic pollutants (POPs). The class of compounds, collectively known as dioxins, has received widespread attention and attracted a great interest in research, following the accidental release of the most toxic, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at Seveso in 1976. Currently, TCDD is mainly formed during anthropogenic activity (steel industry, incinerator, etc.) and over 90% of human dioxin exposure comes from the diet via fatty food (dairy products, meat, fish, etc.) due to the lipophilic nature of dioxins. TCDD accumulates in animal fat and is described as an endocrine disruptor with anti-estrogenic effects *in vivo*. It also presents an anti-estrogenic activity *in vitro*. Since environmental substances are described to be involved in the obesity development, many endocrine disruptors are shown to be associated to an obesogenic effect in reference to molecules that inappropriately regulate lipid metabolism and adipogenesis to promote obesity. Indeed, TCDD is an energy metabolism disruptor. An acute TCDD exposure (industrial accidents or use of Herbicide Orange as a chemical weapon) causes a wasting syndrome characterized by a loss of body weight accompanied by a decrease in adipose tissue mass in humans. However, in human, TCDD exposure is mainly at chronic low doses. Few data are available regards to this exposure about the TCDD effect on energy metabolism. The objective of this work was to study the obesogenic effect of TCDD due to a low dose chronic exposure. C57BL6/J male and female adult mice were fed with a high fat diet (HFD) and exposed or not to TCDD at 1 µg/body weight/week during 32 weeks. Body weight and fat mass were checked. Cholesterol, triglycerides, non-esterified fatty acids, glucose and insulin plasma levels were determined. Transcriptional expression of dioxin and metabolism target genes were measured by RT-qPCR. At physiological level, treated male and female mice exhibited a body weight gain (+8.90±1.09% and +8.04±1.08% respectively) correlated to a fat mass increase (+13.47±1.13% and +10.94±1.13% respectively). The liver weight to body weight ratio increased whatever the sex. When females were exposed to TCDD, hepatic triglyceride content and visceral adipose tissue significantly increased (+41.46±1.00% and +13.55±1.35% respectively) in contrast to males. TCDD failed to induce any change in studied plasma parameters compared to HFD controls. At molecular level, the liver weight to body weight ratio increase could be linked to the Scd1 mRNA overexpression observed in both sex. Similarly, in adipose tissue, the ATGL mRNA expression was linked to visceral adipose tissue mass variations in male and female mice. To conclude, we demonstrated for the first time that chronic exposure to TCDD at a low dose appears to potentiate a high fat diet obesogenic effect in adult mice. However, the underlying mechanisms appear to be sex-dependent.

The belly fat study – an intervention to improve metabolic health in subjects with abdominal obesity

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Health can be defined as the capacity of an organism to keep metabolic balance in an ever changing environment and especially in response to a wide range of stressors. The capacity to respond to dietary challenges is called 'phenotypic flexibility' and might reflect the functioning of metabolic organs, such as liver, muscle, adipose tissue and gut. In the Belly Fat study we compared the effects of two different weight loss diets differing in nutrient quality on phenotypic flexibility. 110 healthy overweight males and females aged 40-70y were randomly assigned to either one of two 12wks dietary advice intervention groups or a control group. Dietary advice was provided in two variants; a Western-type and a Targeted caloric restricted diet (both -30en%). The Western-type diet contained saturated fat, simple carbohydrates and protein from both animal and vegetable sources. The targeted diet aimed to improve organ health and was enriched in unsaturated fatty acids, complex carbohydrates and protein from vegetable sources such as soy. The control group did not receive any dietary advice and was instructed to maintain their habitual diet. Before and after the intervention we quantified liver fat and abdominal fat distribution by ^{1}H-MRS/MRI and applied a mixed meal challenge test (912 kcal; 18 g protein, 76 g carbohydrates, 60 g fat) to gain insight on phenotypic flexibility. Blood samples were collected before and up to 6 hours after the mixed meal challenge. We also measured vascular function and collected adipose tissue biopsies. 100 participants completed the study, 10 participants dropped out (1 in Western diet, 6 in targeted diet and 3 in control group). Weight loss was achieved in the Western-type diet group (-6.3±3.9 kg, $P<0.01$) and in the Targeted diet group (-8.4±3.2 kg, $P<0.01$) The control group remained weight stable. Both intervention diets resulted in a significant decrease in liver fat, HbA1c, fasting glucose, insulin and triglycerides with no changes in the control group. No differences between the targeted and western group were observed for those measures. The targeted diet resulted in a decrease in fasting total cholesterol levels compared to both the western-type diet and the control group. Both intervention diets resulted in a decreased insulin and glucose response curve after the mixed meal challenge with no changes in the control group. No differences between the targeted and western group were observed for those measures. The western intervention resulted in a decreased triglyceride response curve with no changes in the targeted and control group. A 12 week caloric restriction diet of -30en%, independent of diet quality, resulted in an improvement in several classical measurements of metabolic health. Dietary quality improved total cholesterol levels. Vascular measures, metabolomics and adipose tissue transcriptomics are pending and will help us to understand these findings.

