Mechanisms of a long-life health

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Book of abstracts

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– NuGOweek 2015 –

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Welcome

Welcome to NuGOweek 2015 in Barcelona!

NuGOweek 2015 is the 12th edition of the successful annual conference and will take place in Barcelona from the 7th to 9th of September 2015.

NuGOweek is a unique event that stimulates the interactions between researchers, initiates collaborations and leads to developments in molecular nutrition, personalised nutrition, nutrigenomics, nutrigenetics and nutritional systems biology. This year's NuGOweek will focus on the 'Mechanisms of a long-life health'. Nutritional lifestyles have a great impact on our overall health and well-being and there is increasing evidence that unhealthy dietary patterns are one of the major risk factors for cardio vascular diseases, metabolic syndrome, diabetes or cancer. Around this topic the Scientific Committee has organized scientific sessions on Inflammation, immunology, and gut health; The role of genetics in the delivery of personalised nutrition; Effective interventions, personalised diets and sustainable choices; Genotype phenotype interactions; and Mechanisms and interventions in obesity and metabolic health. During three exciting days excellent talks will be given by international experts in the fields and three satellite meetings will be held on on-going European projects. For our young investigators we offer a post-graduate course on 'Nutrigenomics studies in humans: From epidemiology to intervention' which will be held right before the official start of NuGOweek 2015. Young investigators will be given the opportunity to present their results in the 'young investigators session' and we encourage them and all other participants to interact and exchange information during multiple opportunities like poster sessions, lunch and dinner, the welcome reception or during coffee breaks between scientific sessions.

On behalf of the Organizing Committee we would like to thank our sponsors and all people that helped to organise NuGOweek 2015 and would like to welcome you here in Barcelona, our vibrant city at the Catalan coast. We are looking forward to this unique meeting with excellent science, exciting discussions and inspiring topics and we hope you will have an enjoyable stay.

Cristina Andres-Lacueva

Scientific Committee

Prof Cristina Andres-Lacueva	Universitat de Barcelona
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Prof José López Miranda	Universidad de Córdoba
Prof Andreu Palou	Universitat de les Illes Balears
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Scientific programme

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Session 2. Inflammation, immunology, and gut health

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Long-life health: an integrated effort of science and people

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Numerous genome-wide association studies have provided us with knowledge of genes associated with the most common age-related diseases and their risk factors (i.e. CVD, diabetes, plasma lipids, obesity, blood pressure, cancer). These genes and many others to be uncovered using newer approaches (i.e. exome and whole genome sequencing) reveal our genetic make-up which include millions of genetic variants that have been accumulating generation after generation. Whereas these variants define our predisposition to common diseases, the expression of this legacy depends on the environment to which we are exposed and our own habits. Multiple reports describing gene-environment interactions support this concept and whereas these interactions have included the analysis of environmental factors such as tobacco smoking, physical activity, drugs, etc., the most commonly researched environmental factor has been diet. Hence, there are hundreds of studies showing statistically significant interactions between genetic polymorphisms and various components of the diet modulating disease risk factors and even disease itself. More recently, emphasis has been placed on the association between epigenetic marks (i.e. methylation, microRNAs) and disease. Better knowledge of these relationships is relevant given the fact that whereas the genetic sequence cannot be easily modified, the epigenetic marks are susceptible to change when exposed to a different environment. This provides a potential mechanism by which we can trump our legacy with our current behavior and thus change our future disease risk. In general we may say that, despite the fact that great heterogeneity still exists in the methodology and results obtained by nutrigenetic studies, the epidemiological design and the evidence level of these studies continue to improve by incorporating more randomized controlled intervention trials. The results of these studies are crucial to obtain a higher level of scientific evidence and to bring the findings of these gene(epigene)-environmental interactions into personalized medicine and effective disease prevention.

Diet, metabolites, and 'western lifestyle' inflammatory diseases

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Recent studies highlight the profound effects of diet, gut microbiota, and bacterial metabolites on various systems, including obesity, inflammation, and other 'western lifestyle' diseases. Diet affects the composition and diversity of the gut microbiota, which in turn regulates gut homeostasis. There are 2 main mechanisms- bacterial or dietary metabolites engage 'metabolite-sensing' G-protein coupled receptors (GPCRs). For instance short chain fatty acids (SCFAs) bind GPR43, GPR41 and GPR109A. SCFAs also influence gene transcription and epigenetics through inhibition of histone deacetylases (HDACs) which regulate the function of certain transcription factors, notably FoxP3. It is becoming clear that numerous metabolites regulate immunity, in particular SCFAs, omega-3 fatty acids, and tryptophan metabolites. Insufficient intake of fiber or other healthy foodstuffs likely affects gut microbial ecology and the production of particular metabolites, which alters immune regulation and development of inflammatory disorders. These dietary effects may occur in utero, during lactation, and at other times through life, and involve both immediate type effects through metabolite-sensing GPCRs, or epigenetic effects that manifest over decades or generations.

Diet, intestinal microbial communities and host health

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The human large intestine is home to an extremely abundant and diverse collection of microbes, which are collectively termed the intestinal microbiota. Each individual harbours a distinct collection of microbial species, but the majority of gut bacteria are anaerobes that ferment dietary nutrients into short chain fatty acids (SCFAs), and gases. Our indigenous microbes play a number of key roles in the maintenance of host health, including aiding digestion of otherwise indigestible dietary compounds, immune system regulation, and protection against invading pathogens. Conversely, an imbalance or disruption in the composition and/or activity of the intestinal microbiota (termed 'dysbiosis') has been implicated in a number of ailments, ranging from intestinal conditions like inflammatory bowel diseases and colorectal cancer to non-intestinal disorders such as metabolic syndrome and atopy. The composition and activity of the intestinal microbiota appears to be heavily impacted by host diet, with non-digestible carbohydrates providing the major energy source for many intestinal bacteria. In turn, much evidence now suggests that many of potential risks and benefits to host health that are associated with particular diets may be at least partially mediated via the microbiota. As such, there is now accumulating interest in modulating the composition of the human intestinal microbiota via changes in diet to improve host health. The volume and composition of dietary macronutrients such as carbohydrates has a major impact on the composition of the intestinal microbiota, and on SCFA production. SCFAs have been shown to have a range of health benefits, including maintenance of gut epithelial barrier function, appetite control, inhibition of inflammation, and antimicrobial effects on invading pathogens. Increased fibre consumption, which leads to enhanced SCFA production by gut bacteria, is therefore just one example of the interlinked relationship between diet, microbiota and host health. There are specialist substrate-attached bacteria that associate with insoluble fibre particles, and these species may therefore play a key role in the release of energy from insoluble substrates. Furthermore, some intestinal microbiota species can release and transform dietary plant phenolics, and many of these compounds have potent anti-oxidant and anti-inflammatory activities. Conversely, diets that are low in fibre and high in animal fats and protein can result in microbiota formation of damaging products such as trimethylamine oxide, nitroso-compounds and hydrogen sulfide. Deceleration in transit time as a result of reduced fibre consumption may also potentially increase the exposure time of the gut epithelium to the deleterious compounds formed as a result of microbial protein fermentation. Controlled dietary intervention studies have demonstrated that there is typically a large degree of inter-individual variation in the bacterial groups that respond to dietary changes. As such, the response to dietary intervention may be dependent to at least some extent on the original composition of a given individual's microbiota, and holistic approaches to personalized nutrition may in future also have to incorporate this information.

Effects of non-digestible carbohydrates on colorectal health via modulation of WNT signalling

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Epidemiological and experimental evidence suggests that non-digestible carbohydrates (NDCs) such as resistant starch (RS) are protective against colorectal cancer (CRC). This results primarily from the production of butyrate, a short-chain fatty acid with beneficial effects including anti-inflammatory properties, by colonic bacteria. It has been proposed that one of the mechanisms via which butyrate protects against CRC is by modulation of WNT signalling, a pathway central to the regulation of processes, e.g. cell proliferation and differentiation, in the colorectum but that is frequently hyperactive in CRC. This study aimed to investigate the effects of supplementing healthy participants with NDCs on colorectal health via effects on WNT signalling and on two of its functional outcomes, proliferation and apoptosis. We hypothesised that NDC supplementation would increase colonic butyrate concentrations and modulate WNT signalling positively. Using a 2×2 factorial, double-blind RCT design, 75 participants were supplemented with RS and/or polydextrose (PD) or placebo for 7 weeks. Rectal mucosal biopsies were collected pre- and post-intervention and used to quantify the expression of 12 WNT pathway-related genes by qPCR. The effects of the intervention on the epigenetic regulation of SFRP1, a WNT pathway antagonist frequently silenced in CRC, via DNA methylation and microRNA (miRNA) expression was assessed by pyrosequencing and qPCR respectively. Colonic crypt cell proliferative state was measured following whole crypt microdissection of Schiff's reagent-stained crypts and apoptosis was examined by quantifying the expression of two regulators of apoptosis, BAX and BCL-2. Supplementation with RS significantly reduced the expression of CTNNB1, encoding β-catenin (a key component of the WNT pathway) (P=0.045), and c-MYC (a target gene of WNT signalling) (P=0.037), which suggests reduced WNT pathway activity. However, RS and/or PD significantly downregulated two WNT antagonists, SFRP1 and SFRP2, which would be expected to increase WNT activity. RS and PD did not alter SFRP1 methylation or the expression of eight miRNAs predicted to regulate SFRP1, suggesting that changes in gene expression resulted from additional mechanisms e.g. altered histone marks. Participants given RS had significantly increased total crypt cell proliferation (P=0.030) but RS did not alter the proportion of proliferating cells in the top half of the crypt (a marker of crypt health) indicating that neither RS nor PD had adverse effects on proliferation. RS and PD did not modify apoptosis levels through alterations of BAX and BCL-2 expression. These findings suggest that the increased cell proliferation with RS may have resulted from induction of signalling via the WNT pathway. However, these effects were not mediated via alterations in SFRP1 methylation or expression of miRNAs predicted to regulate SFRP1. Funding: BBSRC Diet and Health Research Industry Club (grant number BB/H005013/1).

Coffee inhibits nuclear factor-kappaB in prostate cancer cells and xenografts

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Chronic inflammation contributes to prostate cancer and the transcription factor Nuclear Factor-kappa B (NF-κB) is constitutively active in most prostate cancers. We examine the effects of coffee on NF-κB and on the regulation of selected genes in human derived prostate cancer cells (PC3) and in PC3 xenografts in athymic nude mice. PC3 cells stably transduced with a NF-κB-luciferase reporter were used both in vitro and for xenografts. NF-κB activity was measured by reporter assays, DNA-binding and in vivo imaging. Gene expression was measured in PC3 cells, xenografts and tumor microenvironment by Low-Density Arrays. Western blotting was used to quantify apoptosis. Coffee inhibited TNFα induced NF-κB activity and DNA-binding in PC3 cells. Also, coffee increased apoptosis and modulated expression of a number of inflammation- and cancer-related genes in TNFα treated PC3 cells. In vivo imaging revealed a 31% lower NF-κB-luciferase activation in the xenografts of the mice receiving 5% coffee compared to control mice. Interestingly, we observed major changes in gene expression in the PC3 cells in xenografts as compared to PC3 cells in vitro. In PC3 xenografts, genes related to inflammation, apoptosis and cytoprotection were down-regulated in mice receiving coffee, and coffee also affected the gene expression in the xenograft microenvironment. Our data demonstrate that coffee inhibits NF-κB activity in PC3 cells in vitro and in xenografts. Furthermore, coffee modulates transcription of genes related to prostate cancer and inflammation. Our results are the first to suggest mechanistic links between coffee consumption and prostate cancer in an experimental mouse model.

Plasma proteome biomarkers of inflammation: potential mediators of early childhood health and growth

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Inflammation comprises an orchestrated, systemic response, usually to non-self agents, involving complex host defense and tissue repair mechanisms. Often, inflammation is clinically characterized by elevation of only one or a few acute reactants, such plasma α-1-acid glycoprotein (AGP) or C-reactive protein (CRP), although hundreds of interpretable biomarkers may exist. We hypothesized that untargeted quantitative proteomics can offer a novel, unbiased approach to discover biomarkers of inflammation, including molecular mediators of metabolic consequence of inflammation, and that detected inflammatory mediators could be associated with child growth status. We applied quantitative proteomics to profile plasma proteome of 500 Nepalese children 6-8 years of age using isobaric tag for relative and absolute quantification (iTRAQ). We sought to detect and quantify plasma proteins that covary with plasma AGP concentration, as elevated CRP was not common in this population. We further assessed associations between continuous anthropometric indicators of child growth and drew on the relevant inflammation and growth biomarker literature to infer potential roles of associated proteins. About 10% (n=99) of a total of 982 proteins that were detectable in >10% of children were associated with AGP, passing a family-wise error rate (FWER) threshold of 0.1%. Proteins that were positively associated with AGP included acute phase reactants such as CRP, serum amyloids, complement components, protease inhibitors, transport proteins with anti-oxidative activity, and many intracellular signal transduction molecules. Negatively associated proteins were of both hepatic and extra-hepatic origin. Hepatic-derived proteins included plasma transporters for lipids/cholesterol and micronutrients (vitamin A, E and calcium), insulin-like growth factors (IGFs) and sex hormones. Negative correlates of extra-hepatic origin included structural and functional components of extracellular matrix (ECM) such as collagens, non-collagenous glycoproteins, proteoglycans, and cell-cell/ECM adhesion molecules. In separate analyses, several IGF-related, sex hormone-binding and ECM-related proteins were also strongly associated with child height, weight, arm muscle area, arm fat area, and subscapular skin-fold thickness (all passing FWER threshold of 5%). Using population data, our findings reveal a vast plasma proteome consisting of functional biomarkers of host defense, coagulation, nutrient and hormonal metabolism and tissue remodeling. We speculate that chronic inflammation may contribute to suboptimal growth and body composition in undernourished South Asian child populations. Grant funding: Bill and Melinda Gates Foundation (OPP5241 and GH614).

Mice lacking TLR4 or CD14 are not protected against high-fat diet induced obesity

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The gut microbiota has been proposed to increase body weight and body fat in high fat diet fed mice through increased systemic exposure to low levels of gut derived bacterial lipopolysaccharide (LPS), defined as metabolic endotoxemia. Chronic low-grade inflammation resulting from LPS activation of Toll like receptor-4 (TLR4) has been implicated as a mechanism linking the gut microbiota to body weight. The hypothalamus is a region of the brain where inflammation has been implicated in disrupting energy homeostasis and has been proposed as a factor in obesity development. Gut derived systemic LPS is a potential mechanism linking the microbiota and increased body weight through induction of hypothalamic inflammation. This study investigated whether mice lacking TLR4, or its co-receptor CD14, are protected from diet induced obesity and have reduced hypothalamic inflammation, compared to wild-type mice. Mice were fed high-fat or low-fat diets and body weight, body composition, and food intakes measured. LPS exposure was assessed by measuring serum lipopolysaccharide binding protein (LBP). Denaturing gradient gel electrophoresis (DGGE) was used to identify differences in caecal microbiota composition. Gene expression changes in the hypothalamus were investigated using microarray analysis. Mice lacking TLR4 or CD14 were not protected against obesity. There was no difference in high-fat diet induced increases in body weight and body fat in either TLR4 or CD14 null mice compared to wild-type controls. Food intake was not different in high-fat diet fed TLR4 or CD14 null mice compared to wild-type controls. This was despite increased serum LBP in high-fat diet mice indicating increased systemic exposure to gut derived LPS. Diet and genotype influence caecal microbiota composition but this did not influence obesity susceptibility. Analysis of the hypothalamus did not show evidence for increased inflammatory gene expression in either wild-type mice, or mice lacking TLR4, or CD14. Mice lacking TLR4 signalling are not protected against high-fat diet induced obesity. This study does not support the role for gut-derived LPS or hypothalamic inflammation as causes of increased body weight or body fat.

Chronic supplementation with proanthocyanidins protects from diet-induced intestinal alterations

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In the last years, increased attention has been paid to the link between an altered intestinal function and higher incidence of metabolic disorders, such as obesity. Recent scientific advances point out that a high-fat diet (HFD) is able to alter the gut microbiota composition, which reciprocally leads excessive energy harvesting and storage. Moreover, microbial imbalance increases gut permeability, leading to metabolic endotoxemia, inflammation, and in most cases insulin resistance. Therefore, identifying selective natural compounds, which can modulate intestinal inflammation and barrier function, is postulated as a strong therapeutic strategy to prevent obesity epidemic. The present study aims to determine and compare in obese rats the possible protective effect of a grape seed proanthocyanidin extract (GSPE) on obesity-associated intestinal alterations in response to a cafeteria diet (Cd). Thirty-six-week-old, female Wistar rats were used to analyse the role of a GSPE chronic treatment with a low, moderate or high dose in HFD-induced inflammation. ROS production and MPO activity were measured as markers of oxidative stress. Also, the gene expression of TNF-α, iNOS and Emr-1 were quantified to evaluate the stage of intestinal inflammation. In parallel, the barrier integrity was assessed quantifying the gene expression of Occludin-1 and ZO-1 and measuring the plasma LPS associated with defective barrier integrity. In addition, linear regression and principal component analysis (PCA) were used to assess possible relationships among the variables evaluated. GSPE protects from intestinal oxidative damage in obese rats decreasing ROS levels and MPO activity, without substantial differences among the doses. GSPE does not affect TNF-α and Emr-1 expression in the ileum, but in contrast, the supplementation with moderate and high doses significantly decrease iNOS expression compared to Cd group. Regarding to the integrity of the intestinal barrier, results show that GSPE significantly increases ZO-1 expression respect Cd animals, but no changes are observed in Occludin-1 gene expression. Further, the correlation analysis and PCA support a strong relationship between the intestinal inflammation and oxidative stress with anthropometric and metabolic variables that define the altered metabolic state in obesity. In conclusion, the present study provides the evidence of the ameliorative effect of a GSPE extract, rich in proanthocyanidins, on high-fat diet-induced intestinal alterations in rats, including the reduction of intestinal inflammation, oxidative stress and suggesting a prevention of barrier defect. Based in these findings, our data suggest that nutritional and/or therapeutic interventions focused on intestine health and modulation of intestinal permeability should be extensively explored in the context of obesity.

Intestinal metabolite profile of maslinic acid after its repeated oral administration to rats

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Maslinic acid is a secondary metabolite widely distributed in the plant kingdom and found in a number of edible vegetables and fruits regularly consumed in the Mediterranean region. Although this pentacyclic triterpene is being recognized as a bioactive compound due to its health protecting activities and lack of harmful effects, we have previously reported a low oral bioavailability together with a poor intestinal absorption. These processes could increase the amount of maslinic acid reaching the large intestine, thus favouring its potential chemopreventive activity against colon cancer. Therefore, we aimed at quantifying maslinic acid and identifying its derivatives in the colon content of orally administered rats. Adult Sprague-Dawley rats received daily oral doses (10 mg/kg) of the pentacyclic triterpene over 49 days. The colon content was collected, mixed with methanol 80% that contained betulinic acid as internal standard, prior to being finely grinded with a Polytron homogenizer. Samples were vortex-mixed, centrifuged and the supernatant was filtered before being transferred to an amber vial. Chromatographic separation was performed in a reversed-phase C_{18} column with a gradient elution of water/acetonitrile (0.8 ml/min). Quantification of maslinic acid was carried out in a single quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source operating in the negative mode. The metabolite profiling in the colon was performed by HPLC-APCI-LTQ-Orbitrap-MS and data was acquired using the full scan mode (m/z 100-1000). Derivatives were selected on the basis of their accurate mass (<5 mDa) and isotopic pattern. After 24 h of the last administration of maslinic acid, the analysis of the colon content demonstrated that this compound reached the colon at the concentration of 90.7±36.9 nmol/g. HPLC-APCI-LTQ-Orbitrap-MS determination not only revealed the presence of maslinic acid ($C_{30}H_{48}O_4$), with a retention time of 9.19 min and an accurate mass (m/z) of 471.3480, but also of 13 metabolites. These derivatives could be grouped into five categories: 3 monohydroxylated derivatives ($C_{30}H_{48}O_5$; 487.3427) (M1–M3), 3 monohydroxylated and dehydrogenated metabolites ($C_{30}H_{46}O_5$; 485.3272) (M4–M6), 3 dihydroxylated compounds ($C_{30}H_{48}O_6$; 503.3375) (M7–M9), 3 dihydroxylated and dehydrogenated derivatives ($C_{30}H_{46}O_6$; 501.3217) (M10–M12) and a single dehydrogenated metabolite ($C_{30}H_{46}O_4$; 469.3320) (M13). M2 was the major compound, accounting for almost 60% of total analytes, followed by maslinic acid and M1, representing 15% and 10%, respectively. After the repeated oral administration of maslinic acid, several phase I metabolites were found in the colon content, being the most abundant the monohydroxylated ones, while no phase II derivatives were detected. Supported by grants AGL2013-41188 from MEC (Spain), and 2014SGR1221 from Generalitat de Catalunya.

Procyanidins and DHA suppress inflammation and boost immune system in cafeteria diet-fed rats

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Nutrition can be considered as a two-side coin: although an imbalance in the energy content is associated with obesity, a healthier state can be promoted through the intake of immunologically active compounds. Thus, the aim of this study was to determine the immunomodulatory properties of grape seed procyanidin extract (GSPE) or docosahexaenoic acid (DHA) in cafeteria (CAF) diet-fed rats. Rats were fed either a standard diet (STD) or a CAF diet for 13 weeks. During the last 3 weeks the group fed with CAF diet was divided depending on CAF diet supplementation with GSPE and/or DHA. The healthy properties of GSPE and DHA diet supplementation include the suppression of diet-induced inflammation by promoting a phenotypic switch in the molecular and cell profile of mesenteric adipose tissue, the boost of immune system through the modulation of cell-mediated immunity, involving macrophages and T lymphocyte subsets, and the stimulation of the thymus and spleen functionality to counteract the obesity-induced weakened immune response. Moreover, the combination of GSPE and DHA bioactivities potentiate the immunomodulatory properties of each compound administered individually.

Metabolic and proteomic assessment in pediatric patients with systemic lupus erythematosus

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Lupus erythematosus (SLE) patients have a higher risk to develop cardiovascular complications. Therefore, the study of risk factors must be assessed early in the course of the disease. To describe and compare metabolites, frequency of polymorphism of MTHFR enzyme, anthropometric measurements, food intake and proteomic analysis in pediatric SLE patients and in healthy controls. 19 female adolescents with SLE and 39 healthy volunteers were recruited. We evaluated SLEDAI, SLICC, BMI, weight, height, waist circumference (WC), 24-hours recalls, serum levels of homocysteine, vitamin B12, folate, TNF-α, high sensitivity C reactive protein (hsCRP) by chemiluminescent technique, monocyte chemoattractant protein-1 (MCP-1), adiponectin, leptin, ghrelin by ELISA, lipid profile by colorimetric methods and plasma proteomic by Shotgun proteomics with Isobaric Tag for Relative and Absolute Quantitation (iTRAQ) in both groups. The subjects were genotyped for MTHFR C677T and A1298C polymorphisms by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). We compared SLE and control group by ANCOVA adjusting for age, BMI, vitamins intake and genotypes or haplotypes when applicable. Qui-square, Pearson correlations were also done in statistical analysis. Polymorphisms were tested for Hardy-Weinberg equilibrium. For proteomic assessment, we did k-cluster statistical analyses to separate SLE and control groups into two extreme clusters with better and worst metabolic profile according to homocysteine, TNF-α, hsCRP and folate plasma levels. SLE patients presented higher BMI, WC, homocysteine, triglycerides, TNF-α, hsCRP levels and lower plasma folate when compared to controls. Significantly higher frequency of the (mutant) 677T was found in SLE than in control group. We found 10 proteins with significant different expression between control cluster with better metabolic profile (CCBMP) and lupus cluster with better (LCBMP) and worst metabolic profile (LCWMP) (alpha-2-macroglobulin; alpha-1-antitripsin, apoliprotein AI, apoliprotein E, ceruloplasmin, complement C3, fibrinogen α chain, haptoglobin, hemopexin and serumtransferrin). Eight proteins were higher expressed in LCBMP and lower expressed in LCWMP compared with CCBMP. These proteins that were lower expressed in LCWMP were correlated with a higher risk for cardiovascular disease. Our results may sign some future cardiovascular risk factors for cardiovascular disease in SLE pediatric patients and possible associations between nutritional status and risk factors. Proteomic results showed higher acute phase proteins and pro-inflammatory proteins expressed in SLE pediatric patients, which is an expected result, but the fact that these protein were statistically correlated with distinct metabolic groups give us better input to understand the complexity of systems biology interactions in SLE pediatric patients. This study was sponsored by FAPESP (number 2011/16141-7).

Discovering the gastrointestinal metabolome through a validated HRMS-based approach

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Metabolomics can be applied to many biological matrices. Feces is only rarely used but can be obtained easily and non-invasively and provides insights about the complex interactions between the gut microbiota and the host, while reflecting dietary input. Currently, only few reports are available on high-resolution mass spectrometry (HRMS)-based metabolomic analysis of feces. Moreover, none of these studies take into consideration whether the analytical method is 'fit-for-purpose'? This study intended to unravel the gastrointestinal metabolome of Inflammatory Bowel Disease (IBD) patients by means of validated fecal metabolomic fingerprinting through UHPLC-Orbitrap-HRMS. To ensure the holistic nature of our method, 114 authentic analytical standards were included. Additionally, chemometric optimization of the extraction procedure was implemented. Next, a validation was performed on quality control samples (a pool of representative bulk samples), thereby monitoring linearity, precision (i.e. instrumental, inter-assay and intra-assay precision) and recovery. The newly developed untargeted fingerprinting approach was subsequently employed to analyze our cohort that consisted of 10 healthy volunteers and 13 IBD patients. Finally, a multivariate data analysis strategy was undertaken to differentiate between the various disease phenotypes. Chemometric optimization relied on a sequential strategy of fractional factorial design (FFD) followed by response surface model (RSM). During the FFD experiment seven representatives of metabolite classes (amino acid, carbohydrate, N-compound, bile acid, short chain fatty acid, monocarboxylic acid and amine) were monitored. The following parameters appeared to exert a significant ($P<0.05$) influence on the extraction yield: mass, pre-extraction with water, extraction volume and repeating extraction. The RSM provided statistical proof that 200 mg freeze-dried feces supplemented with 4 ml of water was required. Applying an aqueous dilution (1:3) suppressed matrix interferences, resulting in an increased detection of metabolites. Validation of the untargeted detection method was performed with QC samples. Monitoring seven endogenous compounds revealed excellent linearity ($R^2>0.99$), recoveries (97.2-104.8%) and all components of precision obtained CVs below the FDA recommended level of 15%. The differential analysis of the metabolic fingerprints obtained from the sample cohort revealed 9553 monoisotopic ions. The intensities of QC samples were used to construct a correction trace for the dataset prior to performing multivariate statistics. Classification of the different (disease) phenotypes relied on the predictive power of the models. The validity characteristics of the PLS-DA plots $R^2(X)$, $R^2(Y)$, $Q^2(Y)$ were 0.51, 0.84, 0.53 respectively for the positive, and 0.51, 0.99, 0.80 for the negative ions, thereby confirming its predictive power and suitability ($P_{ANOVA}<0.05$). Per disease phenotype a clear individual cluster was observed. In total, 84.2% and 89.1% of these ions, in positive and negative ion mode respectively, could be putatively identified.

Does addition of genetic information facilitate further dietary change: lessons from Food4Me?

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Optimal nutritional choices are linked with better health but many conventional 'one size fits all' interventions to improve diet have limited effect. Advances in the cost and time efficiency of genome sequencing and enhanced ability to extract information of interest, e.g. disease risk, have fuelled interest in the use of personal genetics. Genotypic information might help to identify each individual's optimum dietary pattern and to motivate better eating habits but the effectiveness of genetics-based information in facilitating behaviour change is unclear. In the Food4Me Study we tested the hypothesis that providing personalized nutrition (PN) advice based on information on individual diet and lifestyle, with/without phenotype or genotype-based advice would promote larger, more appropriate, and sustained changes in dietary behaviour. We recruited 1,607 adults (mean age 39.8 years, 59% female and mean BMI 25.5 kg/m^2) from 7 European countries to an internet-delivered intervention and randomized them to: (1) conventional dietary advice (control) or to PN advice based on: (2) individual baseline diet; (3) individual baseline diet plus phenotype (anthropometry and blood biomarkers); or (4) individual baseline diet plus phenotype plus genotype (5 diet-responsive genetic variants). Outcomes were dietary intake, anthropometry and blood biomarkers measured at baseline and after 3 and 6 months intervention. 1,269 participants completed the study. Following a 6-month intervention, participants randomized to PN consumed significantly less energy, red meat, salt, and saturated fat, increased folate intake and had higher Healthy Eating Index scores than those randomized to the Control arm. There was no evidence that including phenotypic and genotypic information enhanced the effectiveness of the PN advice. This study revealed the efficacy of personalisation in driving bigger dietary change and demonstrated that the internet is an effective vehicle for delivering PN to large numbers of people across multiple countries. This work was supported by the EU FP7 [265494].

Generation of knowledge base for diet-gene interactions: the Food4me Gene cards

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Nutrigenetic research studies the effects of the individual`s genetic variation on the response to nutrients in the context of health and resulting consequences for nutrient requirements. A practical application of nutrigenetics is to use personal genetic information to guide dietary choices as a key component in personalised nutrition. Currently, like most applied genetics approaches, nutrigenetics is unregulated and there are no defined standards beyond some commercially adopted codes of practice. Only a few official nutrition-related professional bodies have embraced the subject and, consequently, there is a lack of educational resources and guidance for those wishing to implement the outcomes of nutrigenetic research e.g. dieticians, nutritionists and genetic counsellors. It is essential that all personalised nutrigenetic advice and information are based on clear evidence of benefit, grounded in a careful and defensible interpretation of nutrigenetic research studies. As part of the Food4me project, a 'global nutrigenetics knowledge network' was established involving the major players in the area of molecular aspects of personalized nutrition. Its purpose is to collate all relevant information on genetic variations involved in the nutrient-health relationships and deliver guidelines for the evaluation of evidence of diet gene interactions: (1) to provide information about nutrigenetic testing in practice; (2) to investigate the interpretations and claims by nutrigenetic tests; (3) to provide a framework of guidelines on how to assess the evidence for scientific validity and health utility for a nutrigenetic advice; and (4) to create the Nutritional Gene cards series – based on the developed framework a Nutritional Genecard will assess the evidence for a particular gene-diet/lifestyle interaction The data is collected in a database the 'Food4me knowledge database (FKD)'. This database accessible through food4me.tno.nl, which stores details on intake, biomarkers, health outcome and SNPs as well as their interactions. If enough data on a gene-diet interaction is available the data will be published as nutrition gene card. Alongside the data in the database on this gene will be made publically accessible. The basis of the nutrition gene cards is a framework that assesses the quality of the evidence for personalised nutrition advice based on genotype. This framework is described in a guidelines paper. The first step is to establish the scientific validity of the reported gene(s) × diet interactions and to determine the likelihood that the predicted outcomes will be consistent and reproducible. Validity itself is essential but not sufficient and, where it has been established, the second step requires the formal assessment of the personal health utility of the individualized advice. The fundamental requirement of a nutrigenetic test, as with any health-related test, states that the results should clearly indicate a diet-related action that is to be beneficial in relation to a concrete aspect of health or performance.

Computer-assisted genotype-specific nutrition guidance

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Responses to nutrition factors vary from one person to the next. Numerous genetic variants are now known that modify the response to specific nutritional exposures in a predictable way. The question then becomes how to make practical use of such information. The rapidly growing number of evidence-supported nutrient-gene interactions makes it increasingly difficult to manage them in clinical practice. Computer support is becoming essential for effective nutrition guidance. The first step needs to define individual nutrition targets. We have to know how much energy a particular client needs and how much each macronutrient class should contribute to total energy. We also need to understand the requirements for more than twenty key electrolytes, minerals, vitamins, and food groups. These target definitions are like the ones used in current dietetic practice, but are much more explicit due to the underlying specific rule sets for genotypes, haplotypes or other genetic variants. An online program (https://nutriscope.net) captures standard anthropometric data and lifestyle characteristics. Genetic information is downloaded from popular sites without storing or displaying genotypes. The program then uses the available information to generate a list of personalized nutrition intake targets. A narrative section outlines specific food needs and intolerances. The next challenge is then to translate the various intake targets into concrete dietary advice for an individual client or patient. This is easy enough for rules that concern a limited range of food items at a time, such as caffeinated beverages. It gets more difficult when the affected targets, such as carbohydrates, sodium, iron, and folate, are present in a wide range of foods. The real challenge comes when an individual has to comply with many rules. The search space becomes unavoidably vast and is no longer amenable to manual efforts. Computer-supported meal-planning can help. Our Personal Online Nutrition Guidance (PONG) program calculates for each targeted nutrient and food group pairs of polynomial distance functions, one for positive deviations and another one for negative deviations. The structure of each polynomial function is set to suit the characteristics of the item. For example, the function for vitamin B12 intake below the individual's target needs to generate a sizeable numeric value even for a moderate deficit, whereas an excess should not be penalized by much. For the sodium distance functions, it has to be just the other way around. The sum of the weighted individual results for a daily menu indicates how closely the composition meets the targets for an individual client. The program offers alternatives for individual food items on the menu, sorted by goodness of fit. Selectable food items include home-cooked dishes with links to popular recipe sites for detailed preparation instructions. Such computer-based resources will get nutrition professionals themselves acquainted with the use of genetics for personalized nutrition, even as they are providing sound advice to their clients and patients.

Nutritional interventions for weight management based on the genotype

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The human genome sequencing and the emerging knowledge about nutritional genomics and nutriomics are contributing to boost personalized nutrition. The scientific progresses in omics technologies, the improved resources to detect individual genetic diversity as well as the rising demands to explain personal variations concerning nutritional requirements and differential responses to dietary intake are generating new ways for nutritional individualization and dietary optimization based on the genotype. Thus, better outcomes in terms of health care can be reached if nutrient intake is customized for each human being considering not only the personal phenotype or clinical signs and other individual's features such as age, gender, physical activity, allergies/intolerances, food preferences/dislikes and social status, but also assessing the genetic make-up to more precisely tailor dietary advices to own needs. The use of new generation technologies involves the efficient and massive detection of single-nucleotide polymorphisms (SNPs) as well as the identification of novel candidate genes and uncovered mutations putatively implicated in subject´s differences in nutritional requirements, gene-nutrient interactions or dietary recommendations characterizing personalized nutrition. Genome-wide association studies have found a number of genetic variants that are associated with complex diseases including excessive body weight and adiposity traits, which are contributing to better understand the molecular mechanisms involved in obesity. Interestingly, recent investigations have reported genetic SNPs that can be associated with overweight risk in human beings and in ethnic groups or with the role of specific genes in weight loss. As an example, the fat mass and obesity associated gene has an impact in fat deposition because carriers of one or more mutated alleles show a 1.5 higher body weight per allele. Other SNPs in obesity candidate genes e.g. MC4R, TCP7L2, PLIN or TFAP2B may affect weight loss in genetically predisposed subjects, who may show differential inheritance-mediated responses not only depending on energy restriction but also on macronutrient distribution of the hypocaloric diet. The Direct-to-Consumer genetic tests, whose availability and demands are steadily growing, can be considered commonest tools concerning gene screening for personalized nutrition, although ethical/legal issues and interpretation restraints needs to be urgently addressed to help health professionals to provide more personalized nutritional advices, to prescribe sound customized diets and to assist in the transformation of current public health recommendations on individualized principles. Summing up, personalized nutrition has a promising future in health care based on personal phenotype data and genetic characters, with specific benefits for a more focused treatment and prevention of obesity. An integrative view of the human genome together with the expected advances in nutrigenomics/nutrigenetics and in bioinformatics analyses as well as a reduction in the costs of omics analytical technologies will allow a widespread and successful use of these genetic tools when devised for body weight and obesity management within a personalized nutrition perspective.

Consumer acceptance of DNA-based dietary advices: are we there yet? – results from a population survey

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The recent development related to nutrigenetics brought this science to the next level with individuals being able to have rapidly access to their personal genetic information via Direct-to-Consumer tests (DTC-tests) available online or through healthcare professionals. This new genotype-based information could potentially be the premise to newly targeted dietary advices, commonly referred to as DNA-based dietary advices or personalized nutrition. The objectives of the present study were to provide an overall picture of the current situation of the use of nutrigenetic testing in the general population of the province of Quebec (Canada) and to address the perceived limits of nutrigenetics. 2,238 Canadian residents from the province of Quebec (Canada), 18 years of age or older (mean age=38.3±14.9), were recruited via social networks and from the Laval University employee/student lists. They completed an online survey that included 37 questions, most of which were closed-ended questions. Two questions were added for validation purposes. A total of 1535 individuals completed the survey and 110 individuals were excluded for not having answered properly to validation items, bringing the total to 1,425 individuals (252 men (17.7%) and 1,173 women (82.3%)). Using a qualitative approach, common themes were extracted using NVivo software v10.2.0. 90.7% (n=1,292) of participants reported to be ready to follow a personalized diet based on the results of a nutrigenetic test. The remaining 9.3% (n=133) were questioned about reasons why they would not be willing to follow personalized dietary advices based on genetic makeup. The restrictions associated with the diet (25.6%), the fact that they do not want to follow any diet (12.8%), that they already have a suitable diet (9.8%), the pleasure of eating (10.5%), the absence of health problems and illness (8.3%), personal food preferences (8.3%), and the negative impact of diet on psychological aspects such as self-control and guilt (8.3%) were among the answers most often quoted by the participants. Globally, a large proportion of Canadians affirmed to be ready for DNA-based dietary advices. On the other hand, a small proportion of Canadians would not adhere to DNA-based dietary advices mostly because of hedonic reasons. Freedom of choice is one of the key determinants of consumer acceptance of nutrigenetics. Advices should be provided to conform to an individual's food preferences and, consequently, should potentially avoid promoting a restrictive dietary approach.

Modifiable risk: genetics at the intersection of chronic inflammation and cardiovascular disease

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Chronic inflammation is often observed as both a pre-condition and exacerbating agent of metabolic syndrome (MetS) and cardiovascular diseases (CVD). A number of genetic variants, mainly single nucleotide polymorphisms (SNPs) have been identified and are associated with MetS, CVD and their sub-clinical components. In addition, some genetic variants support gene-environment (G×E) interactions where the disease risk conferred by the SNP is dependent upon behavioral or environmental factors. Studies show that genetic variants and markers of chronic inflammation, but data on G×Es are scarce. Thus, our work aims to identify G×E interactions affecting any of a set of 26 different inflammation biomarkers. We attempt this in two ways. One, interrogating over 9000 genetic associations for co-associations to any of 26 inflammation phenotypes and 79 cardiometabolic phenotypes (obesity anthropometrics, glycemic traits, lipids, blood pressure (BP) and CVD) identified a number of loci with associations shared between the two phenotype groups. Clustering of the inflammation biomarkers based upon frequency of co-association with the 79 cardiometabolic phenotypes produced two primary and several secondary groups. One primary group, the TNF-IL6 cluster, is characterized by associations with CVD traits and a paucity of such to anthropometrics, BP and glycemic measures. In contrast, the adiponectin-CRP-fibrinogen cluster, is marked by an abundance of associations with anthropometric and glycemic traits and few CVD co-associations. Associations to blood lipids are common to both these clusters. Genes with variants that drive the co-associations are under investigation for G×E effects. Two, a parallel component to our inflammation G×E discovery involves determining differences in risk allele frequencies for inflammation biomarkers across 26 world populations, by examining data on 189 SNPs in the 1000 Genomes Project. We note that populations with East Asian ancestry have highest added genetic burden of risk of chronic inflammation, and those with ancestry in Europe or the Americas have the most reduced burden of risk, compared to world averages. An important contrast is observed in the comparison of Spain and Japan, two populations at extremes of total genetic risk for chronic inflammation. C-reactive protein and fibrinogen appear to have opposite genetic contributions to the protective and additive risk effects associated with their respective SNPs. Little difference is seen in the same comparison for adiponectin. Identification of populations with a high burden of genetic risk of chronic inflammation, in conjunction with novel G×E interactions described above, can identify both vulnerable groups as well as potential strategies for the targeted reduction of the impact of certain genetic variants on the progression of inflammation-facilitated cardiometabolic dysfunction.

Effective dietary interventions for an aging population

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The proportion of the world population's that is in the older age categories is expanding. As this shift occurs more emphasis needs to be directed at promoting those dietary patterns that are associated with achieving and maintaining optimal health outcomes, healthy body weights and forestalls the onset of chronic diseases. Establishing healthy dietary habits early in life is a critical determinant of subsequent health status. The current dietary recommendations for older adults, for the most part, are consistent with those throughout adulthood. One change that occurs with advancing years is a decline in energy requirements, due in part to a decrease in physical activity and metabolic rate, and an increase in the proportions of fat to lean muscle mass. In contrast, nutrient requirements either remain unchanged or increase. Together these changes can pose a challenge to meeting nutrient requirements. A greater emphasis needs to be placed on choosing nutrient dense foods. This can most efficiently be achieved by focusing on the entire diet rather than individual nutrients. For example, such advise as adhere to a dietary pattern rich in vegetables, fruits, whole grains, legumes (beans), low and fat-free dairy products, fish, poultry and unprocessed lean meats, and unmodified liquid vegetable oils; and limited in free sugars, particularly sugar-sweetened beverages, and solid fats. Critical for long term adherence this approach to dietary guidance is flexible and can be adapted to individuals' personal preferences and traditions. As the trend continues for more food to be prepared and/or consumed outside the home it is important to emphasize the importance of adhering to a healthy dietary pattern where ever food is procured. Increasingly, with respect to older adults, attention needs to be given to monitoring nutrient supplement use, particularly to avoid overconsumption. For this age group current data suggests those who are more likely to use nutrient supplements are those who have dietary and lifestyle characteristics most closely associated with lower rather than higher risk for nutrient insufficiency. As individuals age, in some cases, attention needs to be given to adapting living environments to maintain the ability to acquire and prepare familiar foods. Changes in social situations can alter food intake and should be monitored on a regular basis to assure the maintenance of high diet quality. In the 21st century definitions for old age and expectations for the period of time individuals can remain active, productive and independent continues to expand. There are no indications that a person is too old to derive benefits from maintaining or even improving in their diet quality and other lifestyle behaviors.

Food patterns that are nutrient-rich, sustainable, affordable, and appealing

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The creation of optimal food patterns that best meet health, environmental, and societal constraints demands the creation of new metrics and measures. First, energy density of foods is defined in terms of calories per gram. Second, nutrient density of foods is defined in terms of nutrients per calorie or nutrients per serving. Third, affordability of foods is defined in terms of calories or nutrients per unit cost. The environmental impact of foods has been measured largely in terms of greenhouse gas emissions (GHGEs) and the water footprint associated with production and processing, though additional climate-related measures are rapidly becoming available. Frequency of consumption in the population is one measure of food acceptance. These metrics can be applied to individual foods, to composite meals or menus, or to the total diet. It is vital to note that the FAO definition of sustainable diets includes not only the environmental costs of food production and processing but also the foods' impact on health and the overall economic viability of the food system. Creating personalized diets that are healthy, sustainable, affordable, and appealing needs to meet these often-conflicting demands. First, many nutrient-rich foods carry a higher environmental cost; by contrast some environmentally friendly low-cost foods with low GHGE footprint have minimal nutritional value. Second, empty calories cost less, whereas the more nutrient-rich foods cost more. To complicate matters, grains, fats and sweets taste better and cost less, whereas many of the recommended healthier foods are both less preferred and cost more. Diet optimization techniques have resorted to modeling and linear programming to assess the impact of food choices on diet quality and health. The new nutrition science draws heavily on epidemiology, sociology and economics.

Health effects of consuming farmed salmon raised on different feeding regimes in healthy subjects

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Aquaculture has the potential to reduce pressure on wild fish stocks whilst meeting the dietary needs of the population for omega-3 fatty acids and other key nutrients such as vitamin D. However, due to sustainability considerations farmed fish may have to be raised on diets containing some vegetable oils which may reduce its omega-3 content. We investigated the health effects of consuming Scottish farmed salmon raised on different feeding regimes in healthy subjects. Salmon were grown on feeds containing either high (HPUFA) or more sustainable (SPUFA) levels of omega-3 fatty acids, resulting in an EPA+DHA content of 2.1 or 0.9 g/100 g, respectively. In a randomised parallel controlled trial, 51 subjects consumed either 2 portions/week of HPUFA salmon (n=17), 2 portions/week of SPUFA salmon (n=17) or no additional salmon (n=17) as part of their habitual diet, for 18 weeks. Blood samples were collected at t=0, 9 and 18 weeks of intervention to measure the omega-3 index and markers of lipoprotein metabolism, insulin sensitivity, inflammation, oxidative stress and micronutrient availability. After 18 weeks of intervention, the omega-3 index was significantly higher (>2%) in subjects consuming 2 portions/week of HPUFA or SPUFA salmon, compared with no salmon (both $P<0.05$). The omega-3 index significantly correlated with levels of serum 25OH-vitamin D3 in all subjects ($r=0.33$, $P<0.05$). Plasma triglyceride levels were significantly lower in subjects consuming SPUFA salmon after 18 weeks ($P<0.05$), whereas pulse was significantly lower in subjects consuming HPUFA salmon after 9 weeks ($P<0.01$), compared with no salmon. Consumption of HPUFA and SPUFA salmon did not significantly affect micronutrient status in the subjects. As long as consumers eat 2 portions of oily fish per week, the beneficial effects of consuming salmon grown on traditional high fish meal fish oil feeds, or salmon grown on feeds where part of the fish oil is replaced by vegetable oil, are comparable. Funded by the Scottish Government (RESAS)

Medium-chain saturated fatty acids from dairy affect adipose tissue gene expression profiles

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Dairy products contain large amounts of medium-chain saturated fatty acids (MC-SFA), which have been shown to reduce total body fat. The DairyHealth study studied the long-term effects of milk protein and milk fat with a low or high content of MC-SFA on postprandial lipemia in abdominally obese participants. In this 12 week, randomized, double-blind, diet intervention study, participants consumed 60 g milk protein (whey or casein) and 63 g milk fat (high MC-SFA or low MC-SFA) daily in a 2 by 2 factorial design. After intervention, all diet groups gained weight, but only the low MC-SFA groups had increased total body fat. The high MC-SFA groups were protected against the body fat gain. To explore the molecular mechanisms behind this effect of MC-SFAs on body fat, we used microarrays to measure whole genome gene expression in adipose tissue in a subpopulation of 12 participants (6 of the casein + low MC-SFA group and 6 of the casein + high MC-SFA group) before and after intervention. Gene expression of several selected genes was measured in the full study population using qPCR. High MC-SFA intake resulted in downregulation of gene expression of pathways related to complement system and inflammation, whereas gene expression of pathways related to citric acid cycle, electron transport chain and triglyceride biosynthesis were upregulated. Our results show that MC-SFAs have a marked effect on adipose tissue gene expression. We identified several pathways that are potentially related to the beneficial effects of MC-SFAs on body fat percentage.

Methionine restriction as a healthy ageing strategy, is it safe and plausible in humans?

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Methionine is a highly conserved essential amino acid necessary for normal growth the development in mammals with several important biological roles including protein synthesis, DNA and protein methylation and polyamine biosynthesis. It has been established that methionine restriction extends life in multiple organisms including rodents, drosophila, nematodes and yeast. In rodents, the life extending effects of methionine restriction are independent of any effect of caloric restriction. It has been suggested that low fat vegan diets, which can be low in methionine, may slow human ageing by improving insulin sensitivity and lowing hepatic production of IGF-1. Methionine content tends to be higher in protein rich foods including, flesh foods, egg, specific nuts (e.g. Brazil nuts) and beans (e.g. Soybeans) but is lowest in vegetables and fruits. A methionine restricted dietary pattern for human consumption is therefore rich in fruits and vegetables and contains fewer meat options than a standard Western diet. Diets which are lower in protein have been associated with lower incidence of cancers and overall decreased mortality when compared to higher protein diets in those aged 50-65 years. Here, we describe a novel methionine restricted dietary pattern which has been formulated using whole foods to provide 50% carbohydrate, 15% protein and 35% total fat with <8% saturated fat and 17% monounsaturated fats. We conducted a randomised cross-over design pilot clinical trial (n=20) with 4 week dietary intervention phases to test the safety and feasibility of a methionine restricted diet compared to a standard Western diet. Diets were matched for total energy, dietary fats and fibre. Dietary methionine differed by 40% between diets. Participants were aged 50-70 years old, healthy with no history of serious disease, no history of smoking, not currently consuming a vegetarian or vegan diet and not currently on medications and/or supplements which may alter circulating levels of methionine and B vitamins. Seventeen participants completed the trial with a difference of 46% in dietary methionine intake achieved between diet groups. Compared to the standard Western diet, participants consuming the methionine restricted diet lost weight (-1.956±0.3400 kg; P<0.0001) and had increased circulating levels of serum folate (+5.425±1.438 nM; P=0.0018). Plasma methionine decreased to a greater extent in those consuming the standard Western diet (-4.939±0.9144 μM; P<0.0001) compared to those consuming the methionine restricted diet (-3.827±1.110 μM; P=0.0036). Taken together, these data suggest that a methionine restricted dietary pattern is a plausible nutritional regimen for consumption and that plasma methionine is not an accurate indicator of methionine status in the short term. Further, longer term studies are required to determine whether methionine restriction improves insulin sensitivity and lowers IGF-1 in humans aged 50-65 years.

Metabolomic biomarkers of adherence to Mediterranean Diet in subjects at high cardiovascular risk

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The traditional Mediterranean Diet (MedDiet) is a dietary pattern that in recent years has received an increasing interest due to its beneficial effects in human health and its inverse association with total mortality. The PREDIMED (Prevención con Dieta Mediterránea) study has been a prominent intervention trial that has gathered strong evidence about the beneficial effects of MedDiet not only on primary prevention of cardiovascular disease but also in lowering several cardiovascular risk factors, and these benefits are more pronounced in individuals with a higher adherence to MedDiet. The p14 score measures the adherence of MedDiet and was developed and validated in the PREDIMED study. It measures the compliance of typically contained foods in the MedDiet such as olive oil, fruits and vegetables and nuts among others. The aim of this study was to determine the urinary biomarkers associated to MedDiet adherence measured by the p14 score in individuals at high cardiovascular risk from the PREDIMED study. A total of 161 free-living men (55-80 y) and women (60-80 y), were included. Inclusion criteria were: having diabetes mellitus or having three or more major cardiovascular risk factors. Spot urine samples were collected at baseline and analyzed by an untargeted ^{1}H-NMR based metabolomics approach. The normalized NMR signals were analyzed in a multivariable linear regression model by every one of the 14-items and adjusting for potential confounders. Metabolites were identified by comparison of significant signals in each multivariate analysis with validated ^{1}H-NMR databases as well as existing literature. Metabolites that discriminated between items of the p14 score were identified and the sense of its secretion was observed in accordance with specific food items. In this way, lower values of anserine, carnosine and 3-methylhistidine were observed in individuals with low intake of meat and meat products; whereas higher values of proline-betaine and trigonelline were in accordance with high consumption of fruits and vegetables. Similarly some metabolites derived from microbiota metabolism were found to be related to different food items. Furthermore, other endogenous metabolites such as monoscharides, amino acids and derivatives, intermediary metabolites of TCA, ketone bodies and some minor metabolites of fatty acids suggest a modulatory effect of foods included in MedDiet. In conclusion, the identified metabolites could be proposed as biomarkers of specific foods belonging to MedDiet score and their global profile as the biomarker of the MedDiet adherence.

Identification of late responders to dietary weight reduction and maintenance – a two year study

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Clinical studies suggest that minimal, sustained weight loss of 5% to 10% can reduce or eliminate obesity-related disorders. However, research has shown that weight loss is difficult to achieve, and weight maintenance is even a greater challenge. Weight loss process is characterized by a rapid initial weight loss, reaching maximum weight reduction at the first 6 months, followed by a gradual weight regain. The majority of people fail to lose weight at the first 6 months, defined as unsuccessful and usually are not followed-up (beyond the 6 month period) and thus there is lack of data regarding their weight reduction and maintenance outcomes. Our purpose was to identify factors associated with successful late weight reduction and maintenance among overweight and obese adults who failed to achieve initial weight reduction success. Medical computerized files of 5,254 participants who failed to achieve ≥5% weight reduction after initial 6 months were retrospectively analyzed to identify predictors associated with late successful weight reduction and maintenance (≥5% at first and second years, respectively). Over 40 independent variables were analyzed. Main outcome was percentage of weight change. Significant predictors of late success in weight reduction were; more visits to a dietitian, higher baseline BMI, and any initial weight reduction (0-5%) (OR=3.69, compared with participants who initially gained weight). The use of Insulin (OR=0.499) and the presence of hypertension (OR=0.75) were significantly correlated with failure to reduce weight. Predictors for late maintenance were: more visits to a dietitian, higher baseline BMI, any initial weight reduction, younger age, not being treated with insulin (OR=0.316) and more weighings (OR=1.68). Substantial sub-group of obese and overweight patients reduces weigh at slower rate than the defined success time frame of 6 months. Significant specific predictors were identified. Diabetic and hypertensive patients are in a significant higher risk of failure to reduce and maintain weight. Using regression models, we calculated the probability for successful late weight reduction

Metabolomics to discover and validate biomarkers of protein intake

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Protein rich diets improve body weight regulations and are thus believed to play a role in combating the global obesity epidemic. Meat intake, on the other hand, especially red meat, has been associated with a higher incidence of several chronic diseases, including diabetes and colorectal cancer. These associations are, however, based on observational studies where the dietary assessment tools are far from being robust and objective. We therefore aim to unravel novel urinary biomarkers of meat intake and to validate both general and meat type-specific markers in different controlled and less-controlled study settings. A randomized, controlled, single-blinded, cross-over meal study will be conducted in healthy volunteers. Each subject will be randomized to a sequence of four test meals: white meat (chicken), red meat (beef), pink meat (pork) and iso-caloric control (egg white protein or casein). A standardized dinner will be given prior to the intervention day. Urine samples will be collected at baseline and at 1, 2, 4, 6, 12, 24 and 48 h following the intervention. Through untargeted LC-ESI-qTOF-MS metabolomics and multivariate analysis (PCA, PLS-DA) we will explore the dietary exposures and discriminate the metabolites that may act as biomarkers of intake. These will thereafter be identified and validated in two different studies: (1) PREVIEW[1], a 3 year multicenter intervention trial with up to 2,500 volunteers in 8 different countries, where the focus is to investigate the effects of different types of diets (high/low protein content) on the development of type-2 diabetes, in pre-diabetic overweight subjects and (2) CWS[2], a study in pre- and post-menopausal women where the overall goal is to investigate the effect of physical activity on symptoms related to female health. In both of these studies we will use the previously identified markers for (1) validation in different populations covering sexes, nationalities, age groups, and protein intake levels, and (2) estimate meat intake and meat source at the individual level. Although several compounds have been already proposed as biomarkers for meat intake (e.g. 1- and 3-methylhistidine), they may not specifically differentiate the source or their long-term exposure. Short-term meal studies have been shown as good settings for initial biomarker discovery; however, the applicability of the biomarkers has to be validated in less controlled study settings. We hypothesize that an untargeted metabolomics approach will unravel new biomarkers of general and (animal-) specific protein intake. [1]PREVention of diabetes through lifestyle Intervention and population studies in Europe and around the World, WP1, EU FP7 project. [2]Copenhagen Women Study, WP2, University of Copenhagen-initiated project.