Whole-blood gene expression profiles in large-scale epidemiological studies: What do they tell?

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In nutrigenomics, gene expression profiling is used to investigate transcriptional mechanisms associated with nutrients and diet. Blood samples collected in the framework of dietary interventions and epidemiological studies allow the use of humans as the model system, as opposed to using cell lines or animal models. We reviewed recent publications in the field of gene expression profiling, based on a systematic literature search focusing on studies from the last five years and including studies that investigate either single nutrients, foods, food groups or dietary patterns. Findings highlight the role of inflammatory processes as key mediators of the association between diet and disease, and point to the relevance of using blood as the target tissue in nutrigenomics. However, recurring challenges include study design issues, practical and statistical challenges, as well as biological interpretation of the results. Many of the current studies have small sample size, and given the nature of gene expression data their conclusions have limited impact. These challenges should be addressed by future nutrigenomics studies in order to increase their relevance and validity. We conclude that the published literature is characteristic of a new research field where sample sizes are small and reported findings may not always be confirmed by larger and more well-designed studies. Still, transcriptomic data is accumulating which contributes to the understanding of the processes underlying the health impact of diet. The reported findings help to combine and confirm *in vitro* and epidemiological data, and point to new associations that deserve the attention of future studies. However, based on the available literature few conclusions about etiological associations can be made.

Tumor-suppressing effects of *Moringa oleifera* leaves powder (MLP) on colorectal polyps

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Colorectal cancer (CRC) is the third most common cancer and the third leading cause of deaths in the Philippines today. It is characterized by genetic, epigenetic and phenotypic changes. A number of bioactive compounds in foods have been reported to exert anti-carcinogenic effects in humans. However, the mechanisms of action of these compounds have to be elucidated first using animal models. The study focused on MLP as an effective post-initiation tumor-suppressor on the development of colorectal polyps in rat models. Specifically, the study aimed to evaluate the effects of MLP on the mutations of TGFβ, K-ras, β-catenin among azoxymethane (AOM)-induced rats and morphological alterations. The study followed a randomized controlled experimental design. The rats were randomized into four groups and were given the following diet. Groups 1 and 2 had basal diet (negative control and positive controls, respectively); Group 3 and 4 induced with cancer had basal diet with varying concentration of MLP. The study detected and characterized the genes involved in AOM mutagenesis, such as K-ras, TGFβ and β-catenin mutations. Six months post injection of AOM, an evident aberration in DNA sequences was observed in the K-ras gene but no distinct base change occurred for TGFβ and β-catenin genes. Comparative sequence analysis of rats after 11 months of feeding showed a decrease in mutation frequency, with reference to the negative and positive control. Morphological analyses through immunohistochemistry clearly identified a decreasing trend in fluorescence from Group 2 to Groups 3 & 4 while minimal fluorescence was observed in Group 1. The magnitude of the fluorescence for the developed color is proportional to amount of cell proliferation. The findings from the AOM CRC model provided relevant insights into the unexpected effects of chemoprotective phytochemicals present in MLP against chemical-induced carcinogenesis. Our results are the first to suggest mechanistic links between MLP consumption and colorectal cancer in an experimental rat model. In particular, the study showed that a diet consisting of MLP consistently reduced the mutation frequency of K-ras oncogene in rats after 11 months of intervention. Identification of the tumor suppressing effects of MLP may provide an opportunity for its potential use as part of a functional food matrix or nutraceutical for colorectal cancer management.

Vitamin d and prostate cancer: is there a relationship?