Brazilian healthy eating index score

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Nutrition plays an important role in human metabolism and focuses on preventing disease and maintaining health. Therefore, understanding interactions between genes and nutrition becomes a crucial task. The objectives were to describe data of diet quality from Brazilian children and adolescent's participants in three moments of a study that analyzes the response (genomic, proteomic, lipidomic, metabolomic) to a multivitamin/multimineral supplement in individuals, using a n-of-1 study design and systems nutrition. The study was performed in schools in the outskirts of Ribeirão Preto (São Paulo, Brazil), in two consecutive years (2013-2014), both with identical methodology and with repeated or new participants. It was used three 24-hour recalls in subjects from 9 to 13 years old to calculate the scores of the Revised Brazilian Healthy Eating Index (BHEI-R). The study design is a crossover N-of-1 intervention study trial based on the determination of OMICS at three times: at baseline, after 6 weeks of supplementation of micronutrients and after 6 weeks without supplementation. The BHEI-R, adapted from the Health Eating Index 2005, was used to reflect global quality of diets through adequacy, moderation and variety. The scores assigned to each of the twelve food components are summed together and the total rate classifies the quality of the diet. The maximum amount reached by the BHEI-R is 100 points (scores <51 = "inadequate diet;" scores between 51 and 80 = "diet requiring modifications;" and scores >80 ="healthy diet). Mann-Whitney test, and ANCOVA analyzes, adjusting for age, pubertal status and gender, were used. Health eating index scores were derived from 24-hour recalls. Due to outliers related to underreporters and overreporters, the participants who ate lower than 0.79× basal metabolic rate and higher than 2.4× basal metabolic rate were excluded from this analysis. The same patterns were found for food choices between the two years. In 2013, n=89 and 2014, n=79. ANOVA, P>0.05 for all parameters. Total BHEI-R indicates inadequate diets in both years. 2013: 49.33 (37.33-75); 2014: 48.33 (36.33-75.67) (P=0.59). The quality of the diet of children and adolescents studied showed inadequate fruits, vegetables, legumes and milk intake compromising their future health. Therefore, an individualized dietary intervention based on nutritional needs can be a very useful tool for preventing or treating chronic diseases. This study has been sponsored by Nestlé Institute of Health Science.

Effect of plant-based diets on inflammatory profiles: a systematic review and meta-analysis

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Plant-based diets characterized by high consumption of monounsaturated fatty acids, vegetables, fruits, wholegrain cereals, legumes, low-fat dairy products, fish and moderate alcohol intake have been widely reported to be associated with an improved inflammatory profile, thereby reducing cause-specific and overall mortality. Inflammatory biomarkers may act as mediators between dietary types and metabolic disease risk. We therefore aimed to evaluate the effect of plant-based dietary interventions on adiponectin as a robust marker of insulin sensitivity, inflammation and adipocyte function. We were particularly interested to evaluate whether any potential effects would be independent of changes in weight status. A literature search was conducted using databases such as Medline and Embase in order to identify intervention trials investigating the effect of plant-based diets on adiponectin concentrations in adults. Inclusion criteria were: plant-based dietary intervention trials, age >18 years, and availability of information on adiponectin measurements in the intervention and control groups. Overall 7 intervention trials published until June 20th 2015 were found eligible for the meta-analysis. Pooled estimates of effect size using a random effects model as weighted mean differences (WMDs) and 95% confidence intervals (Cis) for the effects of plant-based diets versus control intervention diets were estimated. The between-study heterogeneity was evaluated based on I^2 statistics. Plant-based diets in comparison to control diets were associated with a modest, albeit statistically significant increase in adiponectin concentrations (pooled WMD estimate: 1.24 (95% CI -0.38-2.11) µg/ml). However, a high degree of heterogeneity among studies included in the meta-analysis was observed (I^2=96.3% [94.3%; 97.6%]). As important sources of heterogeneity were identified studies with short intervention periods. When the meta-analysis was conducted among studies with an intervention period of more than 1 year (n=3), the heterogeneity was diminished (pooled WMD: 2.10 [95%CI: 1.95-2.25]; I^2=14.6% [0%; 91.1%]). In order to evaluate whether the observed effects were independent of weight status, we restricted the meta-analysis to studies that reported control for changes in weight (n=3). In this analysis, the effect of plant-based diet on adiponectin concentrations was no longer statistically significant (pooled WMD: 1.10 [-0.93-3.13]; I^2=42.9% [0%; 82.8%]. The present meta-analysis of intervention trials suggests that plant-based diets moderately increase circulating concentrations of adiponectin supporting benefits of diet on inflammation and adipocyte function. Whether increase in adiponectin is independent of effects of plant-based diet in weight reduction merits further investigation. Further randomized control trials with longer intervention periods and careful evaluation of weight changes over time are warranted in the future in order to confirm these findings.

Lipidomic evaluation of lipid mediators in a healthy population fed with farmed salmon

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Consumption of fish and its omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) protect against stroke and lowers the risk of mortality from coronary heart disease. Omega-3 and omega-6 fatty acids form a wide variety of oxidised products that can be beneficial or detrimental to health. In this study, we investigated the effects of salmon consumption on levels of fatty acids and novel lipid mediators derived from omega-3 and omega-6 fatty acids in healthy subjects. Salmon were grown on feeds containing either high (HPUFA) or more sustainable (SPUFA) levels of omega-3 fatty acids, resulting in an EPA+DHA content of 2.1 or 0.9 g/100 g, respectively. In a randomised parallel controlled trial, 51 healthy subjects consumed 2 portions/week of HPUFA salmon (n=17), 2 portions/week of SPUFA salmon (n=17) or no additional salmon (n=17) as part of their habitual diet, for 18 weeks. Blood samples were collected at t=0 and 18 weeks of intervention to measure red blood cell fatty acids and urinary profiles of bioactive lipid mediators mainly generated enzymatically including hydroxyeicosatetraenoic acid products (HETEs), hydroxydocosahexaenoic acid products (HDoHEs) and neuroprotectin, and non-enzymatically including isoprostanes (IsoP), phytoprostane (PhytoP), isofurans (IsoF) and phytofuran (PhytoF). Analyses were performed on GC-MS, GC-MS/MS and LC-MS/MS lipidomics platforms. After 18 weeks of intervention, the omega-3 index was significantly higher (>2%) in subjects consuming 2 portions/week of HPUFA or SPUFA salmon, compared with no salmon (both $P<0.05$), whereas levels of linoleic acid and arachidonic acid were significantly lower ($P<0.05$). Urinary levels of 9(S)-HETE, a monohydroxy fatty acid produced by the enzymatic oxidation of arachidonic acid, were significantly increased in subjects consuming SPUFA salmon, compared with no salmon. Levels of two non-enzymatically produced lipid mediators from arachidonic acid, 12-F_{2t}-IsoP and 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP were more decreased after consumption of HPUFA salmon compared with consumption of SPUFA salmon. Lipid mediators of α-linolenic acid (9-L_1-PhytoP, 16-F_1-PhytoP and ent-16-(RS)-13-epi-ST-δ14-9-PhytoF) were lower after consumption of HPUFA and SPUFA compared with no salmon. Consumption of two portions of salmon per week for 18 weeks resulted in significantly higher levels of EPA and DHA in red blood cell membranes at the cost of linoleic and arachidonic acid. This effect appears to reduce oxidative stress but also modified levels of omega-3 lipid mediators derived from α-linolenic acid without altering those derived from EPA and DHA.

Integration of biochemical markers and metabolomics for selecting responders in intervention study

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Novel omics technologies provide enormous potential to develop personalized nutrition strategies by allowing us to recognize and embrace the differences of individuals' responses to various factors. Among above technologies, the data obtained from metabolomics can be integrated with conventional health-related markers to identify possible responders so as to prognosticate our capacity to respond to a nutritional intervention or to cope with physiological stresses. However, recent human intervention studies have provided some insights into prediction of food consumption, but not yet of intervention-induced changes in the phenotype and function. Therefore, the present study attempted to explore a concept for predicting physiological functions in response to intervention by integrating biochemical markers to metabolomics profiling. This study was a part of a human nutritional intervention study, which was investigated the antioxidant and anti-inflammatory properties of black raspberries and registered at the WHO International Clinical Trials Registry Platform (No. KCT0000644). In a four-week, randomized, double-blind, placebo-controlled, parallel-design study, sixty-seven sedentary and overweight/obese adults (23≤body mass index (kg/m^2) <30) were recruited and randomly allocated to Korean or North American black raspberries, or placebo. ^{1}H nuclear magnetic resonance-based metabolome profiling in blood and urine were applied. Korean black raspberry (KBR) consumption significantly improved oxidative stress and inflammation status than North American black raspberry consumption. After KBR consumption, eighteen metabolites significantly responded and seven metabolites were associated with biochemical markers. A two-metabolite set (glycine and N-phenylacetylglycine) had the strongest prognostic power to screen possible responders against oxidative stress and low-grade inflammation (the area under the ROC curve=0.778). These findings help propose the most likely prognostic markers for selecting favorable responders to intervention in monitoring individual antioxidant and anti-inflammatory capacity.
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Integrated mass spectrometry and multivariate modelling to validate food-derived urinary biomarkers

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Accurate measurement of habitual food intake, which is an essential component of much health-related research, is challenging. Dietary data recorded by self-assessment tools, such as Food Frequency Questionnaires (FFQs) and diet diaries, can be subject to participant bias. Several reports have described the analysis, in human biofluids, of specific metabolites known to be present in individual foods. Such chemicals could potentially provide direct biomarkers of dietary exposure, however, the biotransformation of many dietary metabolites is often complex and to date putative biochemical markers are available for only a relatively small number of specific foods and food components. The M.A.I.N. (Metabolomics at Aberystwyth, Imperial and Newcastle) Study is using a non-targeted metabolomic approach to discover and validate new and existing urine biomarkers indicative of exposure to different foods, which will ultimately be integrated into a diagnostic population screening method in order to objectively measure food exposure. Fasting and post-prandial spot urine samples were collected after participants consumed specific food and drinks in their habitual settings over a three day period. Urine collections were investigated with a range of metabolomic techniques, starting with Flow Infusion-High Resolution Fingerprinting (FIE-HRMS) using Orbitrap Mass Spectrometry (MS) coupled with multivariate classification and feature selection using Random Forest, to evaluate the richness of the urine types as well as the likelihood of biomarker discovery. Multivariate analysis of such high mass resolution data is computationally intensive, therefore all metabolomics workflows were fully integrated with a High Performance Computer providing the ability for more in-depth modelling, quicker processing times and robust validation of processing/model parameters. Ultra High Performance Liquid Chromatography-High Resolution MS (UHPLC-HRMS) using both Reverse Phase C18 and Hydophillic Interaction Chromatography (HILIC), together with tandem MS were used to further elucidate potential biomarkers and to validate pre-existing biomarkers. The combination of chromatography, accurate mass and tandem mass spectrometry (MSn) allowed us to accurately elucidate potential biomarkers after modelling, without the needed for extensive targeted studies. We are currently validating these biomarkers by quantification, using chemical standards where possible, in biofluid samples obtained from controlled clinical studies and free-living habitual individuals taking part in independent cohort studies.

Relationship between nutrient supplementation, inflamatory profile and genetic damage in obese women

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Obesity is a multifactorial disease including complexes interactions between genetic and environmental factors, and it is associated with increased risk for metabolic syndrome, type 2 diabetes mellitus and heart diseases. This study aimed to assess the relationship between micronutrient intake, genetic damage and cytokines blood concentration in morbid obese women (n=30; BMI=45.89±6.8) compared to healthy eutrophic women (BMI=21.56±1.55). The supplementation consisted of two DRI (Dietary References Intakes) per day, during 8 weeks before and 24 weeks after bariatric surgery. Obese women presented improvement of folic acid (7.73±2.0 vs 13.9±6.98), vitamin E (14.0±3.22 vs 16.84±3.65) and B12 (226.04±79.67 vs 320.96±271.2) 24 weeks after bariatric surgery. No difference was detected for other micronutrients such as vitamins A and C, selenium, iron and zinc after bariatric surgery. The comet and micronucleus assays were used to assess, respectively, DNA and cytogenetic damage in PBMCs. Obese women presented significantly higher amount of DNA damage (53.40±22.19) than the eutrophic (22.2±19.7) subjects. However, after micronutrient supplementation there was a decrease of DNA damage ($P<0.05$) in obese women, but the amount continued different from control. Same result was observed in the micronucleus test. Significant ($P<0.05$) decrease of cytokines (IFN-γ, IL-8, e TNF-α) was detected in obese women after surgery and micronutrients supplementation. In conclusion, our data demonstrated that micronutrient supplementation may decrease genetic damage in morbid obese women.

Biomarkers of nuts exposure: diet and host-microbial interaction in people with cardiometabolic risk

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Tree nuts have a recognized role in the improvement of components of the metabolic syndrome (MetS), but the mechanisms implied are not fully characterized. Part of these effects are recently ascribed to the metabolism of polyphenols highly abundant in nuts, which in turn necessarily require a functional diet and host-microbial interaction. Through a LC-ESI-qToF-MS-driven untargeted metabolomic approach, we first identified the most discriminant biomarkers of nuts exposure in 12-week mixed nuts intervention study (30 g/d) involving subjects with MetS. Urolithin A glucuronide, namely a product of the gut microbiota-host cometabolism of ellagitannins contained in walnuts, was the most discriminative dietary biomarker (nivel I identification; ROC AUC=89.6% (80.8-98.4)]) despite the inter-individual variation expected. Plasma levels of urolithin A glucuronide also showed a significant inverse correlation with abdominal adiposity (waist circumference: r=-0.550; waist-hip ratio: r=-0.409) and impaired glycemic control (FI: r=-0.414; HOMA-IR: r=-0.417) of the subjects at baseline, while positively associated with the reduction of adiposity following nuts intake. These data suggested the presence of a more urolithin prone-to-produce microbiota, and therefore a higher exposure to bioactive metabolites, in subjects with less severe MetS traits known to be associated with gut microbial dysbiosis. The generated hypothesis were verified by independently applying a similar metabolomics approach to a subcohort of the epidemiologic InChianti study (n=191, M/F, ≥65 years), where subjects differed for the frequency of nut intake [stratified into No Consumers (n=72), Sporadic Consumers (<2.9 g/d, n=72), Weekly Consumers (≥2.9 g/d, n=47)] but also for the metabolic health (38.22% MetS incidence, equally distributed among the intake groups). Significant changes in circulating medium-chain dicarboxylic acids (nivel I identification), recognized as alternative energy substrates particularly relevant in the case of glycemic control impairment, were also associated with nut consumption, in both studies, giving a striking example of unexplored metabolic mechanisms potentially implied in their health effects. This work confirmed how untargeted metabolomics is a valuable tool for identifying new biomarkers of exposure and for linking exposure to clinical effects, and stressed the role of a healthy gut microbial community in modulating the production and bioavailability of healthy bioactives from dietary polyphenols.

Effect of caffeine on attention and alertness measured in a home-setting, using cognition tests

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There is an increasing interest among nutritional researchers to perform web-based intervention studies instead of testing subjects in a clinical setting. Many tests for measuring particular parameters for nutritional studies are commonly available in drug stores as well as online. Here we present such a study, in which we reproduced the effect of caffeine on attention and alertness in an at-home setting. The study is aimed to reproduce the effect of caffeine on attention and alertness using a web-based study environment of subjects at home. Study design: The study is designed as a randomized placebo-controlled double blind cross-over study. Healthy volunteers consumed a cup of coffee after an overnight fast. Coffee was prepared from a sachet containing either regular coffee or decaf coffee. Each intervention was given twice. Before and one hour after coffee consumption subjects performed on-line cognitive tests at home which measured alertness and attention, established by three computerized tests provided by Quantified Mind. Each test was performed for 5 minutes. Recruitment of subjects via internet was fast and efficient. Within two weeks about 100 subjects applied of whom 70 were eligible. Of these 70 subjects, 53 complete test sessions were obtained, indicating that they were able to perform the Do It Yourself tests at home correctly. The three cognition tests conducted at home showed the same improvement in cognitive performance with caffeine as found in controlled studies in a metabolic ward. The study showed that the effects of caffeine consumption on a cognition test in an at-home setting revealed similar results as in a controlled setting. This type of study is a fast and easy way to demonstrate effectiveness of a supplement and may therefore be interesting to evaluate life-style and nutritional interventions, e.g. by food industry.

High intake of diet antioxidants reduces cardiovascular risk

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The cardiovascular diseases (CVD) are the leading causes of death according the recent WHO data, and the dietary intervention demonstrated the efficacy to protect against CVD risk factors. This study, part of European study ATHENA, aimed to study the beneficial effects of antioxidants on human health. We investigated the dietary intake of anthocyanin, vitamin C, vitamin E and beta-carotene in correlation to the main cardiovascular risk parameters (BMI, waist circumference, cholesterol LDL and HDL, LDL/HDL ratio, triglycerides) in 493 Caucasian subjects divided in two groups: Group 1 (low antioxidants intake) vs Group 2 (high antioxidants intake). The analysis showed that BMI, waist circumference, cholesterol LDL and LDL/HDL ratio were lower in Group 2 vs Group 1. Multiple linear regressions demonstrated that a high vitamin C intake, in the Group 2 was associated to lower LDL (P=0.0015), and a high beta-carotene intake correlated with higher HDL (P=0.026). The consumption of both nutrients correlated with a lower LDL/HDL ratio, waist circumference, and triglycerides (P=0.005, P=0.033, P=0.037 respectively). Furthermore, high anthocyanin consumption was correlated with lower triglycerides and waist circumference (P=0.01). However, when three nutrients were analyzed together, was observed only the correlation with lower BMI also (P=0.024). No evidences were found for the vitamin E. In conclusion, a diet with high intake of antioxidants is linked to a reduced CVD risk parameters, with major influence of vitamin C and beta-carotene.

Multiple saliva sampling and metabolome recognition accuracy after standardized lifestyle

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Different nutritional habits are reflected in the small-molecule composition of body fluids. Urine contains a clear individual metabolic signature although embedded within a large daily variability. Given the potential of metabolomics to monitor disease onset from deviations from the 'healthy' metabolic state, we have reported on the effectiveness of a standardized lifestyle in reducing the 'metabolic' noise using daily multiple urine sampling, which had to be confirmed for saliva. We analysed the impact of the total number of saliva samples on the recognition accuracy and report on the extent to which the saliva metabolome can be normalized by our standardized lifestyle/dietary protocols. Design. Saliva was collected from 24 (5 men, 19 women) healthy volunteers over a period of 10 days: phase I, day 1-7 in a real life situation; phase II, day 8-10 in a standardized diet and day 10 plus exercise program. Data on dietary intake and physical activity have been analysed by a nation specific software and monitored by published protocols. Saliva samples have been analyzed by 1H NMR followed by multivariate statistics. The individual fingerprint emerged and consolidated with increasing the number of samples and reaches ~100% cross-validated accuracy for about 40 samples. The main results of the fingerprinting approach are: individual recognition is very good, although slightly less accurate than that obtained from the urine samples. As in the case of urine, also for saliva the first collection of the day differs significantly from samples collected at different times during the day. This type of behavior identifies a circadian behavior also for saliva. The differences between phase I and the standardized phase are smaller than in the case of urine. This finding demonstrates that urine is more sensitive than saliva to diet. Gender discrimination is very poor. The metabolites that mainly contribute to the individual recognition are: beta-hidroxybutyrate, propionate, isobutyrate, acetate. Almost all metabolites identified in the analysis are present in statistically different amounts (either higher or lower) in the first sample of the day, with respect to the other samples of the day (P-values<0.005). Exceptions are ethanol, methanol, histidine, TMAO, isopropanol. Saliva contains a well-defined individual signature and appears to be little affected by changes in lifestyle such as diet and physical exercise. Due to the stability of its metabolic profile, saliva is in principle suitable for biomedical studies aimed at disease diagnosis, prognosis and evaluation of medical intervention.

A metabolomics approach to discover dietary biomarkers in human urine from a free-living population

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Dietary exposure and nutritional status have a huge impact on the health and well-being of individuals and populations. Obtaining objective, self-reported dietary intake data from individuals is challenging because of the complexity of dietary patterns, the limitations of conventional dietary assessment tools (e.g. Food Frequency Questionnaires), bias and mis-reporting which hinders research efforts to link specific foods to health outcomes. The M.A.I.N. (Metabolomics at Aberystwyth, Imperial and Newcastle) Study aims to develop and validate an objective approach to measuring food intake based on a simplified, robust, diagnostic population screening method using a combination of a panel of known and newly validated urinary biomarkers and machine learning technology. Thirty-six healthy adults (15 males) aged 19-77 years with a mean BMI of 23.7 (SD 3.15) kg/m^2 were recruited. Foods most commonly eaten by the British population were identified and organised into a three-day menu plan; several foods appeared twice or three times, but in different formulations. To assess the effect of timing of food consumption, participants were randomised to one of 12 3×3 Latin squares. Participants were given the food and drinks to consume at home over a three day period with an additional 'unhealthy' meal provided at the beginning of the study. Participants collected urine samples at pre-determined and spot times to assess the most data rich, and reproducible, urine sample type and the urine sampling time which caused least disruption to volunteers' daily activities. There was 78-100% compliance with menu items across the study. Participants were most compliant with collection of first morning void (FMV) urines and least compliant with the collection of fasting urine samples (96% and 81% samples returned respectively). Overall, high dietary compliance suggests the study design was robust and participants found that FMV urines were easiest to collect routinely. Using Flow Infusion-High Resolution Fingerprinting (FIE-HRMS) metabolite fingerprinting, FMV urines proved to be a data-rich urine sample type. Urine collections are now being investigated with a combination of targeted and non-targeted metabolomic approaches to capture and quantify biomarkers of foods and drinks associated with both healthy and unhealthy dietary choices. Work is also ongoing to refine urine sampling protocols which are acceptable and reliable for participant self-collection in the community and which would be feasible for implementation in large-scale studies and surveys. Funded by the UK Medical Research Council (Grant No. MR/J010308/1).

Systems health: refined phenotyping and new molecular biomarkers

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At the Nestlé Institute of Health Sciences we are developing a systems biology approach to better understand the interplay between genes, diet and lifestyle so that we can act upon healthy ageing and disease prevention with a focus on metabolic, cognitive and intestinal health. Metabolic phenotypes and nutritional interventions are characterized in human clinical studies, which are not only performed in the classical case/ control- but also in the longitudinal design, incorporating challenges to homeostasis. Mainly deep sequencing-based functional genomics and nuclear magnetic resonance- (NMR-) plus mass spectrometry-based proteomics and metabonomics as well as micronutrient analysis are delivering holistic mechanistic insights into host and gut microbial metabolism across life span. Ultimately, we translate this basic science into personalized solutions at the interface between food, pharma and diagnostics. My group, the Molecular Biomarkers Core, has meanwhile established an integrated laboratory combining highly versatile and flexible biomarker/mechanism discovery/validation platforms like mass spectrometry with highly sensitive, targeted diagnostics based on e.g. cooperative ELISAs. We complement this by partnering with external expertise in e.g. DNA aptamer-based screening. We term this 'molecular phenotyping' as an in-depth complement to classical phenotyping such as body composition and blood chemistry. This approach generates the necessary systems view on metabolic trajectories of natural human cell lines, animal models and, most importantly, human subjects. The emphasis lies on 'trajectory' as we monitor these biological systems over time, with every entity functioning as its own case/ control pair, rather than comparing a case to a control group by taking omics snapshots at a given moment in time. A further, innovative angle of our research into metabolic, cognitive and intestinal health is to assess metabolic elasticity and flexibility rather than comparing systems at homeostasis: challenging biological systems repeatedly over time and monitoring their (failure of) restoration of homeostasis is giving us early insights into deviations from healthy metabolic trajectories and opens windows of preventive opportunity. In a nutshell, my Biomarkers Core fulfils three main roles: omics technology development and leverage; molecular phenotyping in clinical studies; and driving research projects in gastrointestinal, metabolic and brain health plus healthy ageing. We are doing this at both wet- and dry-lab level, i.e. based on our expertise in bioanalytical chemistry, instrumentation, and chemometrics/ biomathematics/ bioinformatics.

Interaction of riboflavin with MTHFR genotype in relation to hypertension

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Hypertension (i.e. systolic/diastolic blood pressure of 140/90 mmHg or greater) affects ~1 billion adults globally, and is the leading cause of mortality worldwide. It carries a 3-fold increased risk of developing cardiovascular disease (CVD), while effective treatment of hypertension is proven to reduce CVD events, particularly stroke. Apart from the known nutrition and lifestyle factors involved, genetic factors are considered important in the development and progression of hypertension. Recent genome-wide association studies (GWAS), one of which included data from almost 35,000 individuals, identified the gene encoding the folate-metabolising enzyme, methylenetetrahydrofolate reductase (MTHFR) among 8 loci associated with blood pressure. Consistent with GWAS evidence implicating the MTHFR gene, recent meta-analyses of observational studies show an increased risk of hypertension in individuals homozygous for the 677C→T polymorphism in MTHFR. The frequency of the homozygous MTHFR 677TT genotype is reported to be 10% worldwide, but can be as high as 20% (Northern China) to 32% (Mexico). Riboflavin (vitamin B2) in the form of FAD acts as a cofactor for MTHFR and we have been studying its modulating role in relation to this polymorphism. The variant enzyme is known from molecular studies to become inactive as a result of having an increased propensity to dissociate from FAD, but our earlier work suggested that supplementation with low-dose riboflavin could stabilise MTHFR activity in vivo in homozygous individuals. In recent years we showed that CVD patients with the relevant MTHFR 677TT genotype (compared to CC or CT genotypes) had significantly higher blood pressure, and that blood pressure was highly responsive to riboflavin intervention, specifically in the TT genotype group. Further investigations confirmed this gene-nutrient interaction in hypertensive patients (with and without overt CVD), and furthermore showed that the blood pressure lowering effect of riboflavin in the TT genotype group was independent of the number and type of antihypertensive drugs that they were taking. Our evidence to date suggests that the blood pressure phenotype is modifiable by restoring the activity of the variant MTHFR enzyme through improving riboflavin status. Thus riboflavin, targeted specifically at individuals with the MTHFR 677TT genotype, may offer a personalised non-drug treatment, or preventative strategy, for hypertension.

A randomized controlled trial of a gluten-free weight loss diet in HLA DQ2 or D8 positive subjects

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We know that the vast majority of people with biopsy-proven celiac disease carry either HLA-DQ2 or HLA-DQ8 genes, but these 'celiac disease genes' appear in about 35-40% of the overall population. Having the genes does not mean necessarily they will develop celiac disease, it simply means they have a genetic potential to do so. Some observations have reported that the presence of DQ2 or DQ8 may be linked to weight, irrespective of the celiac disease status. The HLA DQ2 and DQ8 proteins are involved in presenting gluten derived peptides to the immune system. We analyzed 221 patients who were genotyped with a nutrigenetic panel, including the HLA genes (Eurogenetica – NutriGene+). Of a total of 221 patients, 215 were overweight or obese and 69 patients were found to have at least one allele for DQ2 or DQ8. Of these, 64 were overweight and were randomly divided into two groups. Both groups were prescribed a 1,800 kcal/day diet and one group was gluten free. Patients were monitored, both weight and BIA, at one month, 3 months and 6 months. The differences between both groups were significant at 3 months and at 6 months, and the gluten-free group showed a significant greater weight loss compared to patients with gluten included in the diet (at 3 m: 14 vs 8% and 6 m: 25 vs 14%). Also the rate of weight loss over the 6 months period was significantly greater in the gluten free group. In conclusion, a gluten-free diet in patients with at least one risk allele for gluten sensitivity correlated to a significantly higher weight loss in patients with weight management problems than a standard diet.

Glucokinase polymorphism interacts with low-fat dairy to influence to glucose-insulin homeostasis

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Meta-analyses studies have associated the consumption of high-fat (HF) and low-fat (LF) dairy products to improvements in glycaemia as well as risk factors associated to type 2 diabetes (T2D). However, a recent review of dairy product intervention studies on insulin sensitivity demonstrated mixed results: 4 studies showing improved insulin sensitivity, 1 showing worsened values, and 5 showing no effect. This may be partly due to genetic variability in the population studied. Glucokinase (GCK) is a key regulator of glucose disposal and storage in both liver and pancreatic beta-cells, and responds to increases in circulating glucose concentration by initiating a signalling cascade that results in insulin secretion. Studies have associated single-nucleotide polymorphisms (SNPs) in GCK gene with impaired glucose regulation and increased risk of T2D. The objective of the study is to investigate the gene–diet interaction effects between SNPs within GCK gene and dairy product consumption on variables related to glucose-insulin homeostasis. Materials and Dietary data using a validated food frequency questionnaire together with fasting blood samples were collected from 210 healthy French Canadians. Dairy products were evaluated as HF (>2%) and LF (<2%) servings per day, then dichotomized into high- and low- intake based on population median. Insulin resistance was calculated using the homeostatic model of the assessment of insulin resistance (HOMA-IR). Thirteen SNPs covering 86% of the known genetic variability within GCK gene were genotyped using TAQMAN methodology. More than one-third (42%) of individuals did not meet the minimum recommendations for dairy intake from Canada's Food Guide (<2 servings of dairy products/day). LF dairy intakes were inversely correlated with fasting plasma glucose level (r=-0.1985, P=0.0042; r=-0.1957, P=0.0048, respectively), adjusted for age, sex and BMI. No correlations were observed between dairy intakes and plasma insulin or HOMA-IR levels. The ANOVA model was used to test for the effects of the GCK genotypes, dairy intake, and the genotypes by dairy intake interaction on glycemic parameters, adjusted for age, sex and BMI. No interaction effects were observed with HF dairy products. We identified a significant interaction between the rs758989 with LF dairy intake on HOMA-IR (P interaction=0.0006). Specifically, homozygotes of the A allele of rs758989 together with LF dairy products (<1.22 portions/day) had a higher HOMA-IR compared with other genotypes or high dairy consumers. These results indicate that the intake of LF dairy products may influence glucose-insulin homeostasis in individuals with specific SNPs related to the risk factors of T2D. Replication studies are needed. Grant: CIHR (MOP229488) and FRQ-S.

MTHFR C677T polymorphism affects normotensive diastolic blood pressure independently of blood lipids

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Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism was found to be associated with hypertension. High blood pressure (BP) is a major risk factor for cardiovascular disease, gestational hypertension and high risk pregnancy. BP is a complex trait strongly associated with blood lipid parameters. However, studies of the effect of MTHFR C677T polymorphism on BP levels independently of blood lipids are scarce. Our objective was to analyze and quantify the effect of MTHFR C677T polymorphism on normotensive BP independently of blood lipids. MTHFR C677T genotyping was done for 151 Israeli women attending the genetics clinic at Soroka Medical Center. Biochemical (blood lipids) and BP data were extracted from Medical Center records. BP was regarded as a continuous parameter using Analysis of Covariance (ANCOVA) and post hoc Tukey's HSD analysis. The frequencies of genotypes CC, TT and CT were 41, 12 and 47%, respectively. A significant ($P<0.0001$) association was found between genotype and diastolic BP (DBP) when adjusted to BMI and age. Mean DBP was significantly lower for CC than for TT genotypes (71.2 vs 78.7 mmHg), however the difference between the heterozygotes (73.9 mmHg) and the other two genotypes was not significant. Cholesterol, LDLcalc and homocysteine blood levels significantly contributed to the effect of MTHFR C677T polymorphism on the DBP trait. There was also significant association between genotype and folic acid levels. MTHFR C677T polymorphism significantly affects DBP in Israeli women, independently of blood lipids. Each C to T substitution is associated with a mean 3.4 mmHg increase in DBP.

Association of BDNF val66met polymorphism with obesity and type 2 diabetes in Sri Lankans

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Brain derived neurotrophic factor (BDNF) is a key player in the pathways controlling energy homeostasis and so is of great interest in the study of feeding behaviour and obesity. Depletion of BDNF levels is associated with increased food intake, weight gain, hyperinsulinaemia and hyperglycaemia. BDNF levels are lower in obesity, type 2 diabetes and metabolic syndrome. The BDNF rs6265 SNP results from a valine to methionine substitution. The variant T (Met) allele results in reduced BDNF production. The association between BDNF rs6265 with obesity and diabetes has been reported in a number of ethnic groups, with mixed results. The role of this polymorphism in obesity and diabetes in Sri Lankan populations is unreported to date. We conducted a population based study to investigate any association of BDNF rs6265 with obesity measures of BMI, WC and WHR as well as type 2 diabetes risk in Sri Lankans representative of the adult general population. 535 subjects (age 18-70 years) were recruited through multi-stage random sampling. DNA was genotyped by Taqman assays. A dominant model of inheritance was assumed (wild type CC vs TT+CT genotypes) due to the low number of homozygous variant allele carriers. Multiple regression was used to study the association between genotype and linear obesity measures; logistic regression was used to study association with diabetes risk. The mean BMI values for the study population were 24.7±3.8 kg/m^2 for males and 25.1±4.3 kg/m^2 for females. Females represented 60.9% and diabetics represented 17.4% of the total population. BDNF SNP was associated with BMI in males (B(SE)=1.19(0.56), P=0.035) with CC genotypes carriers recording higher mean BMI compared to the variant allele (CT+TT) genotype carriers. In females the association was marginally insignificant with risk genotype carriers recording higher BMI values compared to the CC carriers (P=0.07). No significant associations were found for WC or WHR measures. The presence of variant genotypes (CT+TT) was associated with a lower risk of type 2 diabetes (OR 0.41 95% CI (0.18-0.97), P=0.04) in males. This association was not observed following adjustment for BMI (P=0.07). No association with diabetes risk was observed in females. Our findings suggest a potential influence of the BDNF rs6265 polymorphism in the development of obesity in Sri Lankans. The strength and direction of the association with BMI was gender dependent with the variant allele carriers having lower BMI in males and paradoxically higher BMI in females. Though the reason for this gender specific association is not immediately clear from our study, the potential interactions of the variant allele with dietary (e.g. fat intake) or environmental factors (e.g smoking) is possible and should be the focus of future studies. Replication in a larger cohort would consolidate our findings to better explain the association between BDNF and type 2 diabetes in Sri Lankans.

Metabolites of one-carbon metabolism: beneficial effects of the Mediterranean diet – SNPs associations

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One-carbon metabolism (OCM) pathway interconnects DNA methylation and DNA synthesis, which are critical biological processes that prevent carcinogenesis. In addition, since a number of OCM-related metabolites are known antioxidants, the OCM pathway plays a key role in the protection against oxidative stress. Furthermore, both OCM and oxidative stress are important pathways for studying gene-diet interactions and also evaluating dietary effects. A case-control study of Cypriot women (MASTOS) has shown that the Mediterranean diet (MD) can decrease breast cancer (BC) risk. The prominent molecular mechanisms, however, through which the MD influences BC risk, remain to be elucidated. We previously showed that single nucleotide polymorphisms (SNPs) found in two OCM genes (MTHFR 677C>T, rs1801133; 1298A>C, rs1801131 and MTR 2756A>G, rs1805087) and in two oxidative stress-related genes (MnSOD p.Val16Ala, rs4880 and CAT -262C>T, rs1001179), could modulate the association between BC and MD. Here, we have employed a rapid ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method to determine the levels of a range of OCM-related metabolites in the serum of healthy Cypriot women. The aim was to further investigate the association between SNPs, MD and BC and thus, to gain further insights into the underlying molecular mechanisms. Serum samples from the MASTOS study were divided into two groups (low and high) based on their adherence to the MD, which was previously determined as a dietary pattern rich in vegetables, fruit, legumes, fish and olive oil. Three hundred thirty nine (339) samples in total were examined and the levels of eight OCM-related metabolites were determined using external calibration curves. UPLC-MS/MS analysis was performed using an Acquity I-Class system coupled to a Xevo-TQD MS system (Waters, UK). Adjusted coefficient values were determined by linear regression analysis. Women who carried the MnSOD Val/Val alleles and had a high adherence to MD were found to have statistically significant higher levels of riboflavin, S-adenosyl-homocysteine and methionine, and lower levels of cystathionine, compared to those with a low adherence. Moreover, MTR 2756AA carriers with a high adherence to MD presented significantly lower levels of S-adenosyl-homocysteine, cysteine and glu-cysteine. Further, carriers of the CAT -262CC alleles with a high adherence to MD were also found to exhibit significantly lower levels of glu-cysteine. We conclude that the observed differences in the levels of OCM-related metabolites examined here could be the result of a synergistic effect between the wild type alleles of the aforementioned SNPs and the MD. This study suggests a key role of OCM-related metabolites and enzymes encoded by the MnSOD, CAT and MTR SNPs, in the OCM and oxidative stress pathways, respectively, via which the MD exerts its beneficial health effects.

A nutrition systems approach to analyze gene-nutrient interaction in children: preliminary data

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Despite the increase in overweight and obesity among children and adolescents in Brazil, nutritional deficiencies are still a concern. Although seems paradoxical, micronutrient deficiencies are linked to the risk of overweight or obesity. The objectives are: (1) to describe population data of a study that analyzes the response (genomic, proteomic, lipidomics) of metabolites to a supplemental multivitamin/multiminerals in individuals, using a n-of-1 study design and a systems nutrition approach; (2) to determine genetic ancestry in a community based participatory research of Brazilian children and adolescents. The study is a n-of-1 trial based on the determination of metabolomic in three times, at baseline (M1), after 6 weeks of supplementation of vitamins (M2) and after 6 weeks without supplementation (M3). The study was performed in two consecutive years (2013 and 2014) in the same community based participatory research, both with identical methodology and with repeated or new participants. Comparing the three times in each year, we can determine how the individual reacts to changes in vitamin supplementation intake. A total of 136 patients completed the study in 2013 and 135 in 2014. There was significant reduction after the intervention (M2) for glycemia, LDL-C and total cholesterol concentration in 2013. Same significant change was observed for LDL-C and total cholesterol between M1 and M2 in 2014. A new effect appeared for the two lipoproteins with a significant decrease in concentration between M2 and M3. While triglycerides, HDL and VLDL cholesterols were not affected by the intervention in 2013 sample, analysis led to different conclusions in 2014, with significant changes between M2 and M3 for HDL cholesterol (decreased concentration) and between M1 and M2 for triglycerides and VLDL cholesterol (increased concentration). In 2014, glycemia concentration decreased significantly between first and second time points like in 2013. Our results show that children that participated in the present study have genetic background from European, African and Amerindian population. In average, 21% genetic background from Subsaharian Africa, 5% from North Africa, 8% from Middle East, 2% from Eastern Asia, 49% from Europe, 7% from Central America and 8% from South America. In 2013, intervention with Nestrovit intakes led to change in glycemia, LDL and total cholesterols. These effects were replicated in 2014, considering the whole sample. When 2014 sample was divided in a validation and replication sample, a decrease in glycemia was still observed.

Novel congenic models of nutrigenetic interactions with high-sucrose diet

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The association between dietary sugars and obesity, several chronic diseases and range of cardiometabolic risk factors has been repeatedly observed. However, the susceptibility to these detrimental metabolic effects is substantially modulated by genetic factors. Rat chromosome 4 (RNO4) comprises several regions frequently associated with metabolic syndrome features. In a process of positional cloning of metabolic-syndrome related loci we derived three new double congenic strains by introgressing limited rat chromosome 4 regions including Npy, Pparg, Il-6, and Cd36 genes from spontaneously hypertensive rat (SHR) into BN-Lx (Brown Norway) genomic background: BN-Lx.SHR4(Npy), BN-Lx.SHR4(Pparg) and BN-Lx.SHR4(Il6-Cd36). In this study, we have tested whether the genes within the differential segments are involved in nutrigenetic interactions involving high-sucrose diet. We defined the spans of respective differential segments using 79 polymorphic microsatellite markers. Then we compared in silico the genomic sequences of the differential segments in the three double congenic strains (SHR vs BN-Lx). 16-week-old male rats (n=6/strain) were fed standard diet for 15 weeks (STD, control groups) or STD followed by 14 days of high-sucrose diet (HSD, 70% calories as sucrose). We assessed comprehensively the morphometric and metabolic profiles of all groups including glucose tolerance tests, levels of adiponectin and concentrations of triglycerides and cholesterol in 20 lipoprotein fractions. Two-way ANOVA with STRAIN and DIET as major factors was used. We identified significant nutrigenetic interactions in levels of adiponectin, cholesterol and triacylglycerol concentrations in multiple lipoprotein fractions, fasting glycemia, glucose tolerance and adrenal weight. For instance, the levels of adiponectin after HSD fell by more than third in BN-Lx and in BN-Lx.SHR4(Npy) (P=0.006 and P=0.0002, respectively) while it did not change in BN-Lx.SHR4(Il6-Cd36) and BN-Lx.SHR4(Pparg) (STRAIN × DIET interaction P=0.03). Fasting glycemia increased in all strains upon HSD administration, but to a different degree (STRAIN × DIET interaction P=0.00007), the most contrasting being 46% rise in BN-Lx (4.6±0.1 to 6.7±0.2 mmol/l, P=0.0001) and 86% rise in BN-Lx.SHR4(Il6-Cd36) (3.7±0.1 to 6.9±0.1 mmol/l, P=0.0001). We observed strain-specific shifts in the triacylgycerol concentrations in small LDL and large HDL particles and cholesterol levels in large LDL and HDL particles. In silico analysis revealed seven possibly and six probably damaging mutations in several genes within the differential segment, including Cd36 in BN-Lx.SHR4(Il6-Cd36), Tmem176a in BN-Lx.SHR4(Npy) and Ankrd26 in BN-Lx.SHR4(Pparg). We have isolated several regions of rat chromosome 4 affecting the susceptibility to HSD-induced metabolic derangements. Using in silico analysis, we identified sequence variants potentially responsible for the observed nutrigenetic interactions with high-sucrose diet. Supported by GAUK 132415, 434313

Effect of energy restriction on gene expression during metabolic challenge tests in human PBMCs

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Health has recently been redefined as an organism's ability to adapt and implement control in light of physical, emotional, and social challenges of life. Within the NutriTech project health is defined as 'phenotypic flexibility': the capacity to adapt to the continuously changing environment in time and space. Metabolic challenges to study phenotypic flexibility are the oral glucose tolerance test (OGTT) and the mixed meal test (MMT). Energy restriction (ER), the consumption of less energy without malnutrition, is hypothesised to increase health and has been used as a model to investigate the response to metabolic challenges in different health-states. We aimed to study phenotypic flexibility by means of whole genome transcriptional response in human peripheral blood mononuclear cells (PBMCs) upon an OGTT and a MMT challenge after 20% energy restriction. As ER is expected to result in a healthier state, we expect an improved response to metabolic challenges and a change towards a healthier gene expression profile. 72 healthy, overweight men and women, aged 50-65, were subjected to an OGTT and a MMT, before and after a 12 week intervention with either a 20% ER diet or a control diet. Total RNA was isolated from PBMCs during the OGTT at time points: 0, 30, 60 and 120 min; and during the MMT at time points: 0, 60, 120, 240 and 360 min. PBMC RNA of all time points was used to evaluate whole genome gene expression response using Affymetrix microarrays. Data of a total number of 1247 microarrays are currently analysed. Results will be available at the time of the conference.

High consumption of saturated fat intake modulates the effect of FTO on insulin resistance

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The genetic and environmental factors contributing to obesity and insulin resistance are widely explored. Genetic variation in the fat mass and obesity-associated gene, FTO, modulates obesity risk and also metabolic markers associated with type 2 diabetes mellitus (T2D). However, the effects of interplay between FTO variation and nutrient consumption on disease risk is not well understood. In this study we investigated how dietary macronutrients influence the association between FTO genotype and T2D-related metabolic markers. We investigated associations between the FTO SNPs, rs8050136 (A/C) and rs17817449 (G/T) and quantitative trait measures in a cross-sectional population sample from Chile (n=409, 56% females). These quantitative traits included anthropometric (BMI, waist circumference (WC), body fat), metabolic (glucose, insulin, HOMA-IR, lipid profile and liver function), social and lifestyle variables, including dietary intake and physical activity. Both FTO SNPs were in perfect linkage disequilibrium, therefore we report the results for the SNP rs17817449 (G/T) only. FTO genotype was significantly associated with body weight (β:3.1 kg SE:0.8 per copy of the risk allele, P=0.0002), BMI (β:0.95 kg/m^2 SE:0.2, P=0.0009), insulin (β:3.46 pmol/l SE:0.5, P<0.0001) and HOMA-IR (β:0.84 SE:0.13, P=0.0001) but not saturated fat (β:0.41 g SE:0.93, P=0.657). We found an 'FTO × saturated fat' interaction effect on HOMA-IR (P=0.003), such that the effect of FTO genotype on HOMA-IR was significantly greater for those individuals with a higher intake of saturated fat (β:1.23 SE:0.20, P<0.0001) compared to those with lower intake (β:0.45 SE:0.20, P=0.025). All results were adjusted for age, sex, environment, ethnicity, smoking and socio-economic status. These findings provide evidence that the effects of FTO genotype on T2D risk markers are modulated by saturated fat intake and, specifically, that higher intakes of saturated fat exacerbate the effect of the FTO risk allele on HOMA-IR, a marker of insulin sensitivity and T2D risk.

Acylcarnitines as markers of phenotypic flexibility

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Acylcarnitines are involved in the transport of fatty acids into the mitochondria and can be produced from acyl-CoA derived from the degradation of fatty acids and aminoacids. Therefore, acylcarnitines mirror in composition the cellular pool of acyl-CoAs and can also be found in plasma indicating changes in fatty acid and amino acid oxidation, serving as markers of inherited metabolic disorders. The aim of this study was to assess changes in plasma concentration of acylcarnitines in human volunteers during a mixed meal test (MMT) and an oral glucose tolerance test (OGTT) before and after a lifestyle intervention. As part of the Nutritech project, volunteers were challenged with an OGTT and MMT before and after a lifestyle intervention with 20% reduced caloric intake during 12 weeks. Plasma samples were collected at 7 time points during the tests and acylcarnitines measured using the LC-MS/MS based AbsoluteIDQ p180 kit (Biocrates, Innsbruck, Austria). Triacylglycerols were quantitated in 16 different lipoprotein fractions and hepatic fat content assessed using magnetic resonance imaging. As insulin and glucose levels increase during the OGTT and MMT, plasma concentration of acylcarnitine (particularly those derived from fatty acid degradation) rapidly decreases as a consequence of inhibition of lipolysis and beta-oxidation of fatty acids. This decrease in acylcarnitine concentration (lowest values reached at 120 minutes after the start of OGTT or MMT) was followed by a return to its initial concentration when insulin and glucose levels declined. These dynamic changes seem to be linked to insulin sensitivity, since volunteers with faster glucose clearance also showed faster return of acylcarnitine concentrations to their initial values. Based on the kinetics of acylcarnitines during the dietary challenges, it is possible to divide the volunteers in 2 groups – those with a fast or slow return of acylcarnitines to their original concentrations. Simply by using this grouping criterion, differences in other markers of metabolic syndrome became evident between the 2 groups: volunteers who display fast return of acylcarnitine concentrations also have approximately 25% less hepatic lipids and lower concentrations of triacylglycerol in plasma. The caloric restriction improved response to the OGTT only in the volunteers with impaired glucose tolerance and delayed return of acylcarnitine to its initial concentrations. Plasma concentrations of acylcarnitines respond quickly to an OGTT and MMT and their kinetics during the dietary challenges can indicate the status of insulin sensitivity.

The role of food metabolome in biomarker discovery: evidence from clinical and observational studies

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Food intake hold a significant role in the maintenance of good health. Nutrimetabolomics has been proposed as a tool for assessing the changes in metabolome associated with food consumption and/or the effects of a dietary intervention. The main aim of this work was to contribute to the identification of biomarkers related to food ingestion (biomarkers of intake), as well as their potential association with health (biomarkers of effect) in a high risk of cardiovascular disease population from the Mediterranean region through the application of an untargeted HPLC-q-ToF-MS metabolomic approach in nutritional studies with different designs. Results showed that the diet-related differences in urinary metabolome are associated with food digestion, microbiota metabolism and endogenous metabolism. Throughout this work, it was also proved that multi-metabolite models are a precise, specific and accurate measurement of food intake as nutritional biomarkers, which seek to be an objective and accurate tool for determining the dietary exposure. Importantly, the models were composed by metabolites from different pathways, probably as a result of collinearity in the information provided by these compounds. Until now there are very few cases that have attempted to work with combinations of nutritional biomarkers. This represents an important innovation in the field of nutrimetabolomics opening an alternative way for the further discovery of dietary exposure biomarkers as determinants of compliance in long-term intervention trials conducted in free-living individuals, as it is considered in epidemiological studies that examine associations between diet and health. Discriminating metabolites of metabolic fingerprint were also replicated among the studies with different design. The replication of biomarkers allows the level of evidence from observed associations to be increased, as has been suggested for genomic studies The results also confirmed that a non-targeted metabolomics approach allows to access unexplored pathways that are affected by diet. Thus, the identification of endogenous markers leads to new hypotheses to decipher the relationship between diet and health.

Mapping of the DNA adductome to study the genotoxic effects of red meat consumption

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DNA adductomics studies the formation of altered DNA nucleobases due to exposure to endo- or exogenous genotoxins. The best analytical tool for DNA adductomic studies is High Resolution Mass Spectrometry (HRMS) as it enables an 'omics' approach. Recently, an Ultra High Performance Liquid Chromatography-HRMS method was developed and validated successfully to study the presence of both targeted (O^6-carboxymethylguanine (O^6-CMG), O^6-methylguanine, pyrimido[1,2-a]purin-10(1H)-one and α-methyl-γ-hydroxy-1,N2-propanoguanine) and untargeted DNA adducts in in vitro and in vivo DNA samples. To aid with the complex process of omics data interpretation, an in-house diet-related DNA adduct database was constructed in parallel. At the time, this state-of-the-art methodology and database enable investigation of the possible genotoxic effects of red meat consumption. Indeed, red and processed meat consumption have been linked to an increased colorectal cancer (CRC) risk, although, at the time, the exact underlying mechanism(s) still elude(s) us. The most prominent hypotheses suggest that red meat consumption induces oxidative stress, lipid peroxidation and formation of N-nitroso compounds (NOCs). To study these hypotheses, both red and white meat preparations (chicken vs beef, both with or without addition of calcium carbonate ($CaCO_3$)) were subjected to in vitro gastrointestinal (GI) digestion. The thereby obtained digestive samples were analyzed for DNA adduct content by the UHPLC-HRMS method. Data interpretation was enabled by use of the database and ToxID™, SPSS®, SIEVE™ and SIMCA™ analysis. The obtained results show that the GI DNA adduct profile is very much dependent on the composition of the individual colonic microbiota. Some DNA adducts types (e.g. O^6-CMG and ethylthymine) are actively formed during colonic digestion of meat, whilst others are not, possibly indicating dilution and the resulting decrease of DNA adduct levels (e.g. carboxythymine) over time. The most anticipated findings of this study encompass the detection of meat preparation-specific DNA adduct formation. For example, O^6-CMG (an NOC-related DNA adduct) levels significantly increased upon digestion of beef compared to chicken. The additional finding that the presence of the formamidopyrimidine-adenine DNA adduct (adduct related to oxidative stress) appeared to be distinctly lower in samples obtained from meat digestions with added $CaCO_3$, is also particularly interesting since calcium is deemed to be a CRC-protective agent. The detection of altering levels of DNA adducts during in vitro colonic digestion of meat indicates the formation of genotoxic agents during in vivo GI digestion. In accordance with previous results, it appears that the colonic microbiota can significantly contribute to the formation of those genotoxins. Moreover, these results signal a plausible basis for the genotoxic effects of red meat and the cancer-protective attributes of calcium.

Urine metabolomic profiling to identify biomarkers of a flavonoid-rich and flavonoid-poor diets

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The present study aims to investigate the dose dependent effects of consuming diets enriched in flavonoid-rich and flavonoid-poor fruits and vegetables on the urine metabolome of adults who had a ≥1.5 fold increased risk of cardiovascular diseases. A single-blind, dose-dependent, parallel randomized controlled dietary intervention was conducted where volunteers were randomly assigned to one of three diets: high flavonoid diet, low flavonoid diet or habitual diet as a control for 18 weeks. High resolution LC-MS untargeted metabolomics was performed using an Orbitrap mass spectrometer. Putative biomarkers which characterize diets with high and low flavonoid content were selected by state-of-the-art data analysis strategies and identified by HR-MS and HR-MS/MS assays. Discrimination between diets was observed by application of two linear mixed models. Valerolactones, phenolic acids were among sixteen biomarkers related to the high flavonoid dietary exposure. Four biomarkers related to the low flavonoid diet belonged to the family of phenolic acids. For the first time abscisic acid glucuronide was reported as a marker of carotenoid consumption. This metabolomic analysis has identified a number of dose dependent biomarkers, which can be used in future observation and intervention studies to assess flavonoids and/or carotenoids intakes and compliance to fruit and vegetable intervention.

A chemogenomic approach finds novel food, chemical and genetic mediators of triglyceride homeostasis

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The high diversity and low concentration of many food chemicals, such as polyphenolics and uncommon fatty acids, present obstacles to the evaluation of their role in human health. Gene-environment interactions, increasingly recognized as important contributors to differences in phenotypes, involve genes that respond to intervention agents (food, lifestyle, drug, etc.) in an allele-specific manner. Here, we present a method for the discovery of novel bioactivity of food compounds based on structural similarity to drugs of known action. Using the ChEMBL API, we developed a table linking drugs to food compounds with a stringent Tanimoto chemical similarity of at least 0.85 (T85), which then suggests potentially comparable bioactivity. Additionally, a list of 37 genes supporting published gene-environment (G×E) interactions affecting serum triglycerides was used to generate a list of drugs known to target those encoded proteins. By filtering our T85 food compound-drug dataset to return only these drugs, a resource was created that links food compounds having potential impact on triglycerides to the genes that may mediate this effect, and do so dependent on genotype. Secondarily but with less assurance, novel G×Es are proposed, which involve specific foods and which are more refined than the vast majority of macronutrient-centric G×Es. The efficacy of this drug-food compound method was verified by exploring specific evidence in the literature in which both the drug and various similar food compounds show experimental effects on triglycerides. Insight into the mechanism of action of these drugs and food compounds was gained through comparison with yeast fitness signatures generated through the analysis of responses to perturbation by small molecules of individual yeast haploinsufficiency lines. With these results, we created a network connecting food groups to the genes upon which they may act. The network highlights the relative importance of each food group as determined by the number of food compounds supporting its proposed effect on triglyceride levels. In principle, this method can be applied to any set of genes to identify potential small molecular effectors arising from specific food chemicals and their food sources.