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The antiproliferative effect of Vitamin D on cancer cells and its ability to induce cell differentiation and suppression of tumor-induced angiogenesis provokes in the last decade enormous research for elucidation of its role in prevention of different types of cancer and in slowing down the malignancy progression. To determine the circulating vitamin D levels in prostate cancer (PCa) patients and to investigate their relationship with various determinants associated with the severity and progression of the disease. A total of 85 males, aged from 52 to 84 years (mean age 66.76±6.25 years), with histologically confirmed PCa, entered the study. Diagnosis of PCa was established by systemic transrectal ultrasound-guided tru-cut prostate biopsies (10 cores at least). Detected tumors were graded using the Gleason grading system. The risk for biochemical recurrence of localized and locally advanced PCa was assessed according to EAU guidelines. Prostate specific antigen (PSA) serum levels were measured immunochemically. Vitamin D assay was performed by a validated LC-MS/MS method. Descriptive statistics, Student's t-test for comparison of means of different parameters, and non-parametric correlation analysis were used for data analysis. The level of significance was set at $P<0.05$. The mean serum 25-hydroxyvitamin D3 (25OHD) levels of the studied patients (42.85±18.88 nmol/l) were lower than the cut-off limit of 50 nmol/l recommended by the European Endocrinological Society. The patients were divided into 3 groups (RG) by the risk for biochemical recurrence of localized and locally advanced PCa. The patients from the highest RG revealed lowest 25OHD (36.64±18.16 nmol/l). Stratification by the tumor grade indicated severe 25OHD insufficiency (37.84±19.71 nmol/l) in patients with highest grade tumors (Gleason score >7). The same trend for a decrease of 25OHD levels with tumor grade and risk for biochemical recurrence was sustained when the season was taken into account. The differences between 25OHD levels in the RG and in the tumor grade groups were more pronounced during summer ($P<0.05$, $P<0.01$). A moderate negative correlation between 25OHD levels and the Gleason score (Spearman $r=-0.51$, $P<0.01$) and between 25OHD and the risk for biochemical recurrence (Spearman $r=-0.60$, $P<0.001$) was established. No associations between 25OHD values and PSA were found. The suboptimal 25OHD levels in PCa patients and the inverse associations between 25OHD, tumor aggressiveness and the risk for biochemical recurrence provoke us to assume that correction of vitamin D status by supplementation with vitamin D preparations might be beneficial in PCa prevention and slowing down its progression.

Nutraceutical properties and glycemic index of pasta enriched with Faba bean flour

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Pasta is an important staple food and it is very popular in several countries. Recent studies have demonstrated that it is a suitable vehicle for the incorporation of bioactive nutrients. Faba bean (*Vicia faba* L.) is one of the major winter-sown legume crops grown in the Mediterranean region, and has considerable importance as a low-cost food rich in proteins and carbohydrates. It has been demonstrated that *V. faba* (VF) bean is also an important source of phytochemical compounds. Aim of the present study was to evaluate the nutritional properties, the total polyphenol content and glycemic index of pasta obtained using 35% VF bean flour. Subjects were served portions of the test food pasta, control pasta with 100% durum wheat semolina (DWS), and a standard food (glucose), on separate occasions, each containing 50 g available carbohydrates. The study demonstrated that protein, fiber content and the ratio amylose/amylopectin were higher in VF pasta compared with reference pasta. Total phenol content in VF pasta was twofold higher compared with DSW pasta (111 mg/100 g vs 50 mg/100 g). Moreover, VF pasta exhibited 20% higher antioxidant capacity compared with DSW pasta and assessed by ORAC assay (1,017 vs 851 μmol TE/100 g). Pasta obtained with VF flour exhibited a lower GI value (40±5) and a lower glycemic load (GL) (20±2) compared with DWS pasta. The results suggest that enrichment with 35% *V. faba* bean has potential health benefits and VF flour can be used as ingredient to prepare added-value products. The lower GI of VF pasta is likely related to the higher fiber content and/or to polyphenols. Our hypothesis is supported by previous studies which have demonstrated that polyphenols exert an effect on glycemic index with a significant inverse correlation between polyphenol content and GI.