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 molecular 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.

Obesity and metabolic flexibility: the key to metabolic health

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The prevalence of obesity and related health complications is increasing worldwide. The obese insulin resistant state is characterized by an inflexible regulation of substrate oxidation (metabolic inflexibility), that is defined as an impaired capacity of skeletal muscle to switch from fat to carbohydrate oxidation during insulin-mediated conditions and an impaired capacity to increase fat oxidation during conditions of a high lipolytic rate like fasting, exercise and increased activity of the sympathetic nervous system. Insulin resistance is characterized a systemic lipid overflow caused by a reduced ability of adipose tissue to store dietary fat (adipose tissue dysfunction). This lipid overflow and the metabolic inflexibility of substrate oxidation may contribute to the accumulation, and/or an altered composition of bioactive lipid metabolites in ectopic tissues, which may be one of the drivers of insulin resistance through interference with insulin signaling. The interorgan cross-talk in metabolic inflexibility, the role of adipose tissue dysfunction, an impaired skeletal muscle lipid turnover as well as the impact of microbial products from the gastro-intestinal tract will be briefly discussed. Focuss will be on dietary intervention strategies that may improve metabolic flexibility. Data will be presented on the impact of dietary fatty acids, polyphenols, short chain fatty acids (from microbial fermentation of dietary fibres) and lifestyle intervention on metabolic health in overweight or obese subjects with a high risk for developing type 2 diabetes mellitus and cardiovascular disease.

Need for a paradigm shift in overweight and obesity management

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In spite of obesity being declared a 21st century pandemic by the World Health Organization, clear opportunities for its diagnosis as well as treatment are being surprisingly missed. The need to treat the consequences of obesity (such as type 2 diabetes, hyperlipidaemia and hypertension) is readily accepted by clinicians, however, the disease itself is often not really tackled. In the short consultation time available in the outpatient setting some physicians may not have the time or skills to address this complex heterogenous problem. Screening gaps may be also signs of 'clinical inertia', an attitude that implies overestimation of care provided, use of weak reasons to avoid intensification of therapy as well as lack of education, and training. Importantly, until recently obesity has not featured strongly in medical training underlining the need to bring the topic of both overweight and obesity more into the focus of the medical curriculum and for enhancing the awareness of medical students of their underlying complex aetiology. Both overweight and obesity require a coordinated care from a multidisciplinary group combining individualized dietary advice, exercise and behavioural programmes, with some patients requiring the added support of pharmacotherapy or surgery. Traditionally, the futility and the recidivism rate have been used by physicians to justify passive attitudes. Although obesity is a particularly challenging medical condition to treat due to its complex aetiology and multifactorial nature, well-recognized effective approaches for the treatment of obesity have been identified. While prevention is essential even before excess weight sets in, it is imperative to remember that moderate (5-10%) weight loss, which is achievable using a variety of interventions, yields clinical benefits and, thus, should be encouraged in patients studied in an individualized way.

The impact of milk derived bioactives on glycemic management

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Recently, extensive research has suggested that food derived bioactive peptides may be beneficial for human health. Milk derived peptides have been reported to have potential benefits for reducing the risk of type 2 diabetes. The objectives of this study were to (1) investigate the effect of milk-derived bioactives on insulin secretion from pancreatic β-cells in vitro and in an ob/ob mouse model and (2) ascertain the effects in humans. The effect of the bioactive on β-cell insulin secretion was established. Additionally, metabolic profiling and functional assays including intracellular calcium and plasma membrane potential (PMP) were performed in BRIN-BD11 cells following treatment with the bioactive. The bioactive was tested in an animal model and glucose tolerance tests (GTTs) were performed pre and post a 12 week intervention. The acute effect of the bioactive was tested in healthy human volunteers. Participants (n=62; mean (±SD) age 53 (±7) years, BMI 31.3 (±5) kg/m^2) attended the laboratory on two separate occasions at least 7 days apart. On each occasion, participants were provided with a fixed breakfast containing 70 g carbohydrate, along with a 10% (w/v) solution of either a casein hydrolysate or a sodium caseinate (intact control protein) in a randomised, crossover fashion. Blood samples were collected in the fasted state and periodically over 2 hours postprandially and were analysed for a range of biomarkers. Acute insulin secretion from BRIN-BD11 cells was significantly increased in the presence of 1 mg/ml bioactive ($P=2.22\times10^{-5}$). Intracellular calcium and PMP of BRIN-BD11 cells were not changed. Metabolomics analysis revealed significant increases in C18:1n9, C20:5n3 and MUFA, succinate and decreases in GABA and sn-glycero-3-phosphocholine. Acute treatment with the bioactive improved glucose tolerance with a significant reduction in AUC during a GTT ($P<0.01$) in the ob/ob model. In human subjects, acute ingestion of the casein hydrolysate enhanced postprandial insulin secretion and reduced non-esterified fatty acids in comparison to the intact protein ($P<0.05$). The enhanced insulin secretion resulted in a reduction in postprandial glucose levels ($P<0.05$). The results illustrated that the milk-derived bioactive has a positive effect on insulin secretion from β-cells both in vitro and in humans. The bioactive promoted insulin secretion through Ca^{2+}-independent pathway and significantly altered the metabolic profile. Identification of this novel hydrolysate provides an opportunity for potential use as part of a functional food matrix or nutraceutical for glycemic management. Further work is underway to examine the systemic availability of the hydrolysate and to incorporate such a bioactive into food matrices. This study was funded by CSC-UCD and Food for Health Ireland.

Herbal extract reduced energy intake by modulating gastrointestinal hormones in overweight women

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Obesity has become a serious public health problem in many countries worldwide. The genesis of obesity is multifactorial and involves genetic, neurological, dietary habits, physical activity, socio-cultural conditions and endocrine factors. Peptide hormones produced from the gastrointestinal tract in response to nutritional intake, such as ghrelin and glucagon-like peptide-1 (GLP-1), are considered to be major regulators of appetite. It has been shown that higher plasma ghrelin levels is observed in many disorders of metabolism and weight, On the other hand, GLP-1 is an anorexigenic peptide produced in response to food intake. Regulators of appetite and the homeostatic food intake are thus of high interest. The aim of this study was to evaluate the effects of an herbal extract product containing Yerba Mate, Guarana and Damiana (YGD) on food intake, ghrelin and GLP-1 levels in overweight and obese women. The study included 30 sedentary women with BMI ranging from 25-39.9 kg/m^2 in an acute randomized single-blinded placebo-controlled cross-over trial. Blood samples were taken at baseline and 15, 30, 60, 120, 180 minutes after fixed breakfast (494.50 kcal, 52.67% carbohydrate, 12.91% protein and 34.5% fat) or lunch (632.05 kcal, 61.67% carbohydrate, 16.97% protein and 21.44% fat). The amount of food intake was estimated by subtracting leftover weight from the portion weight determined for each item taken. On each testing day, participants received placebo or tablets containing YGD. Body composition was measured at baseline of intervention by dual energy X-ray absorptiometry (DXA). Acylated ghrelin and GLP-1 were analyzed using a magnetic bead-based multiplex kit. Although there was no difference in energy intake at breakfast, at lunch the energy intake was significantly lower (-43.28±13.05 kcal; P=0.005) in YGD group. Similarly, previous studies have shown that a proprietary formulation of YGD resulted in significant reduction in food intake and in significant weight loss. A higher reduction of glucose concentration was observed between 90 and 210 minutes after breakfast in YGD group (P=0.03). Concerning the effect of supplementation on acylated ghrelin levels, it was observed that YGD reduced significantly its levels 60, 150 and 210 minutes after lunch (P=0.04). In addition, the YGD increased significantly the GLP-1 concentration 45, 60, 90, 150 and 210 minutes after breakfast (P=0.04). Similar results were also detected after lunch. In summary, our data showed that South American herbal extract containing Yerba Mate, Guarana and Damiana were capable to reduce energy and macronutrient intake by reducing acylated ghrelin and increasing GLP-1 in overweight and obese women. These results support the beneficial effects of YGD in assisting weight management.

Grape-seed proanthocyanidins decrease triglycerides in HepG2 by a sirtuin-dependent mechanism

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The incidence of nonalcoholic fatty liver disease (NAFLD) is increasing rapidly due to the prevalence of obesity. Early hypotheses proposed that sirtuins (SIRT) could potentiate oxidative metabolism in liver and protect against hepatic fat accumulation by directly enhancing the transcriptional activity of a series of critical metabolic regulators. Naturally-occurring dietary polyphenols have been shown to modulate SIRT1 deacetylase activity in several in vivo and in vitro studies. Here, we evaluated whether similar effects could be reached by proanthocyanidins (PAC), a family of flavonoids, on several experimentally induced-hepatic steatosis cellular models. Accordingly, HepG2 cells were cultured with palmitate 0.5 mM, glucose 30 mM, palmitate 0.5 mM + glucose 30 mM or glucose 30 mM + fructose 5.5 mM for 48 hours. Then, cells were treated with PAC derived from grape-seed (25, 50, 100 mg/l) for the last 24 hours of incubation. We pioneerly report that PAC significantly decreased intracellular lipid accumulation in a dose-dependent manner in three of the four experimental models. Notably, the decreased levels of triglycerides were already significant with a dose of 25 mg/l when NAFLD was induced with palmitate 0.5 mM + glucose 30 mM, indicating that this dose was the minimal dose associated significantly with an improved protection against hepatic triglyceride accumulation. To assess the effect of PAC on gene expression in the palmitate 0.5 mM + glucose 30 mM model, the relative mRNA levels of key genes on lipogenesis and fatty acid oxidation were determined by RT-qPCR. We observed that PAC down-regulated fatty acid synthase (FAS) and acetyl-coA carboxylase (ACC). In addition, no significant effects were observed in peroxisome proliferator-activated receptor alpha (PPARα) indicating that the delipidating effect of PAC could be mediated by reduced lipogenesis but not by increased fatty acid oxidation. Interestingly, when cells were treated with the SIRT1 inhibitor Sirtinol, the protective effects of PAC treatment were diminished indicating that PAC is capable to protect against NAFLD through a SIRT1-dependent mechanism. In conclusion, our data suggest that PAC may attenuate the development of NAFLD by enhancing SIRT1 activity and, consequently, modulating the expression of key genes involved in lipid homeostasis. Therefore, PAC could be a valid tool to boost SIRT1 activity and its consumption might be a potentially therapeutic approach to prevent or treat hepatic fat accumulation. This work was supported by grant no. AGL2013-40707-R from the Spanish government.

Modulation of the enteroendocrine cell functions by flavan-3-ol

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Grape seed proanthocyanidins extract (GSPE) has been reported to modulate cellular membrane potential and enterohormone secretion in intestinal endocrine cells (STC-1). Furthermore, it has been described that monomeric flavan-3-ols act at different levels of the enteroendocrine cell metabolism, activating bitter receptors (hTAS2R14 and hTAS2R39) and inducing the increase of intracellular Ca^{2+} concentration. Nonetheless, their effects on enterohormone secretion remain unclear. The aim of our study was to evaluate the effect of flavanol monomers and proanthocyanidins on enterohormone secretion by intestinal endocrine cells (STC-1) and the mechanisms involved. The cellular membrane potential (ΔΨcell) and enterohormone secretion from STC-1 were evaluated in response to different doses of compounds that are predominant in GSPE (i.e. (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin gallate (ECg) and dimer B2). The compounds were added to a final concentration of 1, 10, 100 or 200 μM and the ΔΨcell was evaluated using the fluorescent probe $DIBAC_4(3)$, which was monitored with excitation and emission filters set at 493 nm and 516 nm, respectively. The cells were incubated with compounds at the high dose and low dose, 200 μM and 1 μM, to evaluate the secretion of GLP-1 and CCK. The treatment with monomers modulates cellular membrane potential, inducing depolarization at low concentrations that do not lead to a change in enterohormone secretion. In contrast, high concentrations caused membrane hyperpolarization, which led to a decrease of GLP-1 and CCK secretion. A decrease of CCK secretion was triggered by both concentrations of B2, and 200 μM also caused a significant decrease of GLP-1 levels. The in vitro results show that purified forms of flavanol monomers and proanthocyanidins modulate enteroendocrine hormone secretion, inducing at high concentration a decrease of GLP-1 and CCK release under our experimental conditions. Changes in cellular membrane potential may explain this inhibition.

Multigenerational influence of paternal high fat diet on the white adipose tissue

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Obesity, highly associated with the incidence of insulin resistance, is one of major risk factors for various metabolic diseases. Current high prevalence of obesity worldwide is primarily associated with excess calorie intake and physical inactivity. Recently, it has been suggested that diet-induced obesity influences glucose metabolism in the next generation. It is, however, unclear how and what physiological characteristics are inherited from obese parents. In this study, we investigated transgenerational impacts of paternal high fat diet on the inguinal and gonadal white adipose tissue (iWAT and gWAT, respectively) of the male mice and their offspring. Male mice were fed either with control diet (CD) or high fat diet (HFD), called as the founder generation (F0). The female offspring (F1) was bred to produce the third generation (F2). All offspring in both groups were fed with control diet. We analyzed mRNA expression patterns in iWAT and gWAT of F0 CD and HFD and F2 male born to F1 female (CFM, HFM) and F2 female born to F1 female (CFF, HFF). F0 HFD group compared to F0 CD group showed that gene expression of Ppargc1a and Ppara, related with energy expenditure and lipid oxidation, respectively, decreased in the iWAT. Expression of Glut4 gene also decreased, which might be associated with ectopic fat accumulation in obese mice. The gWAT of HFD group showed downregulated expression of Atgl and Hsl gene compared to that of CD group indicating lower lipolysis. Intriguingly, F2 mice showed different patterns of gene expression in WATs according to their grandfather's diet. Expression of Glut4 and Ppargc1a gene was downregulated in iWAT and gWAT, respectively in HFM group compared to CFM group, which are similar to F0. On the contrast, F2 female showed different patterns. Gene expression of Ucp1 and Glut4 was upregulated in the iWAT and gWAT, respectively, in HFF group compared to CFF group. Taken together, paternal high fat diet affects gene expression in iWAT and gWAT not only of the mice that were fed with, but also of the F2 offspring in the sex-dependent manner. The difference may be associated with sex-dependent fat accumulation capacity of the subcutaneous white adipose tissue.

Association between activated cellular immunity and metabolic syndrome

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An emerging field of research recognizes an interplay between activated cellular immunity, inflammation, and impaired metabolism. Central to the 'immunometabolism' theory, inflammation and immune activation are involved in the development of obesity, insulin resistance and potentially also in metabolic syndrome (MetS). However, there is little evidence from population-based epidemiological studies investigating these relations. Therefore, we explored the association of the cellular immune activity biomarker total neopterin with MetS and its components in a sub-sample of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. A total of 845 healthy participants (375 men and 470 women) have been included in the analysis. MetS was defined based on the harmonized definition (Alberti et al. 2009) as having any three of the following five components: (1) abdominal obesity; (2) elevated triglycerides; (3) reduced high-density-lipoprotein (HDL) – cholesterol; (4) elevated blood pressure; and (5) abnormal glucose metabolism. Plasma concentration of total neopterin (7,8-dihydroneopterin + neopterin) was determined by liquid chromatography-tandem mass spectrometry at Bevital A/S, Bergen, Norway. In cross-sectional analysis, using pre-defined cutoffs as per the MetS definition, increased total neopterin was associated with abdominal obesity (OR per 10 nmol/l = 1.58, 95% CI=1.19-2.11) and reduced HDL-cholesterol (OR per 10 nmol/l = 2.27, 95% CI=1.65-3.11). Further adjustment for C-reactive protein – as a measure of chronic low-grade inflammation – attenuated the association between total neopterin and abdominal obesity (OR per 10 nmol/l = 1.28, 95% CI=0.96-1.72). Increased plasma total neopterin was not associated with overall MetS (OR per 10 nmol/l = 1.34, 95% CI=0.96-1.88). The latter association was not modified by age, sex, and general obesity. These data suggest that elevated concentrations of plasma total neopterin – as a biomarker of activated cellular immunity – are associated with abdominal obesity and reduced HDL-cholesterol, thereby supporting the emerging knowledge on the interplay of immune response and metabolism. Future studies are warranted to better understand the potential role of these interrelations in chronic disease development.

Proteomic profile of children and adolescents from different metabolic groups

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In a previous study we performed an intervention crossover study to examine how a child or an adolescent from 9 to 13 years responded to vitamins supplementation (vitamin A, thiamine, riboflavin, pyridoxine, folic acid, vitamin B12, vitamin D, vitamin E, niacin, vitamin C, biotin, pantothenate, calcium, phosphorus, iron, magnesium, zinc) in terms of lipid and glucose profile. The subjects were from schools of Ribeirão Preto (São Paulo, Brazil). The study design was based on measuring and comparing metabolites related to lipids and glycaemia at baseline (moment 1), after six weeks of a micronutrient daily supplementation (moment 2), and following 6 weeks without the daily supplementation (moment 3). Using k-cluster statistical analysis, two reverse metabolic groups were found, based on glucose, total cholesterol, triglycerides, VLDL cholesterol, LDL cholesterol and HDL cholesterol levels. Cluster 1, n=111 subjects, had better glucose and lipid profile when compared to Cluster 2, n=25 subjects (ANOVA $P<0.05$ for all parameters, except for glycemia in moment 1). ANCOVA analyses, comparing variables between the two clusters and adjusting for age, pubertal status, gender, and energy intake showed cluster 1 with better anthropometric, nutrient intake and metabolites results. In cluster 1 and 2, total cholesterol and LDL decreased throughout the study, and in cluster 1 glycemia also decreased from moment 1 to moment 2. The aim was to compare the fold changes (Moment 2/Moment 1 ratio) for protein abundances between cluster 1 and cluster 2. iTRAQ proteomic approach. 4 experiments were performed. Experiment 1 and 2 were identical and they were made with pools composed by plasma samples from 5 participants from each cluster in each moment; experiment 3 and 4 was identical and they were made with pools composed by plasma samples from other 5 participants from each cluster in each moment. Only the results of the proteins that had at least 3 valid results from each protein identified were considered. 20 proteins were found, of which 13 exhibited a fold-change ≥ 1.2 or ≤ 0.8: Alpha-1-antichymotrypsin, Alpha-1-antitrypsin, Alpha-1B-glycoprotein, Antithrombin-III, Kininogen-1, Complement C3, Fibrinogen alpha chain, Fibrinogen beta chain, Fibrinogen gamma chain, Heparin cofactor 2, Ig mu chain C region, Plasma protease C1 inhibitor and Vitamin D-binding protein. They are mainly linked to: lipid and glucose metabolism, acute phase, coagulation, immunity, inflammatory response, hemostasis and vitamin D transport. The differences in plasma protein abundances between cluster 1 and cluster 2 can, at least in part, explain the different results found for lipid, glucose and metabolic profile between cluster 1 and 2. This Project is sponsored by Nestle Institute of Health Science and FAPESP (2012/00783-2).

Seasonal fruit consumption in different photoperiods: Physiological effects

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Nowadays, globalization makes us being closer to different nations and cultures which allow us eating whichever kind of food in any time of the year. According to the Xenohormesis theory, animals can use some plant bioactive compounds as environmental cues in order to adapt their metabolism to upcoming seasonal variations. We hypothesize that fruit consumption can modulate the metabolism of mammals in a season-dependent fashion. The aim of this study was to investigate the physiological effects caused by the consumption of different fruits in different seasons, spring and autumn. Five groups (n=6) of Fisher 344 rats fed standard diet were housed in three light schedules of 6 hours of light per day (SD, n=30), 12 hours of light per day (ND, n=30) or 18 hours of light per day (LD, n=30). After 1 month in these conditions, animals in each photoperiod were orally supplemented with 4 different kinds of lyophilized fruit (100 mg per kg of body weight/day): autumn fruits as orange (T) or grape (R) and spring fruits as apricot (AB) or cherry (CI) for 12 weeks. Another group used as control received the vehicle. Multivariant statistical analyses Principal Component Analysis (PCA) and Random Forest (RF) were performed with 21 variables related to biometrical data and indirect calorimetry tests using the software Metaboanalyst in order to identify physiological and biometrical changes induced by each treatment respect the vehicle in the three different photoperiods. According to PCA and RF analyses, cherry, a spring fruit, induced clear season-dependent physiological changes as suggested the clear separation between SD and LD, while cherry-treated ND animals overlapped the LD group. Orange, an autumn-winter fruit, also showed differential effects in LD and SD, while the ND photoperiod overlapped with both LD and SD. In turn, the photoperiod-dependent effects of AB and R treatments were less clear. The analysis of all the autumn versus all the spring fruits in SD and LD revealed clear photoperiod-dependent differences in the physiological effects exerted by fruits consumption. Lipid oxidation, activity, energy expenditure and weight of fat depots had the most important influence in these differential effects. Fruit consumption causes alterations in animal physiological and biometric parameters depending on the season of fruit administration. These findings might be relevant to understand the role of nutritional habits in the development of metabolic alterations that lead to highly prevalent diseases such as obesity, cardiovascular disease or the metabolic syndrome, in industrialized societies. This work has been supported by the Spanish Ministry of Economy and Competitiveness (MINECO), (AGL2013-49500-EXP, FRUITOBES project).

Effects of yerba mate on regulation of obesity

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The prevalence of obesity is a major public health concern because of the associated weight-related diseases that result in significant morbidity and mortality and a reduced quality of life. Research in the nutrition field has recently aroused considerable interest based on the potential of natural products to counteract obesity. In this sense we have previously published that yerba mate (YM) is an excellent candidate because it's in vitro and in vivo modulation of adipogenesis and obesity, respectively. The mechanisms responsible for YM modulation on obesity, however, are unclear. The goal of current study is to investigate possible mechanisms. We evaluated, using a C2C12 cells, the effects of YM (50; 100; 200; and 300 μg/ml) in the metabolic flux to understand cellular metabolism, and the measurement of oxygen consumption and extracellular acidification using the Seahorse XF Analyzer. A high-fat diet-induced mice model of obesity was used to evaluate the anti-obesity effects of YM (1 g/kg) after 8 weeks of intervention. Indirect calorimetry and food intake were assessed; oxygen consumption (VO_2), CO_2 production (VCO_2), respiratory quotient (RQ) and resting energy expenditure (RER) data were obtained at 6^{th} week. Brown adipose tissue (BAT) and muscle was examined to determine the mRNA levels of uncoupling protein-1 (UCP1), and PPAR-γ coactivator-1α (PGC-1α). Our data shows that, at the highest concentrations (200 and 300 μg/ml), were able to increase the O2 consumption rate (OCR). This data means that YM improves mitochondrial function. The high-fat diet-induced mice model of obesity indicated that, at the end of the study, the regular ingestion of YM extract significantly decreased the final body weight (48.7±6.70 g), when compared with those fed the high-fat diet (55.7±2.73 g). This weight loss was not related to a reduction in food intake. The mice in the high-fat diet–YM group had significantly less epididymal fat than the mice in the high-fat diet group. Indirect calorimetry showed, at 6^{th} week, that YM intervention significantly increased VO_2, VCO_2, RQ and RER in high-fat diet–YM group, compared with high-fat diet group. Moreover, our data showed that a high-fat diet downregulates the expression of PGC-1α and UCP1 in BAT and muscle, which may have decreased energy expenditure and increased diet-induced obesity. The present study also showed that PGC-1α and UCP-1 mRNA levels in both were recovered after YM treatment. It has been shown that during adaptive thermogenesis the mitochondrial respiration increases, and the energy is dissipated to prevent obesity by reducing fat storage. In summary, the data presented showed that YM improves mitochondrial function, in vitro. In addition, the in vivo results indicated that YM upregulated PGC-1α mRNA levels, which regulates mitochondrial biogenesis and respiration by inducing the expression of UCP1, and reducing fat storage.

Pathway and network analysis in adipose tissue related to BMI and glucose tolerance

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Noncommunicable diseases, like hypertension, diabetes, cardiovascular disease and cancer, have a strong relation with obesity. Some metabolic abnormalities associated with type 2 diabetes develop many years before the onset of diabetes and are expected to be preceded by changes in gene expression of related genes in adipose tissue. Adipose tissue is not only important for energy storage but also as an endocrine organ, it can synthesize and secrete a variety of hormones. The most important of these adipokines are leptin, TNF-α, IL-6, resistin, visfatin, and adiponectin. They are known to be involved in the control of sensitivity to insulin and in inflammatory processes. The aim of this study is to find differences in glucose sensitivity related processes in adipose tissue from individuals with different BMIs. Differentially expressed genes were determined with microarray technology and the affected biological processes and their regulation were evaluated with pathway and network analysis. We used a publicly available transcriptomics dataset (GEO: GSE27951) in which gene expression was measured in adipose tissues in three groups, consisting of 6 lean, 10 overweight and 17 obese subjects, with different glucose tolerances. Analyses of individual characteristics confirmed the known strong positive correlation between Hb1Ac and fasting glucose levels ($r=0.76$ and $P<0.001$), moreover we found significant correlations between BMI and fasting glucose and insulin levels ($P=0.026$ and $P=0.003$, respectively). These results confirm the link between glucose and insulin metabolism and obesity. Our first analysis of the transcriptomics data shows that groups divided according glucose and insulin levels are better separated than groups divided according to BMI, and this provides insight in the importance of this metabolism in adipose tissue. Pathway and network analysis were performed with the commonly used open-source tools, PathVisio and Cytoscape, respectively. Identified pathways and networks help us understand the differences in adipose tissue from different individuals and under different metabolic conditions. This understanding is key for early interventions aimed at prevention of obesity related diseases that are directly related with many deaths worldwide every year. This project is supported by CAPES, CNPq and the EU FP7 project MICROGENNET (31.96.42.94 E, www.microgennet.org).

Role of $1,25(OH)_2D_3$ in normal human tissue adipose model: real-time monitoring and cell viability

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In recent years the study of the development of adipocytes has become an area of intense interest because adipocyte differentiation is associated with several pathologies. In light of the global obesity epidemic, there is an increase on the interest of the role of factors potentially changeable in the adipogenesis. As a complex disorder, obesity includes both genetic and environmental factors in its pathogenesis, being the genetic factors possibly modulated by vitamin D ($1,25(OH)_2D_3$) (VitD). The mechanisms of the VitD in preadipocytes and adipocytes is still poorly understood. The aim of this study was to assess the kinetics of proliferation and differentiation of human preadipocytes SGBS cells in mature adipocytes, as well as the role of the VitD during this process, by real time cell analyzer (RTCA), also MTT and proliferation assays and lipids quantification. We used two different concentrations of VitD, 10 nM and 100 nM, commonly studied in murine cells. The VitD, at both concentrations, significantly increased the concentration of lipids in SGBS cells after 16 days of differentiation. While in preadipocytes, the VitD 100 nM decreased the concentration of lipids after 48 hours. The VitD 100 nM showed a more pronounced effect on the SGBS cells than on the concentration of 10 nM, besides this effect being more pronounced in cells treated in preadipocytes or continuously from that stage, or in the period of differentiation. In the MTT assay, the SGBS cells exposed to VitD 100 nM showed absorbance values significantly lower than the controls. For the cells treated continuously since the preadipocytes phase with VitD 10 nM, the absorbance has also shown being significantly lower than the controls after 16 days of differentiation, suggesting an accumulation of events. In the RTCA assay, the treatments led to the amendment of cell impedance at all times, mainly during the first hours, probably due to the Ca^{2+} influx. In contrast to the MTT assay, the VitD group, especially at the concentration of 100 nM, had a higher normalized cell index than the control during adipocytes proliferation stage and after 4 days of differentiation. An inverse response was observed in mature adipocytes (12 and 16 days). This current study shows the action of the VitD in human preadipocytes and adipocytes, and for the first time, it shows that VitD may modulate both the morphology and metabolism of adipose tissue. The observation that the VitD may alter the metabolism of lipogenesis may provide new insights about the possible pathways involved in proliferation, differentiation and lipogenesis in adipocytes and preadipocytes in a normal human adipose tissue model. However, further studies should also be carried out in human preadipocytes and adipocytes to elucidate the possible mechanisms proposed and assess the impact of these on the obesity. Supported by CAPES and CNPq.

Consumption of fruits out of season modify body fat mass and lean mass in rats

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Polyphenol composition in fruit plants provides a chemical signature of the environmental conditions. The chemical signature of each fruit depends on various factors including photoperiod, temperature, recollection season and environmental stress. Several studies have correlated photoperiod alterations (hour day/night) with obesity and it has been reported that several polyphenols could modulate the molecular clock. The objective is to investigate whether the consumption of fruits out of season, or from different geographical origins, or plants without stress can induce an erroneous signal that can stimulate metabolic alterations associated with obesity in a lean rat model and to select the fruits with greater effects for further studies with obesity rat models. A total of 126 Fisher 344 male rats were distributed in 21 groups of six rats each depending on the treatment received and the photoperiod condition. Animals were fed with standard diet and supplemented with vehicle or with one of the following lyophilized fruits: orange from Spain(O), orange from Argentina (OS), apricot (A), grape (G), organic grape (OG) or cherry (CH) for 10 weeks. Animals were housed in three different rooms with three different light:dark periods: Normal Day (12h:12h; ND), Long Day (18h:6h; LD), and Short Day (6h:18h; SD). Body composition parameters (fat mass and lean mass) were analysed by quantitative magnetic resonance measurements (EchoMRI™) one week before sacrifice. At the end of the experimental study, the epididymal (eWAT), retroperitoneal (rWAT), inguinal (iWAT) and mesenteric (mWAT) white adipose tissues, and interscapular brown adipose tissue (iBAT) were removed and weighted. OS and G significantly ($P<0.05$) decreased body fat and increased relative lean mass on ND and LD photoperiods compared to its natural photoperiod. Moreover, OS significantly decreased iWAT mass on the same photoperiods (ND and LD). CH significantly decreased relative lean mass on ND photoperiod, while it had the inverse effect on SD photoperiod, compared to its natural photoperiod. Moreover, CH significantly decreased RWAT mass on ND and SD photoperiods and significantly decreased iBAT mass on SD photoperiod, compared to its natural photoperiod. Short day fruits (OS and G) consumed on different photoperiods (normal day or long day) decrease body fat while increasing relative lean mass. Moreover, cherry, which is a long day fruit, changes the lean mass and RWAT mass when consumed in a different photoperiod. These results suggest that consumption of OS, G and CH out of season may stimulate metabolic alterations leading to the modification of the amount of fat mass and lean mass in rats.

Acylcarnitine profiling in plasma and tissues from mouse models of obesity and diabetes

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In recent years metabolomics approaches have identified various acylcarnitine species with predictive or diagnostic power. Since most analyses are done in plasma or urine, the exact metabolic origin is often not known. We established acylcarnitine profiles from plasma and tissue (liver, muscle, kidney) from mouse models representing various stages of diabetes progression. Leptin signaling-deficient ob/ob and db/db mice at an age of 20 weeks represented early and advanced stages of type II diabetes, with normoglycemia and hyperglycemia, respectively. Streptozotocin-treated insulin-deficient mice treated with a single high dose represented an end-stage of type II diabetes or a type I diabetes. All three models were accompanied by respective wild-type control mice. Specifically, we focused on the various isomeric acylcarnitine species that occur in individual pathways of amino acid- and fatty acid-breakdown. Furthermore, since metabolites with odd-numbered acyl chains gained interest as potential diabetes biomarkers, we determined concentrations of odd-numbered acylcarnitine species ranging between C3 and C17. Results showed increased concentrations of branched-chain amino acid derived acylcarnitine species in all three mouse models both in plasma and tissues. These increases were most pronounced in plasma and muscle tissue of streptozotocin-diabetic mice and clustered according to metabolic pathways of amino acid and fatty acid metabolism. Interestingly, specifically the branched-chain amino acids and related acylcarnitine species were recently reported to have predictive power for diabetes development. The hyperglycemic db/db and streptozotocin-treated mice also displayed increased concentrations of several odd-numbered acylcarnitine species, while long-chain even-numbered acylcarnitine species remained unchanged or were decreased. Metabolites with odd-numbered acyl-chain lengths were also recently proposed as potential biomarkers. For the obese ob/ob and db/db mice, strongest increases were found for mono-unsaturated acylcarnitines palmitoleyl-carnitine (C16:1) and oleyl-carnitine (C18:1) especially in liver, as well as other longer-chain acylcarnitine species. Furthermore, ob/ob and db/db mice display decreases in various medium-chain dicarboxylic acylcarnitines in liver tissue. Specific for db/db mice were alterations in lysine- and tryptophan-derived acylcarnitine species. In summary, while all three models displayed altered concentrations of intermediates in branched-chain amino acid metabolism, with a strong association with muscle tissue in streptozotocin-treated mice, the obese ob/ob and db/db in addition displayed alterations in longer-chain acylcarnitines derived from fatty acids, with a strong association to metabolic alterations in liver.

Genetic and lifestyle factors in metabolic disorders in Mexico

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In Mexico there is a high prevalence of obesity, which is associated with various metabolic disorders such as dyslipidemia, insulin resistance, and hypoadiponectinemia. These conditions are modulated by lifestyle factors (diet and physical inactivity) and genetic variants related to lipid metabolism. To analyze if the presence of metabolic disorders are related with lifestyle factors (diet and physical inactivity) and the polymorphisms Ala54Thr of the FABP2 gene and -493 G/T of the MTTP gene. In a cross-sectional study, a total of 599 unrelated subjects were included. Biochemical tests were determined by dry chemistry on a Vitros 250 Analyzer (Ortho Clinical Diagnostics, Johnson & Johnson Co, Rochester, NY). Serum levels of insulin and adiponectin were determined by an enzyme immunoassay method (ELISA). Insulin resistance (IR) was calculated using the HOMA index. Body composition was analyzed by electrical bioimpedence (In Body 3.0). The food consumption data were analyzed in the Nutrikcal®VO software. Genotyping was performed by allelic discrimination (Taqman, Applied Biosystems) in a termocycler LightCycler 96 (Roche). Statistical analysis was performed in the SPSS Software (version 19). T-student test and chi-square test were used for quantitative and qualitative variables, respectively. Linear regression models, binary logistic regression and univariate general linear model were performed. A p-value <0.05 was considered statistically significant. The 66% of the subjects had abdominal obesity, and 58% physical inactivity. Moreover, 75% of the subjects had an excessive intake of fatty acids, 80% excess in saturated fatty acids, and 66% excess in simple carbohydrates. By contrast, 95% of the subjects had a deficiency in the intake of polyunsaturated fatty acids. Hyperinsulinemia was explained by high intake of simple carbohydrates and obesity (R^2=0.33 P<0.001). Sedentary subjects had a higher prevalence of metabolic disorders than those who practiced physical activity, such as hypoadiponectinemia (59 vs 41%), hypoalphalipoproteinemia (69 vs 31%) and hypertriglyceridemia (66 vs 34%), respectively. In this study, the risk haplogroup (Thr54/-493 T) was associated with an increase of total cholesterol and LDL-c levels (10.32 mg/dl and 9.02 mg/dl respectively; CI 95%, P<0.01). In addition, physical inactivity was related to an increase of LDL-c levels (9.53 mg/dl; CI 95%, P<0.01). Inadequate intake of fatty acids and simple carbohydrates were common among the study population. Abdominal obesity, physical inactivity and the genetic component were associated with metabolic disorders. These findings highlight the importance of design new personalized-nutrition strategies for the prevention and treatment of chronic diseases.

Myostatin in relation to training and its effect on energy metabolism in human skeletal muscle cells

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Myostatin is a secreted peptide mostly known for its inhibitory effect on muscle growth, but it has also been suggested to influence other processes such as insulin signaling and adipocyte differentiation. In this study we investigated the regulation of myostatin in response to acute and long-term exercise and in relation to dysglycemia in skeletal muscle and adipose tissue. We also investigated the metabolic effects of myostatin on cultured primary human muscle cells. We performed an extensive human training intervention study on sedentary men (40-65 y) with either a normal glucose metabolism or dysglycemia. The subjects underwent a 45 min acute bicycle test before and after 12 weeks supervised training. Blood samples and biopsies from m. vastus lateralis and adipose tissue were collected. Total gene expression in biopsies was measured with deep mRNA sequencing. Myostatin mRNA expression was reduced in skeletal muscle after acute as well as long-term exercise and the expression level correlated negatively with insulin sensitivity (GIR, mg/kg/min, r=-0.49, P=0.01). Myostatin was also expressed in adipose tissue and was significantly increased after 12 weeks of training. Furthermore, myostatin expression in adipose tissue tended to correlate positively with GIR (r=0.39, P=0.06) and negatively with body fat percentage (r=-0.41, P=0.04). To study the direct effects of myostatin on energy metabolism, human primary myotubes were incubated with myostatin. This promoted increased basal glucose uptake and lactate production and induced expression of gene indicative of a more glycolytic metabolism. In conclusion, myostatin is differentially regulated in muscle and adipose tissue in relation to training and dysglycemia. Furthermore, myostatin has a role in regulating glucose metabolism in muscle cells.

Proanthocyanidins overexpress POMC and reduce food intake in leptin resistant DIO rats

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Leptin resistance (LR) is a state of elevated leptin levels that fail to suppress appetite and reduce body weight in obesity. To date, various mechanisms have been proposed to explain LR, nonetheless the exact mechanisms in obesity are poorly understood. There are evidences that LR causes alterations in gene expression in CNS, mainly in the hypothalamus. It has been shown that grape seed proanthocyanidins extract (GSPE) improves features of metabolic syndrome. Consequently, the aim of this study was to evaluate the effect of chronic consumption of GSPE on leptin resistance in the hypothalamus. Energy balance including body and fat depots weight, energy expenditure, food intake and respiratory quotient together with leptin receptor ObRb and pSTAT3 protein levels and mRNA expression of the long and short forms of leptin receptor (Obrb and Obra), cytokine signalling-3 (Socs3), protein tyrosine phosphatase (Ptp1b), neuropeptide Y (Npy), agouti related protein (Agrp) and proopiomelanocortin (Pomc) were examined. Three groups of animals (n=7) were fed for 10 weeks either with standard diet (STD), or cafeteria diet (CD). After that, CD rats were supplemented for 21 days with vehicle (CD) or cafeteria diet plus GSPE (25 mg/kg of body weight) (CD+GSPE). Although GSPE treated animals did not show significant differences in physiological parameters, GSPE significantly reduces food intake. Concerning to gene expression, Pomc mRNA was increased in the hypothalamus after the treatment. In addition, treated animals normalized pSTAT3 protein and Socs3 mRNA levels to standard levels in the hypothalamus. These results suggest that proanthocyanidins improve leptin signalling cascade through increment of Pomc mRNA levels in rat hypothalamus which could explain the decrease of food intake. However, further long term studies are needed to elucidate the potential role of GSPE on the improvement of leptin signalling in obesity and metabolic diseases. This work has been supported by the grant AGL2013-40707-R

The effect of age on phenotypic flexibility responses

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Phenotypic flexibility is the ability of an organism to adapt to an external challenge or continuously changing environment. Metabolic adaptation to a disturbance of homeostasis is determined by a series of interconnected physiological processes and molecular mechanisms. The nature of these flexibility responses can determine to what extent an individual can adequately react to external challenges. The onset of many chronic metabolic diseases may result from impairment or loss of flexibility in parts of the system. The objective of this study is to assess whether ageing results in an alteration in phenotypic flexibility. Homeostatic levels of metabolic processes may vary with age, but ageing can also impact on the amplitude of the response to an external challenge, the time taken to reach maximum response, the time required to return to homeostasis and the total exposure (AUC). While homeostatic levels can be measured at a single time-point, a challenge test must be undertaken in order to assess additional responses of phenotypic flexibility over time. This study uses data from the Metabolic Challenge Study (MECHE), on 141 subjects that underwent an oral glucose tolerance test (OGTT). Following a 12 hour overnight fast, individuals underwent a 75 g OGTT. Venous blood samples were taken directly before (0 min) and during the OGTT (10, 20, 30, 60, 90 and 120 min). Full measurement profiles over time are available for ApoB, ApoC2, ApoC3, c-peptide, ferritin, glucose, leptin, insulin, IL6, NEFA, resistin and TNFα. Using linear regression analysis, we assessed the effects of age on both fasting concentrations, and flexibility responses over time (AUC, maximum of curve, minimum of curve) for each of the measured metabolic processes. In order to meet the assumption of normality for model residuals, all AUC responses were log-transformed. The effects of age on the times to reach minimum and maximum values were analysed using an accelerated failure time model, with response equal to the log of the time to maximum (or minimum), and assuming a Weibull distribution. The cohort consisted of 68 males and 73 females, with mean body mass index of 25.6 kg/m^2 and age ranging from 18-60 years with a mean of 32.4 years. Preliminary results show that increasing age was associated with increased fasting concentrations of ApoB, ApoC2, c-peptide, glucose, and ferritin (correlations ranging from 0.23-0.33). There were also corresponding increases in the maximum and minimum amplitudes of these responses with increasing age. Age was positively related to the AUC for ferritin (r=0.23) and c-peptide (r=0.20). The time profile data identified a number of responses that were related to age that had not been identified by fasting data alone. For example, there was a negative relationship between age and the AUC for NEFA (r=-0.22). The minimum amplitude of the curve increased with increasing age for leptin (r=0.19). Older individuals took longer to reach minimum response for ApoC3 (r=0.19) and reached maximum response for TNFα more quickly (r=-0.19). These preliminary results suggest that the subtle effects of age on phenotypic flexibility responses may be overlooked when assessing only fasting concentrations of metabolic responses.

PBMCs of normal- and overweight subjects differentially respond to LPS in Agrimony intervention

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Profiling of peripheral blood mononuclear cells (PBMCs) could serve as less invasive and more direct alternative to tissue biopsies for research purposes. Bacterial lipopolysaccharides (LPS) have been widely used in models studying inflammation response both in vitro and in vivo, as well as the mechanisms of anti-inflammatory activity of variety of substances. Agrimonia eupatoria L. (agrimony) is a poorly studied herb widely used by the Bulgarian traditional medicine. It has been established that the plant is a valuable source of antioxidants, such as polyphenols. Recent intervention study involving healthy volunteers aimed to assess the effect of agrimony infusion with regard to its impact on the inflammatory and antioxidant reactivity of human PBMCs upon ex vivo stimulation with LPS. Subjects were divided into two groups: normal weight individuals with BMI<25 (NW) and overweight – with BMI≥25 (OW). Expression levels of two enzymes related to the antioxidant status (GCLc and SOD1) and two pro-inflammatory cytokines (IL-1β and IL-6) were measured at the start and after the intervention in control and in LPS treated cells. Before the agrimony intake period NW PBMCs responded readily to LPS stimulation, as represented by a strong increase in GCLc ($P<0.001$), IL-1β ($P<0.001$) and IL-6 ($P<0.05$) expressions, demonstrating induction of inflammatory response and flexibility to maintain antioxidant balance. Less prominent was the effect in the OW group. Comparing mRNA levels in LPS treated cells before and after the intervention period it appeared that agrimony tea consumption resulted in significantly lower IL-6 ($P<0.001$) and IL-1β ($P<0.001$) gene expression in the NW group, as well as lower GCLc expression in both NW ($P<0.001$) and OW ($P<0.05$) groups. No significant difference in cytokine mRNA levels of IL-1β and IL-6 was established in LPS stimulated PBMCs of OW group as compared with LPS stimulation before the intervention. Agrimony tea consumption did not affect GCLc, IL-1β and IL-6 expression levels in LPS non-stimulated cells in both groups. LPS treatment did not appear to have impact on SOD1 expression before as well as after the intervention. However, a significant decrease of SOD1 mRNA levels was detected in the untreated PBMCs of the NW group. In conclusion, LPS effectively induced inflammatory response in freshly isolated and cultured PBMCs from NW and OW individuals, gene induction in NW group being more prominent. Agrimony tea consumption suppressed LPS stimulated inflammatory response in PBMCs in NW subjects, confirming the anti-inflammatory potential of the herb. PBMCs isolated from OW subjects remained highly sensitive to LPS stimulation, regardless of the long period of tea consumption. Agrimony consumption resulted also in decreased SOD1 expression in NW subjects, which could be attributed to the antioxidant properties of the herb.

Effect of milk ingredients on glucose and inflammation in overweight subjects

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Postprandial hyperglycemia has been implicated in a low-grade inflammatory response. In this study it was shown that dairy significantly lowers hyperglycemia. A 7-week intervention study, with normal, semi-skimmed milk and a milk composition containing extra whey protein and micronutrients (Lenire composition), was performed to investigate whether these natural ingredients of milk, in higher amounts than normally present, can beneficially affect glucose regulation and low-grade chronic inflammation in elderly, overweight, but apparently healthy subjects. An acute effect on postprandial glucose was demonstrated for both milk and the Lenire composition using the PhenFlex challenge. Drinking semi-skimmed milk before a high calorie meal reduces acute glucose response, without increasing the insulin response. Regular milk is to be preferred to the Lenire composition, which has no additional effect on glucose response reduction, but increases acute insulin response. The longer term intervention showed a small improvement in the postprandial glucose response curve, when milk was consumed before the high calorie meal(Phenflex challenge); without the milk preload, the postprandial glucose response curve was unchanged. The intervention had no effect on fasting concentrations of glucose or insulin, or on postprandial response curves for insulin, there was however a borderline improvement in glycated haemoglobin in the milk group. There was also a weak improvement in most of the inflammation markers after the intervention period, somewhat more pronounced in the milk group. Fasting concentrations of osteoprotegerin and 24 h urinary creatinine were increased after 7 weeks of daily consumption of the Lenire composition, compared to normal milk, suggesting a potential effect on reduction of bone resorption and increase in muscle mass.

Integrating multi-omics data for revealing the effect and mechanisms of Lycium chinense Mill extract

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Oxidative stress and low-grade inflammation have been known as common risk factor of chronic disease. The fruit of Lycium chinense (LC), small red berries in the family Solanaceae, is well-known as a traditional herbal medicine, and nowadays has been widely used as a functional food with variety of beneficial effects including anti-inflammatory activity. LC is rich in anti-oxidants such as carotenoid, and flavonoids that could affect the expression of pro-inflammation cytokines. In this study, we hypothesized that an administration of a LC beverage may exert anti-inflammatory effect in overweight subjects (23≤body mass index<30 kg/m^2) with high LDL-C level (≥130 mg/dl). Fifty-three eligible subjects were randomized to either a LC (n=26) or a placebo (n=27) for 8 weeks in a double-blinded trial. LC beverage was standardized to contain 2 mg of anthocyanin/per serving (80 ml). Transcriptomics analysis of inflammatory mediators and signaling molecules, lipid metabolism, blood cell differentiation, plague formation, coagulation, and antioxidant makers were applied using quantitative real-time reverse transcription-polymerase chain reaction array. Postprandial plasma level of inflammatory cytokines were assessed by proteomics using Luminex assay. In addition, 11 subjects were received the postprandial test after co-administration of a bolus of fat challenge formula and a LC at the final visit. Postprandial blood samples were obtained at 0, 2, 4, 6 and 8 hours after administration. Correlation analysis of transcriptome, proteome, and metabolic parameters responded to LC beverage are underway. The preliminary results described here indicate that daily consumption of a LC beverage may provide a potential health benefits of reducing inflammatory cytokines in subjects overweight and high-level of LDL-C. [This study was supported by grants from RDA (Project No. PJ0084502013) and MSIP through NRF (Bio-synergy Research Project NRF2012M3A9C4048761)]

Seasonal and latitude effects of fruit consumption on biometric parameters and energy intake in rats

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Xenohormesis is a theory that explains how bioactive secondary compounds produced by environmentally stressed plants can warn about deteriorating environmental conditions, allowing the heterotrophs that consume them to prepare for adversity while conditions are still favourable. Consequently, it has been observed that different pathologies, such as obesity, might be caused by an illegitimate stress signalling of food. The aim of this study was to determine whether fruit consumption out of its season can modulate different biometric and physiological parameters in normoweight rats. In addition, the effects of the consumption of one fruit cultivated in different hemispheres on these parameters were also evaluated. 108 male Fisher rats were fed with a standard diet and distributed in three photoperiods in order to emulate season's day length: a normal day (ND, 12h light and 12h darkness), a long day (LD, 18h light and 6h darkness) and a short day (SD, 6h light and 18h darkness). After an adaptation period of one month, the animals of each photoperiod were divided into 6 groups (n=6) depending on the treatment received during 11 weeks. One group received a vehicle whereas the other animals were supplemented with one of the following fruits (100 mg per kg of body weight/day) of different seasons or latitudes: Mediterranean orange and grape (autumn), Mediterranean apricot and cherry (spring) and a South American orange. Compared with the SD rats, an increase of cumulative food intake and body weight gain was observed in grape-supplemented ND rats, and both orange and grape caused a significant increase in relative spleen weight in rats submitted to larger photoperiods. In comparison with LD animals, apricot-supplemented SD rats showed a significant decrease in cumulative food intake that was not accompanied by lower body weight increase. Furthermore, apricot-treated ND rats showed an increase of body weight gain compared with LD rats. Either apricot or cherry increased relative spleen weight in ND rats and apricot also induced a significant increase in testes volume in these animals when compared with the LD group. On the other hand, South American orange treated-rats showed higher cumulative food intake and body weight gain in ND and SD photoperiods than the Mediterranean orange-treated group. The consumption of either winter or spring fruits out of its season, mainly grape from winter and apricot from spring, alters body weight gain, energy intake and relative spleen weight in male Fisher rats. Furthermore, the latitude of origin of the fruit can influence the photoperiodic control of these parameters. The research described here received funding from the Spanish Ministry of Economy and Competitiveness (MINECO), AGL2013-49500-EXP, FRUITOBES project.

Ability of a single meal composition in changing postprandial inflammatory responses

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Changes in dietary habits have contributed to increase obesity rates, an important cardiometabolic risk factor. In contrast to the Western pattern, Mediterranean diet has been associated with cardioprotective effects. It is unknown whether small changes in dietary habits, including Mediterranean food components, could induce metabolic benefits. We investigated the effects of a Brazilian typical breakfast (BRAZ) and a modified breakfast with Mediterranean food (MOD) on fasting and postprandial metabolic profile and pro-inflammatory genes. This crossover clinical trial included 80 overweight individuals with at least one additional cardiovascular risk factor. Participants received one of two isocaloric breakfasts for 4 weeks. Either a BRAZ (coffee, whole fat milk, french bread, butter and mozzarella cheese) or MOD breakfast (coffee, 1% fat milk, whole wheat bread, ricotta cream with olive oil and peanuts) was given in a random order. After a 2-week washout, individuals received the other intervention. Before and after each intervention period, individuals were submitted to a fat tolerance test (FTT) with breakfasts with similar composition to the interventions. Inflammatory markers were assessed by ELISA kit and gene expression was assessed by PCR array in a pooled sample of individuals. Variables before and after each intervention were compared by repeated measures ANOVA. The association between inflammatory and dietary data was assessed using Pearson's correlation coefficient. Participants (51.7±9.5 years) had a mean body mass index of 30.5±4.2 kg/m^2 and waist circumference of 99.7±10.1 cm. At the end of both interventions, participants did not change anthropometry, fasting plasma glucose or triglycerides. Using ANOVA, inflammatory markers showed that BRAZ and MOD breakfast interventions provoked, respectively, contrasting results in fasting E-selectin (13.1±5.0 to 18.1±7.0 vs 14.2±5.9 to 13.2±6.6 ng/ml), TNF-α (3.2±1.3 to 6.1±1.9 vs 3.4±2.1 to 2.7±1.7 pg/ml), IFN-γ (1.5±0.6 to 2.7±0.7 vs 2.0±0.9 to 1.8±1.0 pg/ml), IL-6 (2.3±0.8 to 5.7±1.9 vs 3.1±2.0 to 2.2±1.5 pg/ml) and IL-8 (3.7±2.4 to 4.2±2.6 vs 5.8±4.1 to 3.8±2.5 pg/ml), and also in postprandial responses to FTT (p diet <0.01). Changes in MUFA and PUFA intakes and changes in inflammatory markers were inversely correlated, while changes in saturated fat intake were directly correlated to IFN-γ and IL-6. After the BRAZ and MOD interventions, differences in postprandial relative gene expression (comparing to before intervention) of IL-1α (2.31 vs 0.37), Colony stimulating factor 2 (1.96 vs 0.36) and E-selectin (2.43 vs 0.68), respectively, were observed. Our data indicate that modification of a single meal can improve cardiometabolic risk in the short-term by reducing low-grade inflammation. FTT results show that changing dietary fat may contribute to reduce postprandial inflammation. Further confirmation of our findings should motivate changes in eating habits in non-Mediterranean countries like Brazil.

Expired breath: a non-invasive approach to monitor metabolic status during weight loss

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Human breath can be used as a non-invasive diagnostic of health of the individual, including ageing, inflammation and oxidative stress, lung diseases, and metabolic disorders. Breath analysis of key compounds enables us to interrogate anabolic conditions (after food intake) and catabolic states (fasting) and can be an expressed as a marker of the plasticity of metabolic control. These metabolic processes can be monitored through our ability to measure specific compounds, as indicators of an individual's metabolic state. Here we report on results investigating the dynamics of breath Hydrogen (H_2), Methane (CH_4) [as markers of CHO utilisation] and abundance of natural Carbon 13 (C^{13}) [as a marker of catabolic state] in overweight and obese adults with type-2 diabetes during weight loss on different dietary patterns. Individuals were randomly assigned to consume a hypocaloric, low carbohydrate, low saturated fat diet (LowCHO 14% of energy as carbohydrate, 28% protein, 58% fat) (n=13) or an energy matched high carbohydrate, low fat diet (ModCHO 53% CHO, 17% protein, 30% fat) (n=14) combined with a structured exercise program for 12 weeks (Baseline, weeks 4, 8 and 12). We observed equivocal: weight loss of 10.1% (LowCHO) and 9.8% (ModCHO) and HbA1c reductions of 19.1% (LowCHO) and 15.6% (ModCHO) in both groups. Breath Hydrogen was altered during the intervention (Repeated measures ANOVA: Time P=0.388; Dietxtime P=0.011); The LowCHO group experienced significant reductions in Hydrogen (delta=-8.9 ppm, P=0.013) at week 12 and non significant change in the ModCHO group (delta=+1.7 ppm, P=NS). Methane tended to increase during the intervention in both diets (Time P=0.078, Dietxtime P=0.3). Carbon^{13} enrichment reduced on both diets during calorie restriction; this decrease was larger in LowCHO (P<0.001), as compared with ModCHO (P=0.019) after 4-weeks. These data indicate that changes in metabolism, due to a calorie restricted weight loss diet and lifestyle intervention are measurable and detectable in breath. Non-invasive monitoring technologies could transform how we measure health, how we identify and monitor people most at risk of disease and the way we monitor food intake.