A quality appraisal tool for observational data in nutritional epidemiology

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Sharing nutritional observational data enables joint data exploration with improved statistical power that may answer important research questions in nutritional epidemiology. However, findings of such joint analyses depend on the quality of the original data from the individual included studies. To date, there have been few tools to assess the data quality of observational studies in nutritional epidemiology. Such tools could help guide users to locate data that matches their research needs and evaluate the quality of the findings generated. To develop a quality appraisal tool (QAT) to assess the quality of data from observational studies for implementation in the 'Data Sharing In Nutrition' (DASH-IN) infrastructure. DASH-IN is an open access nutritional data infrastructure facilitating joint data analyses developed by ENPADASI (European Nutritional Phenotype Assessment and Data Sharing Initiative), a collaborative project with 16 consortia consist of a multidisciplinary consortium of scientists from 51 research centres in 9 European countries. Firstly, a literature review was performed to retrieve existing QATs for cohort, case-control and cross-sectional studies. Secondly, two face-to-face workshops were organized to determine the items for inclusion in a QAT. Thirdly, the QAT was discussed in the ENPADASI consortium until consensus was reached. A total of 4,884 distinct papers were retrieved from PubMed by pre-set eligible criteria. From these, 26 papers were included after title, abstract and full-text screening according to pre-set inclusion and exclusion criteria. In total, 26 QATs were selected from 24 eligible papers and 2 sets of recommendations that were obtained from 2 systematic reviews. During the face-to-face meetings, 26 items and 24 items were allocated into a 'study design' or 'measurement' domain, respectively. Related to dietary assessment, 18 items were selected for key dietary assessment methods (e.g. dietary records, 24-hour dietary recall, food frequency questionnaire, screeners, diet history). Consensus on the final list of items and wording will be obtained in the ENPADASI consortium. The QAT provides a minimum set of quality parameters to describe observational data from nutritional epidemiological studies. It is a timely contribution to improve sensitivity of research that uses existing nutritional data. Further research is needed to examine the validity, reliability and inter-rater agreement of the QAT.

Effects of phenolic compounds supplementation on the adipocyte morphology of diet induced obese rats

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The prevalence of obesity is increasing every day in a huge scale, being more pronounced in the developed countries. An excessive increase in adipocyte area by lipid accumulation during obesity can lead to hypertrophy, where cells tend to be dysfunctional and their gene expression becomes irregular. In this context, research about reduction of adipose tissue depots is an important factor to improve obesity state. Dietary polyphenols have been described to counteract some of the effects of a diet induced obesity and several metabolic syndrome disorders. Specifically, a grape seed proanthocyanidin extract (GSPE) has been widely studied for counteracting a number of obesity associated factors. This extract contains different phenolic compounds and among them gallic acid (GA). Resveratrol (RSV) is another phenolic compound that exerts anti-obesity effects according to numerous studies. The aim of this work was to determine the capacity of phenolic compounds supplementation to change adipose tissue morphology at reducing the adipocyte hypertrophy. For this, eight-week-old male Wistar rats were given a standard chow diet (STD, n=6) or a cafeteria diet (CAF). After eight weeks, the CAF rats were divided in four groups (n=6) at supplementing with vehicle, 50 mg/kg of RSV, 6.65 mg/kg of GA or 25 mg/kg of GSPE during 3 more weeks. After their sacrifice, retroperitoneal adipose tissues (rWAT) were excised, paraffin infiltrated and cut into 2 μ sections that were hematoxylin/eosin stained. Images of the adipose sections were analysed to quantify adipocyte size. Total cell number was calculated considering rWAT weight. Results showed that although the weight of rWAT and the adipocyte area increased with CAF diet compared to STD diet, the total cell number were not different between the two groups meaning that CAF induced hypertrophy. Interestingly, although any of the phenolic compound supplementation had an effect at reducing rWAT weight, GSPE reduced adipocyte area and increased total cell number compared to CAF rats. Moreover, RSV also reduced adipocyte area to STD rat levels and showed a tendency to increase cell number, while GA also showed these tendencies not being statistically significant. These results indicate that GA, RSV and GSPE are able to counteract adipocyte hypertrophy induced by obesity, suggesting that adipocytes will be more functional and, therefore, this could explain some of the beneficial effects of phenolic compounds on obesity. Further investigation is ongoing to define molecular mechanisms and other beneficial effects of these compounds to use them as anti-obesity treatments. Work funded by AGL2013-40707- R from Spanish Ministerio de Economía y Competitividad.

Discovery of polymorphisms in the 101 kB vitamin D receptor gene that can affect fragility fracture