Direct and transgenerational effects of chronic high-fat diet on gene expression in hypothalamus

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Hypothalamus is a central organ to sense nutritional status and regulates energy homeostasis. It has been known that various dietary conditions have an effect on neurology systems, mainly in appetite-regulating neurons in the hypothalamus. Here, we present that chronic high-fat diet (HFD) induces transcriptional changes in the hypothalamus, some of which is transmitted into the third generation in a sex-specific manner. C57BL/6J male mice (F0) were fed with HFD for 20 weeks from 5weeks of age, resulting in glucose intolerance and obese phenotypes. To determine whether HFD has transgenerational effects, we obtained F1 and F2 progeny through breeding with out-bred mice fed with control diet and all progenies consumed only control diet during the life. According to results of microarrays, direct consumption of HFD remarkably up-regulated gene expression of solute carrier family 6 (neurotransmitter transporter, dopamine), member 3 (Slc6a3, 2.6-fold) and dopa decarboxylase (Ddc, 1.8-fold) and down-regulated gene expression of oxytocin (Oxt, 1.53-fold) and nuclear receptor subfamily 4, group A, member 1 (Nr4a1, 1.6-fold). The altered expression of Slc6a3 and Oxt was validated by quantitative real-time polymerase chain reaction (qRT-PCR). Intriguingly, functional network analysis of the genes using STRING v10 suggested that HFD influenced hypothalamic protein network through transcriptional changes of dopamine and oxytocin system. The expression changes were also transmitted to F2 male offspring born to F1 female whose fathers consumed HFD. F0 HFD increased both slc6a3 and appetite-regulating genes, including Agouti related protein (Agrp), and Pro-opiomelanocortin (Pomc) in F2 male but not in F2 female. Taken together, these results imply that direct consumption of HFD alters dopamine-related gene expression in hypothalamus and furthermore affects hypothalamic expression in their third generation, which might be associated with metabolic programming.

Effects of CLA, DHA or Anthocyanins on the expression of browning markers in mouse white fat depots

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The prevalence of obesity is growing nowadays, being more pronounced in the developed countries. For this reason, new studies about natural compounds supplementation and reduction of obesity or its risk factors are taking importance. In this context, the research of molecular mechanisms that promote energy expenditure could be one effective therapy against obesity. The browning process, transformation of white adipose tissue (WAT) into brown adipose tissue (BAT), is emerging as one of these strategies because this transformation leads to changes in the adipose tissue genotype and even phenotype and could prevent diet-induced obesity by increasing thermogenesis and energy expenditure. Some of the most important marker genes of browning process are Uncoupling Protein 1 (UCP1), which mediates thermogenesis and under some stimuli is activated in BAT and other white adipose depots as inguinal WAT (iWAT) or retroperitoneal WAT (rWAT), and PR-domain-containing 16 (PRDM16) which is a transcription factor that induces UCP1 activation and brown adiposities differentiation. On the other hand, Receptor-interacting protein 140 (RIP140) is a corepressor of nuclear receptors implicated in browning and microRNA-133a (miR-133a) has been described to repress PRDM16 expression and, as a consequence, it reduces browning gene mRNA levels. The aim of this work was to determine the effects of a supplementation of natural compounds in the browning process. We investigated the role of Conjugated Linoleic Acid (CLA), Docosahexaenoic Acid (DHA) and an Anthocyanin extract (ANT) in twelve-week-old NMRI male mice by supplementing their chow diet with 100 mg/kg day of CLA (n=10), DHA (n=10) or ANT (n=10) during 15 days. Standard control group (STD; n=10) only received the chow diet and the vehicle. After their sacrifice the expression of marker genes of browning was analysed in iWAT and rWAT. In iWAT, miR-133a, a repressor of the browning process, was reduced by both CLA and DHA supplementation, while UCP1 mRNA had the inverse tendency. On the other hand, ANT supplementation overexpressed RIP140, known as a repressor of UCP1 and browning, despite a down-regulation of UCP1 was not evident in iWAT. However, ANT supplementation downregulated significantly UCP1 expression in rWAT. These results suggest that CLA and DHA could be good promoters of browning process and energy expenditure through the modulation of miR-133a, whereas ANT might be a repressor of some browning pathways leading to an anti-browning effect. Further investigation would be necessary to establish the molecular mechanisms of these compounds to make them useful as anti-obesity treatments administrated together with a healthy diet. This study was supported by grants from the Ministerio de Educación y Ciencia of the Spanish Government (Grant No. AGL2013-40707-R) and Universitat Rovira i Virgili – Banco Sanrtander (Grant No. 2014LINE – 08).

Effect of high-heated food on human metabolome

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Advanced glycation end products (AGEs) formed during high-heat cooking of food have been positively correlated to insulin resistance, markers of oxidative stress and inflammation, potentially increasing the risk of developing of diabetes and cardiovascular disease. However, most of these results are still a matter of debate. One of the main limitations in existing studies is that the evaluation of exposure to dietary AGEs derived from heated foods is based only on a very small number of 'representative' markers, such as Nε-carboxymethyl-lysine. In this scenario, the effect of other potentially harmful compounds produced during the heating process cannot be discriminated from those ascribed to AGEs. The application of untargeted metabolomics by ultra-performance liquid chromatography and mass spectroscopy (UPLC-MS) may offer a more comprehensive assessment of markers related to the intake of heated food and their effects. To explore the effect of the intake of the same meal cooked by two different heating processes on the metabolome of overweight healthy individuals. 20 patients were recruited for a crossover meal test with two meals of identical ingredients prepared by roasting (meal A) or steaming (meal B), respectively. Fasting, postprandial, 24 hours and 48 hours samples of plasma and urine were collected and analyzed by UPLC-MS. Data were preprocessed by MZmine2 ver10 and statistical data analysis was performed using Matlab® and PLS-toolbox. Data were normalized and autoscaled prior the analysis. Partial least squares-discriminant analysis (PLS-DA) was applied to discriminate between subjects randomized to meal A or B at each experimental time point. Cross-validation and prediction of an external test-set were used to test the significance of the models. The best discrimination between the two meals has been observed in data from analysis of urine samples, collected between 5 and 24 hours after meal intake. A clear excretion profile could be observed for the most discriminating metabolites, revealing the presence of markers associated with the intake of high heated food. 16 discriminant metabolite features were identified in the positive mode and 25 in the negative mode. In a highly controlled setting intake of the same food heated at two different temperatures led to clear distinction between excreted metabolites.

The belly fat study – a nutritional intervention to improve metabolic health

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Prevention of diet-related diseases requires an accurate estimation of the individual health status, to allow intervening with dietary and life style changes. Optimal 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 individual's capacity to respond to dietary challenges is called 'phenotypic flexibility'. Phenotypic flexibility can be a very important indicator of individual health status, as it might reflect the (dys)functioning of metabolic organs, such as liver, adipose tissue and gut. The objective of the Belly Fat study was to compare the effects of a 12 week intervention of two different weight loss diets that differ in nutrient quality on organ health, predominantly on lipid accumulation in the liver and on phenotypic flexibility. The application of a mixed meal challenge test was used to gain insight in the phenotypic flexibility. 110 healthy overweight males and females aged 40-70 were randomly assigned to either one of two dietary advice intervention groups or a control group. The dietary advice was provided in two variants; a Western-type caloric restricted diet (-30en%) and a targeted caloric restricted diet (-30en%) that specifically aimed to improve organ health and to reduce lipid accumulation in the liver. The targeted diet was assumed to have a more favorable dietary composition than the Western-type diet, being enriched in monounsaturated as well as polyunsaturated fatty acids including n-3 fatty acids. In addition, the diet contained an increased proportion of complex carbohydrates when compared to the Western-type diet. Furthermore, protein from vegetables sources such as soy were included in this diet. The control group did not receive any dietary advice and were instructed to maintain their habitual diet. Before and after the intervention participants were subjected to an 1H-MRS/MRI-scan to quantify liver fat and assess abdominal fat distribution. On a separate study day before and after the intervention, participants consumed the mixed meal challenge after an overnight fast. Prior to consumption of the meal and up to 6 hours after consumption, plasma, serum, PBMCs and adipose tissue samples were collected and several vascular functions were assessed. At the end of this study day, participants consumed an ad libitum lunch, food intake during this meal was used as measure for satiety. The study was finished in April 2015, a total of 10 participants dropped out of the study of which 1 in the Western diet group, 7 in the targeted diet group and 2 in the control group. Analyses are still ongoing but successful weight loss was established in both intervention groups, namely -7.1±3.1 kg in the Western-type diet group and -8.6±3.1 kg in the targeted diet group versus -0.8±1.7 kg in the control group.

Effects of different dietary nicotinamide riboside concentrations on physiological phenotype in mice

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Metabolic flexibility is characterized by the ability to rapidly switch between carbohydrate and fat oxidation. NAD^+ plays a crucial role in both carbohydrate and fat oxidation as an electron donor or a coenzyme. NAD^+ can be converted from different precursors, one of which is nicotinamide riboside (NR). Previous studies have shown that NR supplementation could promote cellular metabolic state. However, whether NR affects metabolic flexibility is still unclear. Our objective was to examine the effects of different dietary NR concentrations on metabolic flexibility in mice fed a high fat diet. Male C57BL/6JRcc mice arrived at 9 weeks old. After 4 weeks of adaptation on a low fat diet (en10%, 30 mg/kg NR), mice were stratified into 5 groups (n=12 per group). Each group was ad libitum fed for 15 weeks with a high fat diet (en40%) containing 0.10% L-tryptophan and either 5, 15, 30, 180 or 900 mg/kg NR. Body weight, lean mass, fat mass and feed intake were measured weekly. After 10 and 14 weeks, indirect calorimetry (InCa) was performed, including a fast-refeeding challenge. Inca data were used to calculate the delta respiratory exchange ratio (RER) when switching from fatty acid to carbohydrate oxidation ($\Delta RER_{FAO\text{-}CHO}$) and switching from carbohydrate to fatty acid oxidation ($\Delta RER_{CHO\text{-}FAO}$), which may reflect metabolic flexibility. In addition, energy expenditure (EE) and activity were measured during InCa. After 15 weeks, mice were sacrificed and blood glucose, and serum triglycerides (TG), non-esterified fatty acid (NEFA), insulin, leptin, adiponectin were analysed. Results showed that $\Delta RER_{FAO\text{-}CHO}$ was greater in the 30 mg/kg NR group than in the 5 mg/kg, 15 mg/kg or 900 mg/kg NR groups at week 14. Also, the $\Delta RER_{CHO\text{-}FAO}$ was greater in 30 mg/kg than that in 5 mg/kg group. At week 10, however, these differences were not visible yet. EE and activity did not differ at week 10 nor week 14. Similarly, we did not find differences in body weight, lean mass, fat mass and cumulative feed intake over a 15-week period. Although there were no differences in serum insulin, leptin, adiponectin, adiponectin/leptin ratio, TG, NEFA or blood glucose, we found significant correlations between adiponectin/leptin ratio and $\Delta RER_{FAO\text{-}CHO}$ or $\Delta RER_{CHO\text{-}FAO}$. Current results suggest that 30 mg/kg of dietary NR intervention can lead to the greatest metabolic flexibility among the five NR treatments, which cannot be explained by energy expenditure and activities. Notably, this benefit on metabolic flexibility occurs after prolonged NR intervention. The association of metabolic flexibility with serum adiponectin/leptin ratio suggests involvement of adipose tissue in metabolic flexibility.

Oral leptin during lactation prevents metabolic disorders in rats due to gestational undernutrition

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Maternal calorie-restriction during gestation in rats has been associated with lasting detrimental effects on the homeostatic control of energy balance and other metabolic alterations in the offspring, particularly when exposed postnatally to obesogenic conditions. Oral leptin supplementation during lactation has been shown to largely revert the perturbations in the structure and function of key organs, such as the hypothalamus and the adipose tissue, which may partially account for the programmed trend towards obesity-related metabolic alterations in later life. In this study we aimed to assess whether leptin supplementation orally taken during lactation may effectively improve the metabolic profile of the adult offspring of calorie-restricted dams during gestation and their response to an obesogenic diet. Three groups of male Wistar rats were studied: the offspring of ad libitum fed dams (controls), the offspring of 20% calorie-restricted rats during days 1-12 of pregnancy (CR), and CR rats supplemented with physiological doses of leptin throughout lactation (CR-Leptin). After weaning, all animals were fed a standard, normal fat diet (SD) until 4 months of age, and then half of the animals of each group were moved to a high-fat, high-sucrose (western) diet (WD) until the age of 6-months. Body weight, body composition, and food intake were followed. Blood parameters and liver triglyceride (TG) content were analyzed at the age of 6 months. Energy expenditure and locomotive activity were also measured in adulthood by using the LabMaster-CaloSys-Calorimetry System (TSE Systems, Bad Homburg, Germany). Results show that CR animals (under SD) displayed greater adiposity index and feed efficiency than controls, despite showing no significant differences in body weight during the studied period. These parameters were normalized to control levels in CR-Leptin animals. CR animals also showed higher insulin resistance index (HOMA-IR), and higher fasting insulin and circulating TG and leptin levels than controls, both under SD and WD, whereas these alterations were not found in CR-Leptin animals. CR-Leptin animals also displayed lower hepatic TG content than CR animals. Besides, CR animals (under both SD and WD), but not CR-Leptin animals, showed lower locomotive activity and nocturnal energy expenditure compared to controls, in favour of an imbalance of energy homeostasis toward lower energy expenditure. They also showed increased values of the respiratory exchange ratio (RER) compared to controls, under both types of diets, which may suggest an impaired ability to oxidize fat. Notably, leptin treatment during lactation partially reversed this difference in energy nutrient oxidation. In conclusion, oral leptin supplementation with physiological doses during the lactation period in rats largely prevents detrimental effects on energy homeostasis and metabolic alterations in adulthood caused by inadequate fetal nutrition.

Discordant phenotypes for obesity and diabetes, new biomarkers and correlation network analysis

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Discordant metabolic phenotypes for obesity and insulin resistance (IR) or type 2 diabetes (T2D) provide a unique and still unexploited opportunity to examine the interrelations between these associated metabolic disorders, diet, and the gut microbiota. We carried out a high-throughput serum metabolic profiling of 64 adult subjects (19/45 M/F, 22-71 y) assigned to 4 final groups based on their BMI [normal weight versus morbidly obese individuals] and on glycemic control markers [normoglycemic insulin sensitive versus hyperglycemic IR] (protocol approved by the local Ethics and Research Committees, Virgen de la Victoria and Carlos Haya Hospitals, Malaga, Spain). A total of 480 very hydrophilic to very hydrophobic biochemically annotated metabolites were (semi)quantified in serum by ESI-MS-driven analysis. After preprocessing (i.e noisy variables removal, missing data imputation by k-NN averaging, data normalization, removal of age and drug-related confounding effects) a total of 44 metabolic variables significantly differed in multi-group comparison (one-way ANOVA P=0.05, q=0.05, R 3.1.2). With the exception of the aliphatic amino acids glycine and glutamate, the metabolic markers were predominantly lipids. Nine of them also ranked within the top-20 variables selected through all the variable selection techniques implemented, thus representing the more robust metabolic phenotype predictors in multi-group discrimination. Although not reaching the statistical significance in multi-group comparison, several interesting relations between individual metabolites and clinical components of the metabolic syndrome were finally unveiled by intra-group correlation network analysis. In the awareness that the information obtained by circulating concentrations of several of the targeted metabolites may be primarily influenced by exogenous factors, such as diet, or related to biosynthesis in the short term (hence subjected to variability), exhaustive information on dietary intake assessment are highly required in future confirmation studies. The integrative analysis of other biological matrices giving more stable biological confirmation of the data proposed should be taken as target in further studies.

1HNMR-based nontargeted metabolomics approach for predicting diabetes prevalence: the PREDIMED study

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Metabolomics is a new tool increasingly applied to discover biomarkers involved in metabolic pathways. To acquire a better picture of metabolic dysfunctions associated with Type 2 diabetes mellitus (T2D), we investigated whether metabolites from a urine 1H-NMR-based nontargeted metabolomic approach may differentiate T2D and nondiabetic participants, at high cardiovascular risk in the PREDIMED study (n=171) at baseline. A total of 31 metabolites were found to be significantly different between diabetic and nondiabetic participants. Subsequently, the significant metabolites were subjected to a stepwise logistic regression analysis to establish a prediction model of T2D prevalence. It was composed of 10 metabolites, which included increases of glucose, cis-aconitate, phenylalanine, pyruvate, lactate, 3-hydroxyisovalerate and trimethylamine-N-oxide and decreased amounts of trigonelline, phenylacetylglutamine and glutamine. This model had an AROC of 94.0% while individual metabolites had lower values (<80%). In addition, the model score was significantly associated with T2D prevalence after adjusting for covariates (odds ratio=2.20; 95% CI: 1.07-4.54; P=0.033). Our findings highlight the altered metabolic fingerprint associated to metabolites related with glycolysis and gluconeogenesis pathways, amino acids including branched-chain amino acids and tricarboxylic acid cycle. This study reinforce the use of metabolomics and prediction models to discover and evaluate the main metabolic pathways altered in T2D, and hereafter to further investigate on its diagnosis and treatment, which supports the development of personalized medicine.

Computer modelling in predicting efficacy and safety of food components that interact with CYP3A

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All organisms, including us humans, are continuously confronted by numerous natural and synthetic chemicals, also known as 'xenobiotics', whether through eating or aspiration. Cytochrome P450 (CYP) enzymes are heme-protein monooxygenases that catalyze oxidative reactions of a broad spectrum of substrates, mostly lipophilic, and hence play a critical role in the metabolism of xenobiotics, such as drugs, environmental pollutants, dietary products, and also steroid hormones and lipids. Members of the CYP3A subfamily are particularly relevant in this respect, due to their abundance in human intestine and liver, and the vast structural diversity of their substrates. Here we report the use of computational tools (e.g. docking in particular) to predict the nature of interaction, i.e. the inhibition of CYP3A4 by selected polyphenolics, including stilbenes, herbals and candidate therapeutics, and to further predict interactions between consumed phytochemicals and prescribed drugs. Chemically modified stilbenes, cell culture and in vitro reactions, served to support the prediction by computer modelling of docking. The concentrations of CYPs were demonstrated to be 'induced' by xenobiotics, whereas the inducer is also a substrate for the induced CYP. Components in foods, drinks, food additives and orally administered medicines were shown to inhibit CYP3A4 activity and, as a result, increase the actual dose of the drug that reaches the blood circulation in its active form, which often causes unfavorable and long-lasting interactions and probably fatal toxicity. Continuous exposure to these compounds, especially those that activate the xenobiotic nuclear receptor PXR (pregnane X receptor), may lead, in a feedback fashion, to increased expression of CYP3A4 in the intestine, making the food–drug interaction even more complex during extended periods of use. While the above suggests that co-administration of drugs and foods that are rich in polyphenols is expected to stimulate undesirable clinical consequences, it is important to develop predictive tools to group and evaluate the potency of xenobiotics in inhibiting and inducing CYP. Integration of results from several enzymatic models and computer approaches promotes comprehensive understanding of these interactions and their potential hazard.

The nutrigenomic response to a 12 months dietary RCT in CRC patients

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We are currently conducting a large multicenter clinical intervention trial in colorectal cancer (CRC) patients (CRC-NORDIET study) aiming at investigating the long-term effects of a dietary intervention on overall survival and disease-free survival. The aim of the present sub-project is to investigate the large scale nutrigenomic response related to dietary treatment effects. The molecular effects of the intervention will be studied in targeted subgroups of the trial population, aiming at discovering molecular patterns that will be important for future prediction of response to dietary interventions in combination to medical therapies to optimize individualized treatment. Men and women aged 50- 80 years old (n=500), diagnosed with primary CRC (Stage I-III) are invited to the randomized controlled, parallel two-arm intervention trial. The periode of intervention last for 12 months, with follow-ups at 6 months and 1, 3, 5, 7, 10 and 15 years after baseline Participants in the diet-intervention group receive an anti-inflammatory and phytochemical-rich intervention, based on the Norwegian food-based dietary guidelines, merging nutritional counselling with a diverse number of health promoting activities. Participants in the control group continue their habitual diet. Both study arms are offered equal advices and monitoring of physical activity. We have established a comprehensive biobank of samples collected in a consistent, precise manner. The biobank is steadily growing as new participants are recruited to the study and includes PAX tubes, plasma and red blood cells (EDTA, citrate, heparin), serum, dried blood spot cards, isolated PBMCs and tumor biopsies. Estimated patient recruitment period will be 2012-2016. By June 2015, 154 patients have been included in the study where 115 and 82 have reached 6 months and 1 year follow-up, respectively. Blood samples for genomics-, epigenomics-, transcriptomics-, proteomics- and metabolomics analysis are collected at all time-points up to 1 year. In addition the study database accumulates data from questionnaires (nutritional status, dietary intake and habits, quality of life, fatigue, comorbidities, etc.) and data on body composition, anthropometric measures, blood pressure, physical function and activity (accelerometer). Furthermore we collect samples for biomarker analysis (compliance to diet, inflammation and oxidative stress) and samples for characterizing tumor. Finally we collect samples to measure the response to an oral glucose tolerance test. We expect high compliance to the Norwegian food-based dietary guidelines due to the unique extensive intervention strategy. The lifestyle intervention may ultimately lead to enhanced overall and disease-free survival and have the potential to improve the overall health and quality of life of colorectal cancer patients. The study will broaden our knowledge about the molecular mechanisms behind both short-time and long-time intervention-induced changes.

Describing food by foodomics

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Food science, which links in a multidisciplinary way a variety of scientific fields, such as chemistry, biology and genetics, agriculture, medicine and veterinary, needs more and more effective post-genomic tools to evaluate the quality of food and its impact on health. In this perspective, food cannot be regarded as static systems, but the food quality is a dynamic property that integrates direct and indirect consequences of human practises and environmental factors. An increasing number of applications assessing the food quality uses the foodomics science to evaluate the influence of the genetic selection and the changes occurring in different growing conditions (for example, organic compared to conventional) and breeding, geographical origin, protocols for food production. Moreover, the foodomics approach, applied to the in vitro digestion of food products, helps to evaluate the food matrix effect on bio-accessibility of nutrients. A broad coverage of foodomics applications, including food products of animal and plant origins, will be presented.

Suplementation with olive oil had an effect on lipid metabolism in metabolic syndrome

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One of the main components of Mediterranean diet is the olive oil (OO). Its consumption has been recognized to modulate oxidative stress and an anti-inflammatory effect, mainly in liver. Generally, supplementation studies in rodent models have been conducted with high concentrations of OO. These content do not represent the consumption in human Western diets. The objective of this study was to evaluate the effect of the intake of low concentrations of OO on circulating lipids, fat deposition and gene expression in liver, muscle an adipose tissue in obese and lean Zucker rats. This is a suitable animal model of human metabolic syndrome. At 2 weeks of age male Zucker Fa/Fa rats (n=15) were randomly assigned to diets. Lean Zucker rats were fed with control diet (1.3% corn oil), obese Zucker rats received isocaloric chow diet with olive oil (OO) (0.5% of total fat) or control diet (corn oil) during 6 weeks. Obese Zucker rats fed with control diet increased levels of LDL, VLDL, cholesterol, triacylglycerols, insulin and fasting glucose as compared with lean Zucker rats in response to control diet. The body weight gain was significantly different between lean rats control and obese and OO diet. Histological analysis of liver, muscle and adipose tissue showed an abnormal accumulation of fatty acids in the obese group fed with the control diet. Supplementation with OO reduced some biochemical parameters of the metabolic syndrome in obese Zucker rats. The intake of OO reduced the expression of leptin, PPARa and FABP4 in abdominal adipose tissue, in comparison with obese Zucker with control diet. Expression of SREBP-1a and AdipoR2, also, decreased in hepatic tissue and muscle, respectively. Beside olive oil supplementation reduced the accumulation of fatty acids in liver and muscle compared with obese Zucker control diet. In conclusion the present study demonstrated that a low concentration of olive oil (similar to human consumption in Western diets) could prevent some parameters of metabolic syndrome through regulation of lipid metabolism.

Medicinal food: From ancient times to present

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'Let food be thy medicine, and medicine be thy food' Hippocrates 460-379 B C. The medicinal properties of certain traditional foods is honey, nuts, spices, olive oil and wine have been recognized since biblical times Medicinal foods from ancient times to the present. Similarly wrapping medication in food or mixing bitter medicine with fruit juices, wine or honey to make it more palatable or easier to swallow. Only in recent years, the FDA has established a 'New class of drugs' MEDICINAL FOOD. The target is being in this case Alzheimer sufferers with difficulty swallowing or memory issues affecting compliance with drug taking. Another benefit to this product is the added nutritional value of the product which can be enhanced to address whatever other deficiency the individual has in a more natural manner. We have patent rights pending for a food product concept combining a high affinity Phosphate chelating agent ($NaHCO_3$), and a modicum of magnesium lactate in a food product which can be (1) a cookie; (2) a bread stick; (3) a corn or potato mix chip, a cup cake, Ice cream/ frozen yogurt. The cookie was tested on 12 Peritoneal Dialysis patients all exhibiting various levels of Hyperparathyroidism and moderately severe hyperphosphatemia, (4.9 to 6.7 mg/dl) The palatability and acceptance has been virtually accepted universally even by those who ordinarily 'do not eat sweets'. The preliminary results of their effectiveness in terms of phosphate control are encouraging. Whether because of better compliance as this is 'new medicine' or because indeed better mixing of the medication with the food bolus results in better chelation of the phosphorous in the gut. These results open the door to more extensive use of 'food medicine' or medicine in food in many areas of modern medicine. More Studies Are thus warranted

The relationship between pancreatic β-cell function and the proteomic signature in healthy adults

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Proteomics in the study of type 2 diabetes mellitus (T2DM) has potential to be a highly useful technique in both improving the incidence of T2DM diagnosis and monitoring the various stages of disease progression. Gaining knowledge of the proteome may also aid in the development of new targets/ lifestyle interventions in the prevention of T2DM. The objectives of this study are to (1) examine the impact of phenotypic parameters on the proteomic signature and (2) uncover protein signatures related to pancreatic β-cell function. This research focuses on data obtained from the Metabolic Challenge Study (MECHE). 214 healthy participants aged between 18-60 years were recruited and randomised to receive an oral glucose tolerance test (OGTT), an oral lipid tolerance test (OLTT) or both, on two separate clinical visits. Both β-cell function adjusted for homeostatic model assessment of insulin resistance (HOMA-IR) and the disposition index (D.I.) were calculated for participants (n=100) who completed an OGTT and had complete proteomic data. The MECHE proteomic dataset contains information on 1129 proteins. The impact of gender, BMI, age and β-cell function measures on the proteomic signature was assessed using principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), orthogonal partial least square discriminant analysis (OPLS-DA) and linear regression. PCA reduced the proteomic data into seven protein patterns. PCA along with PLS-DA and OPLS-DA models showed that gender and BMI had a large effect on the proteomic profile. Out of the seven protein patterns, three were significantly associated with gender and four were significantly associated with BMI; while two protein patterns were significantly associated with ageing. Functions of proteins associated with gender included DNA binding, hormone binding and mineral transport. Examples of proteins strongly associated with BMI were complement component 4, C-reactive protein and APOE-2, E-3 and E-4. Functions which dominated age associated proteins include lipid metabolism, cell signalling and bone mineralisation. Pancreatic β-cell function and HDL cholesterol were significantly negatively associated with protein pattern 4 (β-coefficients of -0.221 and -0.264 respectively), whereas triglycerides and total cholesterol were significantly positively associated (β-coefficients of 0.221 and 0.554 respectively). Proteins which functions include lipid metabolism and immunity influenced protein pattern 4, with APOE proteins dominating the positive loadings. In conclusion this study identified proteins whose levels were influenced by BMI, gender and age. Furthermore, this analysis uncovered a protein signature related to β-cell function. Early detection of decreased pancreatic β-cell function would allow for implementation of nutritional and lifestyle interventions before progression into T2DM status. Future work includes further investigation into protein function and proteomic pathway analysis to identify potential pathways modulated.