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Vitamin D deficiency (VDD) has been implicated as a cause of osteoporotic fractures among post-menopausal women incurring significant health and economic impact. The current standard of care, which includes surgeries, lifestyle modifications, anti-osteoporosis drugs, calcium and vitamin D supplementation, have been consistently shown to be dependent on racial profile. This study aimed to discover genetic variants in the entire 101 kB vitamin D receptor (VDR) gene for osteoporotic fractures in a group of post-menopausal Filipino women using targeted next generation sequencing (TNGS) approach comparing women with and without fragility fractures. The study followed a 1:1 case-control study design. All subjects were seen at the Philippine Orthopedic Center. Blood samples were collected for determination of serum vitamin D, calcium, phosphorus, glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase and as primary source of genomic DNA for VDR gene sequencing. About 0.10-10 ng genomic DNA was used for enrichment of the VDR target gene by applying a custom VDR AmpliSeq™ panel. DNA libraries were constructed using the Ion Ampliseq Library Kit v2.0 and sequenced on the Ion Torrent Personal Genome Machine. Sequencing reads were aligned against the hg19 reference genome. The variant calling was based on the GATK best practice workflow using HaplotypeCaller. Variants were annotated using Annovar tool. The frequencies of the detected variants were compared with dbSNP database. The diseased and healthy group were comparable in terms of age, BMI, fasting plasma glucose, blood urea nitrogen, creatinine, serum vitamin D, serum calcium, serum phosphorus, number of hours of sun exposure per week and physical activity level. Calcium and vitamin D supplements were taken by 8% of case patients compared to 4% of control patients. A total of 1,496 unique variants in the whole 101 kb VDR gene were identified. Novel sequence variations not registered in the dbSNP database were found among cases and controls at a rate of 23.1% and 16.6% of total discovered variants, respectively. Noteworthy is the discovery of two disease-associated novel heterozygous frame shift deletions (P-value <0.05) predicted to result to a truncated protein. Of the regulatory SNPs, one disease-associated rare SNP, rs141114959, showed statistically significant association to low serum 25OHD vitamin D levels (P-value=0.009).Taken together, these findings show the power of using TNGS in identifying sequence variations in a very large gene and the surprising results obtained in this study greatly expand the catalogue of known VDR sequence variants that may represent an important clue in the emergence of osteoporotic fractures. The SNP information will also provide the additional guidance toward a personalized nutritional advice to reach a sufficient vitamin D status in the Filipino population.

Datasharing in nutrition: the pros and cons – how can we optimize research infrastructures to serve the needs of nutritional systems biology

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The ENPADASI project of the Joint Programming Initiative "Healthy Diet for a Healthy Life" (www.enpadasi.eu) aims at establishing a Knowledge Hub by connecting datasets from nutritional intervention/observational studies. To this aim, the Project focuses on developing the DASH-IN Infrastructure, which builds on the Nutritional Phenotype Database (dbNP – www.dbnp.org), originally developed by NuGO and tested as a case study within the EU-FP7 EuroDISH project by combining metabolomics datasets from different intervention studies. The results of this case study showed that standardisation of tools and methods is a major gap to be filled in, if we want to achieve a fully operational, nutrition-specific Research Infrastructure (RI), which is the overall goal of ENPADASI. The project as a whole interfaces biological and bioinformatics expertise and WP2 focuses on solving major obstacles for study comparison through the DASH-IN infrastructure, by improving standardization in the uploading of study designs and datasets, developing the proper tools for minimal requirements and quality appraisal, and finally testing the outcome by running a case study. Within the activities of WP2 we also needed to analyze the perceived pros and cons of datasharing through the DASH-IN infrastructure, and to determine the expectations that will help its further development. For this purpose, an online survey was organized to guide the design of case studies to be performed during the project, and the link was distributed to all 51 ENPADASI partners spread over 9 EU countries. The survey addressed two major questions: (1) Why would you consider sharing data? (2) Why would you consider using shared data? The response rate was 73% and the results showed overall broad interest in datasharing through field-specific RIs, both from scientists employing high throughput -omics technologies, as well as from nutritional epidemiologist and public health nutritionist. Over 50% of the respondents have already experienced deposition of their datasets (mostly published data, raw data and metadata) in a research infrastructure and correspondingly allowed datasharing. As for using shared data, almost 50% of the respondents have had previous experience. The major factor limiting acceptance of datasharing appears to be the fear of others using unpublished data before the Authors themselves. This aspect could possibly be counteracted by introducing a choice in the time of release, which could be determined by the authors themselves upon data upload. On the other hand, major drivers in favor of datasharing appear to be journal policies requesting data deposition prior to publication, and the advantages from shared data analysis. Overall, the perceived added value of sharing data through RIs relates mostly on increased study power and comparative value of studies, thus leading to new insights and novel interpretations. These results represent indeed a good start, and they point at crucial aspects that need to be solved to maximise the advantages of datasharing through nutrition-specific RIs, toward the long-term goal of achieving a fully open access environment supporting top level nutritional science.

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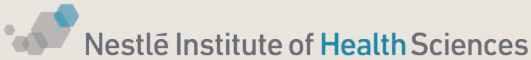
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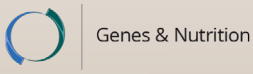
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