Gene expression signatures in blood: responses to dietary intervention

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Diet is critical for maintenance of optimal health and reduced disease risk. Assessment of dietary modulation of health and disease status in humans has been limited to date as a consequence of difficulties in accessing target tissues and observed inter-individual responses to nutrition. The use of human peripheral blood as a means of assessing health and disease status of non-hematologic tissue and organ systems in the human body is gaining momentum. Together with recent innovations in gene expression technologies it is now clear that gene expression profiling of human blood to determine predictive markers associated with health status and modulation by diet is now a feasible prospect to investigate diet-gene interactions. Maintenance of homeostatic regulation in response to nutritional challenges is an indicator of health status. Cell defence systems, immunity, inflammation, redox regulation, metabolism and DNA repair are essential to maintain homeostatic regulation and are impacted by nutrition. Human whole blood presents opportunities to assess cell defence system status in humans and identify food formulations to improve health status. Principal component analysis of blood gene expression profiles reveal characteristic gene expression patterns within study populations of human volunteers and identify cell defence system gene marker changes in gene expression profiles associated with differences in individual volunteers, meal formulations and time. Human blood and the dynamic cell population it contains is a useful biological indicator to determine predictive signatures indicating health status and the impact of exposure to diet. Predictive gene signatures will require functional and biological validation to generate confidence in prediction of health status. However, the data presented indicates that this approach is feasible in monitoring and surveying the impact of diet-gene interactions to generate evidence for effective translation of research on food, drink and health.

EuroDISH: shaping a food, nutrition and health research infrastructure for Europe

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EuroDISH (www.eurodish.eu) is a European ESFRI project directed at describing the available and needed research infrastructure (RI) for food, nutrition and health research in Europe. NuGO is one of the project partners. The initial funded phase was completed summer 2015. The inventory of the needs for RIs was done following the DISH pillars: Determinants, Intake, Status and Health. The main outcomes were summarised in the Milano Milestone, which was presented in the EU Pavilion at the World Expo 2015 in Milan, May 15th, 2015. A first important outcome is that there is a need for overarching research between the DISH pillars. This enables us for instance to understand how psychological factors influence what we eat and in the end affect our health, but receive feedback from satiety signals that are part of our biological status. Infrastructure supporting especially data structures, communication, and analysis needs to enable such overarching research. Besides obviously supporting academics in linking existing scientific approaches and expertise to allow cutting edge research, an overarching European RI will also benefit policy makers, food industry, and societal organisations and professionals in their need for integrated scientific insights to steer, innovate, and advice related to food (products) and health. Furthermore, the EuroDISH inventory concluded that the food, nutrition, and health research field will benefit from access to public and private data, at larger scale and across disciplines, as well as innovative and standardised research tools. Participation in a research community across DISH disciplines and wider participation across countries will enable alignment and more efficient use of funding and resources. An implementation strategy for the needed overarching RI, requires a well-governed E-platform that facilitates and stimulates connection of existing and future initiatives. It also requires support in national roadmaps and strategic agendas, political and financial commitment at Member State level to build national hubs, and commitment at European level to guarantee a sustainable research infrastructure with an ERIC (European Research Infrastructure Consortium) status that is aligned with global initiatives. NuGO's involvement has mainly been in the inventory of requirements for the I and S pillars, for example by organising a workshop and survey during last year's NuGO week in Naples. Several types of studies and data types were identified to be specific or most important for nutritional research: particular study designs like cross-over and challenges, metabolomics, diet-microbiome interactions, and whole genome methylation. For other types of experiments and data processing, the RI for food, nutrition, and health will connect to complementing initiatives, including ELIXIR, ISBE, ECRIN, Biomedbridges, BBMRI, JPI-HDHL (ENPADASI for dbNP), among others, to fully integrate into the European landscape of RI-related consortia.

Folate deficiency and DNA-methyltransferase inhibition modulate guanine-quadruplex frequency

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G-quadruplexes (G4) are highly stable tetra-stranded DNA secondary structures known to mediate gene regulation and to trigger genomic instability events during replication. G4 structural stability can be affected by DNA methylation and oxidation modifications; thus nutrients such as folate that have the ability to alter these processes could potentially modify the genomic occurrence of G4 elements. Hela cells were cultured in a range of folate concentrations or in the presence or absence of 5-aza-2'-deoxycytidine, a DNA-methyltransferase inhibitor. G4 structures were then quantified by immunofluorescence using an automated quantitative imaging system. G4 frequency in Hela cells and nuclei area mean were significantly increased in 20 nM folate medium compared with 200 nM and 2,000 nM folate, as well as in the presence of 5-aza-2'-deoxycytidine when compared to cells non-exposed to 5-aza-2'-deoxycytidine. These changes were exacerbated when pyridostatin, a G4 stabilizing ligand, was added to the culture medium. G4 intensity in Hela cells cultured in deficient folate condition with pyridostatin was highly correlated with DNA damage as measured by γH2AX immunofluorescence (r=0.7). This study showed for the first time that cellular G4 balance is modifiable by low folate concentrations and that these changes may occur as a consequence of DNA hypomethylation. Although the exact mechanism by which these changes occur is unclear, these findings establish the possibility that nutrients could be utilized as a tool for sustaining genome integrity by modifying G4 frequency at a cellular level.

Nrf2 inhibition and Snail-1 activation by caffeine, a nutrigenomic study

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Activation of hepatic stellate cells is crucial in liver fibrosis, which is perpetuated by growth factors and pro-inflammatory molecules. Caffeine has been shown to be able to modify these events in vitro studies. Determine if caffeine prevents experimental liver cirrhosis. Liver cirrhosis was induced in Wistar rats. Animals were treated with caffeine (15 mg/kg/day). The fibrosis degree and inflammatory infiltrate were evaluated and classified by Knodell index. Inflammatory infiltrate was quantified by immunohistochemistry. Gene expression was analyzed by QRT-PCR for Col-1, CTGF, TGF-beta1, TNF-a, IL-1b, IL-6, SOD and CAT. Nrf2 and the activation of Snail-1 was analyzed by western blot. Expression of TNF-a was checked by ELISA, and the activity of SOD and CAT antioxidant enzymes was determined by zymography. Treatment with caffeine decreased fibrosis index. Knodell index also showed lower levels of fibrosis and necroinflammation. Expression of pro-fibrogenic genes CTGF, Col-1 and TGF-b1, and pro-inflammatory genes TNF-a, IL-6 and IL-1b significantly decreased in rats that received caffeine. Caffeine treatment decreased CD11b positive areas. SOD and CAT expression was greater in animals treated with caffeine, and we found a strong correlation with the enzymatic activity. Lower levels of the transcription factor Snail-1 were detected in treated rats, in contrast to NRF2, which increased in the presence of caffeine. Our results suggest a potent effect of caffeine to limit pro-fibrogenic and pro-inflammatory response even in the presence of an inducer of liver cirrhosis. The mechanisms by which caffeine acts involve inhibition of transcipcional factor Snail -1, down-regulation of the expression of pro-fibrogenic genes as well as the activation of transcription factor Nrf2 which in turn activate the antioxidant enzyme systems, resulting in the prevention of inflammation and fibrosis. This results could be applied in different types of fibrosis.

Proteomic analysis of Zn depletion/repletion in the hormone-secreting rat thyroid cell line FRTL5

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The human zinc (Zn) proteome was predicted to represent about 10% of the total human proteome. The micronutrient Zn is indeed known as cofactor for over 300 enzyme activities, as well as an essential component of transcription factors and other structural/regulatory proteins. The predisposing effect of Zn deficiency on risk of developing a wide spectrum of chronic disease has also been reported. In an effort to identify pathways potentially affected by Zn deficiency/excess and playing important roles in disease development, we have recently performed an in silico approach which extended the Zn proteome by also including the Zn-binding protein interactome. Such Zn protein interaction network includes proteins involved in hormone-dependent physiological processes, some of which are known to be regulated by intracellular, vescicular Zn transporters at the level of hormone secretion. We have previously shown that the ZnT8 transporter, primarily expressed by pancreatic beta cells where it regulates insulin secretion, is also present in specific endocrine cell types of pituitary, adrenal glands and thyroid, suggesting a more general role in regulating hormone secretion. Experimental evidence has been provided for a role of Zn in normal thyroid homeostasis, which might involve thyroid hormone biosynthesis. To determine a possible causal relationship between intracellular Zn levels and thyroid function, we set up mild Zn deficiency conditions in the FRTL-5 in vitro cell model, derived from a Fischer rat primary thyroid culture and displaying a primary thyroid cell phenotype in terms of growth properties, thyroid-stimulating hormone (TSH) dependence, expression of TSH receptor and ability to synthesize and secrete thyroglobulin. We then performed proteomic analysis by comparing cells whose intracellular free Zn pool had been depleted by treatment with the specific Zn chelator TPEN, and cells in which Zn concentrations were restored by addition of $ZnSO_4$ in the culture medium. Quantitative Proteomic analysis was carried out on total extracts by using stable isotope dimethyl labelling approach, coupled to nano-HRLC-MS identification. Quantification was performed by comparing the relative abundance of differentially labelled peptides by MaxQuant analysis software. The results identified approximately 500 proteins, about 30% of which were differentially regulated. To compare the three experimental conditions (control, Zn depletion and Zn depletion/repletion), cluster analysis and principal component analysis (PCA) were performed. Preliminary results show differential regulation of about 30 proteins, among which 7 ribosomal proteins were strongly upregulated and voltage-dependent ion channels and histones were strongly downregulated in Zn repleted cells, as compared with Zn depletion alone. Further analysis is in progress to confirm the results and to connect all differentially regulated proteins with specific pathways related to thyroid function.

LC-MS based metabolomics reveals markers of beer intake

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In epidemiological studies, moderate alcohol consumption is shown to have beneficial health effects. To assess the alcohol intake well-established biomarkers (i.e. ethyl-glucuronide and ethyl sulfate) are present. However, novel biomarkers characterizing the exact origin of consumed alcoholic beverage is still lacking. Thus we applied untargeted metabolomics approach for identification plasma and urine metabolic patterns related to beer intake and utilize those (1) to establish novel beer intake biomarkers and (2) to explore for the potential underlying mechanisms of health associated acute effects of beer intake. A meal study – randomized crossover, single-blinded intervention with a study period of 4×3 days – was conducted with three different beers (strong pilsner, regular pilsner and alcohol-free pilsner), and a soft drink. 18 healthy men and women in the age group 18-60 were enrolled in the study. Plasma and urine samples were collected before and at various time points after the intervention (45, 120 and 180 min for plasma and 90 min, 90-180 min and 180 min-24 h for urine). In addition to the samples, test beverages, wort and hops extract used in the production of the beer were analyzed with ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry. Anova simultaneous component analysis (ASCA) was used to isolate the effect of beer and the discriminant metabolites (beers vs soft drink) were selected by partial least squares discriminant analysis (PLSDA). Comparison of markers of beer intake with metabolic profiles of test beers and beer raw materials revealed 14 metabolites originating from hops extract (two in plasma and urine and 12 in urine), ten from wort (four in plasma and seven urine), 25 from fermentation (only in urine) and 41 from human metabolism (one in plasma and 40 in urine). Some examples of hops originating plasma and urine markers are iso-alpha-acids (iso-cohumulone and mixture of iso-humulone and iso-adhumulone). Those are observed as early excreted metabolites in plasma (45 min) whereas in urine they peaked after 90 min. Amino acids, iso/leucine and tyrosine, are identified as wort originating markers of beer intake. As a conclusion, beer intake lead to subtle changes in plasma and urine metabolic profiles, comprised of not only beer components but also metabolites derived from human metabolism.

Vitamin D levels in risk groups of Bulgarian population: preliminary data

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There is mounting evidence in the last 1-2 decades for the so called 'non-mineral' effects of vitamin D and for the connection of its deficiency with a number of chronic conditions, such as chronic kidney disease (CKD), immune disorders, and different cancers. CKD is among the diseases with higher rate in population. It is characterized by low levels of the active form of vitamin D3 in the early stage, prior to detection of any significant changes in plasma calcium, phosphate and parathyroid hormone to be elevated. Recent studies show a positive correlation between the deficiency of the circulating form of vitamin D3 (25OHD), the severity of CKD, the onset and gravity of anemic syndrome in renal failure patients. Recent in vitro studies on erythroid bone marrow cells have shown that 25OHD stimulates their proliferation and the expression of erythropoietin receptor. Studying the dynamics of 25OHD changes and the relation with CKD severity and parameters evaluating calcium metabolism, and erythropoiesis will be a prerequisite for adequate therapeutic measures. Prostate cancer (PCa) is the most common solid malignancy in males. Human prostate cells express vitamin D receptor for the active form of vitamin D (1α,25-dihydroxyvitamin D), which stimulates differentiation and inhibits the proliferation, invasiveness, and metastasis of PCa cells. However, the relationship between vitamin D levels and PCa progression is still a matter of controversy. Aim: To examine the vitamin D levels in two groups of risk patients: with CKD and prostate cancer, and to evaluate the relationship with the disease severity, laboratory and clinical prognostic markers. The first risk group enrolled in the study comprised 40 patients in different stages of CKD. The second group included 52 patients with laboratory (elevated PSA) and/or clinical (abnormal digital rectal examination) suspicion for PCa. Blood samples for vitamin D levels measurement, were collected from each patient. Serum 25-OH vitamin D levels were assayed by LC-MS/MS. The differences between vitamin D levels in various groups were assessed by Student's t-test of variance. All CKD patients were vitamin D deficient (36.63±24.30 nmol/l), a 27% below the cut-off value of 50 nmol/l, defining vitamin D sufficiency. There was no significant difference in 25OHD levels between the overall population, male and female CKD patients. The final pathological results revealed 24 cases of PCa and 28 cases of benign prostate diseases – chronic prostatitis and/or benign prostate hyperplasia (BPH). The statistical analysis showed a clear trend demonstrating lower levels of 25-OH vitamin D among PCa patients, compared to those with BPH (t=1.96). A significant decrease in 25-OH vitamin D levels was established in PCa patients with aggressive, high-grade tumors: those with Gleason score >7 ($P<0.05$). Our results suggest a potential beneficial role of vitamin D in PCa patients. The interrelationships between 25-OH vitamin D levels and various laboratory and clinical parameters, could be used for diagnosis and prognosis of CKD and PCa.

Food metabolome biomarkers associated to dietary patterns in healthy volunteers

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There is a growing interest in nutrition epidemiology to identify biological markers related with the intake of nutrients, foods and dietary patterns associated with prevention of diseases. This study aims at identifying urinary biomarkers to describe the dietary patterns in healthy young individuals. This is an observational study done in the Campus de l'Alimentació-Torribera (University of Barcelona) during the academic course 2014-2015. Forty-four healthy volunteers were included. Inclusion criteria were: non-smokers, healthy and being among 18 and 25 years old. Participants recorded their dietary habits with a validated food frequency questionnaire (FFQ). In two different visits (separated three months), the participants collected two samples of 24-hour. FFQ data were grouped into 28 categories and a k-means cluster analysis (Metaboanalyst 3.0) was used to identify the dietary patterns. Urine was analyzed by LC-q-Tof (Applied Biosystems). Differences in the urinary biomarkers between patterns were analyzed using the metabolomics R package MAIT. K-means analysis identified two dietary patterns, one characterized by significant higher intake of whole grain cereals, fruit, vegetables, pulses, coffee, moderate wine, nuts and dried fruit ($P<0.05$) (Healthier Dietary Pattern, HDP) while the other cluster was characterized by significant higher intake of refined grain cereals, processed meats, snacks and high energy beverages ($P<0.05$) (Unhealthier Dietary Pattern, UDP). Urinary food metabolome showed differences between both dietary patterns. The HDP was characterized by higher urinary excretion of urolithin A glucuronide, a characteristic microbial metabolite of nuts consumption, of 4-hydroxyhippuric acid, a microbial metabolite related to higher intake of vegetables and derived foods, proline betaine, which is related to orange consumption and trigonelline that is a metabolite linked to coffee consumption. The UDP was characterized by higher urinary of tyrosine sulfate, an endogenous metabolite associated to cardiovascular risk factors. In conclusion, these results indicate that HDP and UDP are reflected in the urinary metabolome. Among the HDP biomarkers, it is to note that microbial derived metabolites could be very useful to evaluate food intake in epidemiological studies. Furthermore, UDP biomarkers could be related with future diseases in epidemiological studies.

A new chemical library of food compounds and food-derived metabolites developed in FOODBALL

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FOODBALL (Food Biomarkers Alliance) is a large collaborative project (22 partners from 11 countries) funded by the JPI HDHL (2015-2017) which includes a systematic exploration and validation of nutritional biomarkers to obtain a good coverage of the food intake in different population groups within Europe. One of the aims of FOODBALL is to develop new online resources to facilitate identification of nutritional biomarkers using metabolomics. A major limitation in metabolomics is the lack of commercial standards to validate putative identifications. The FOODBALL chemical library will facilitate the sharing of not easily accessible standards for diet-derived compounds. It will be a virtual library, with compounds stored in the laboratory where they have been isolated. Version 1.0 will be an online catalog of pure compounds and reference materials (food extracts, biofluids from animals fed pure compounds, incubation media from in vitro systems to produce metabolites…) made available by FOODBALL partners and associated collaborators. The catalog will contain the list of available compounds with associated data including elemental formula, monoisotopic mass, solubility, origin, purity, available quantity, storage conditions, stability, links to existing databases, type of spectral data available and contact details of the laboratory offering to share the standard. The catalog will be queryable by compound name and chemical structure. In the final version, which should be available at the end of 2016, spectral data (GC-MS, LC-MS, NMR, UV, IR) collected in standardized formats will be made queryable online. Anyone interested in one compound in the catalog will directly contact the provider. A bilateral negotiation will define the terms of collaboration. A financial compensation in addition to the shipping fees is possible if agreed. Contributors and users will have to respect a charter of good practices. For example the provider will have to indicate on a packing slip the minimum information regarding the appearance of the product, quantity, recommended storage conditions, stability and safety information if available. The acquirer will bear all shipping costs, and will have to share the spectral analyses he has acquired on his own analytical platform. This will continuously enrich the content of the chemical library. The library will allow to post a demand for a non-available compound, to stimulate its synthesis or isolation. The greatest need is for human metabolites of food-derived compounds. The FOODBALL chemical library is a collaborative initiative widely open to new contributors/users. Anyone interested to contribute can contact us.

A unique blend of natural compounds opposes age-related changes in gene expression

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The median age of populations around the globe is increasing and with a greater portion of the population living to an advanced age comes an increase in social and economic burdens. Therefore, strategies to attenuate the negative effects of aging, including supplementation, are of great interest. A unique blend made up of vitamins, fatty acids and phytochemicals was designed to improve 'healthspan' – the length of time that people live without experiencing many of the negative side-effects of aging. To date, caloric restriction (CR), typically a 30-40% reduction in caloric intake compared to ad libitum fed controls is the only intervention consistently demonstrated to improve healthspan across a broad range of species. Therefore, we used calorie restriction as our positive control to study the healthspan promoting effects of ingredients and ingredients blends. The purpose of this study was to test the effects of long-term feeding (18 months) of a blend ingredients not readily available in a healthy diet on age-related changes in gene expression and gene ontology pathways compared to age-matched controls and CR mice; CR was included as a positive control. Four groups of B6C3F1, male mice were studied; N=8 per group. Long-term fed mice consumed one of three diets from 12 to 30 months: (1) Old Controls (OC)(fed AIN93 diet); (2) old CR (OCR)(25% calorie restriction compared to the control group (fed a modified version of AIN93 diet)); and (3) old supplemented (OS)(fed AIN93 diet plus a blend of natural ingredients). The fourth group was made up of young controls (YC)(fed AIN93 diet from 2-5 months of age). Microarray analysis was performed in multiple tissues; gene expression profiling was performed using Affymetrix Mouse Gene 1.0.ST arrays. The dietary supplement effectively opposed a number of age-related changes in gene expression and positively influenced pathways associated with healthspan. Furthermore, the supplement elicited a gene expression profile similar to that of the OCR group. In conclusion, a unique blend positively influenced gene expression related to biomarkers of healthy aging. These effects, elicited by a mid-life nutritional intervention, could have positive implications for healthy human aging or 'healthspan' and warrant further investigation.

Activation of p53 and antioxidant relevant transcriptional response by broccoli in colon cancer cell

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The tumour suppressor protein p53 plays an important role in the regulation of cellular antioxidant defence systems. We investigated if fruit and vegetable extracts, previously shown to regulate the activity of transcription factors nuclear factor kappa beta (NF-kB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2), can also induce cellular defence processes, by enhancing p53-regulated transcription and expression of downstream target genes. Fruit and vegetable samples were extracted using water/methanol. A luciferase-based reporter assay was used to screen for p53-inducing extracts in the colon cancer cell line HCT116. Regulation of p53 target gene expression was analysed using custom made TaqMan Array Microfluidic Cards in HCT116 colorectal cancer cells, wild type and p53 knock out (p53KO), and non-transformed fibroblasts. Of 19 extracts tested, broccoli, onion, tomato, carrot and basil extracts significantly induced p53 transcriptional activity. Broccoli and onion extracts were further tested for their ability to regulate expression of antioxidant and cancer related genes. We found that broccoli, but not onion, induced a transcriptional response in both wild type HCT116 cells and fibroblasts. Although this response was less pronounced in p53KO cells, a set of commonly regulated genes was still induced in all HCT116 cells, suggesting a partially p53-independent response, likely mediated via the Nrf2 pathway. Overall, a greater number of regulated genes, many with importance in metabolism and ROS regulation, was found in wild type colon cancer cells. In conclusion, we identify broccoli as a potent inducer of p53 and relevant gene expression in vitro.

C34T polymorphism of the AMPD1 gene in endurance athletes

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Adenosine monophosphate deaminase (AMPD) is one of the most important regulators of muscle energy metabolism during exercise. AMPD displaces the equilibrium of the myokinase reaction toward ATP production (2 ADP → ATP + AMP) by converting AMP to inosine monophosphate (IMP). An activity of this enzyme, encoded by AMPD1 gene, can be affected by the C34T genetic polymorphism (rs17602729). We studied frequencies of C34T variant in a group of triathletes who were participants of Czechman Triathlon Race 2013 in the Czech Republic. Buccal swap samples were taken from 118 competitors but from only 101 of them we gained complete data including the final race time. Our results showed that frequency CT genotype was overrepresented in a group of athletes who finished the race in 300 min compared to athletes did not (36.1 vs 12.3%; P=0.007). TT genotype was missing in our group of subjects. These results are quite surprising in the light of previously published studies where 34T allele is considered to be relatively unfavorable for sports performance in general. On the other hand our results are consistent with the recently published study Lifanov et al. (2014) showing higher frequencies of 34T allele in soccer players compared to sedentary controls. Thus 34T allele could potentially be useful for long-term endurance activities.

Metabolomic profiling dairy product intake in healthy men

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With a third of the human diet made up of fermented foods, the health-promoting properties of fermented dairy products represent a major area of research with potential implications for public health policy and food industry development. Despite evidence from numerous studies that describe metabolic and immune-modulating properties of fermented dairy products, there remains controversy concerning the validity of these proposed properties, with the mechanisms underpinning their effects not yet well-established. Recently introduced to the field of nutritional studies, metabolomics has already shown its potential to identify biomarkers of food intake and regulated metabolic pathways; its use is thus proposed here to characterize the effects of milk and fermented dairy products consumption on health. In a double blinded cross-over clinical study, fourteen healthy male volunteers were recruited to test a yoghurt containing the widely used probiotic Lactobacillus rhamnosus GG (LGG) and a non-fermented milk as a control. The dynamic response to the acute (single dose of 800 g) and chronic (400 g/day for 2 weeks) ingestion was then evaluated by metabolic profiling of serum and test products using liquid chromatography-mass spectrometry. Prior to analysis, proteins and phospholipids were removed from samples using acetonitrile/formic acid and a phospholipids removal set (Phenomenex Phree®). The metabolome was then measured by liquid chromatography-mass spectrometry (UHPLC/UHR-QTOF), repeatability and signal drift were assessed with the use of quality controls (pool of samples). Peak detection and preprocessing were performed using Progenesis QI® (Waters) and the XCMS R package; statistical analysis was conducted with Systat® and R (v3.2.1) software. This untargeted approach will be combined with nutrikinetic and nutridynamic analyses to correlate the postprandial serum after single dose intake, the fasting serum after two weeks intervention and the test product metabolomes, with the aim to identify specific food biomarkers, their related metabolic pathways and potential associated health outcomes.

Effects of grape seed-derived phenolic compounds on ghrelin secretion

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The hormonal secretion in the enteroendocrine cells is modulated by several pathways, including nutrient sensing mechanisms involving bitter taste receptors (TAS2R). Recently, it has been reported that bitter tasting phenolic compounds such as flavanols specifically activate TAS2R14 and TAS2R39. Nonetheless, the effects and mechanism of action of dietary phenolic compounds on the secretion of the hunger hormone ghrelin have not been studied. In this work, we first studied the effects of gallic acid (GA), monomeric flavanols and proanthocyanidins on in vitro ghrelin secretion, focusing on the role of bitter taste receptor signaling. Thereafter, we studied the effects of the admiministration of a grape-seed proanthocyanidin extract (GSPE) on in vivo ghrelin secretion, food intake and body weight gain in rats. For the in vitro studies, a mouse ghrelinoma cell line (MGN3-1) was used to assess the effects of GA, (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate (ECG), the procyanidins B2, B2 gallate and C1 and GSPE. The specific TAS2R14-39 bitter blocker 4'-fluoro-6-methoxyflavanone and the PLCb-2 inhibitor U-73122 were used in combination with GSPE or ECG to study the role of a bitter-sensing pathway in the observed effects. For the in vivo studies, 38-week-old male Wistar rats were daily gavaged with GSPE during an 8-day treatment. Body weight and food intake were monitored during this period, and rats were sacrificed 24 h after receiving the last dose. Ghrelin gene expression in stomach and duodenum were assayed with TaqMan probes. For both studies, the levels of acylated and total ghrelin were analyzed by radioimmunoassay after a C-18 extraction of the acidified samples. Monomeric flavanols exhibited a stimulatory effect on ghrelin secretion in MGN3-1 cells, being ECG the most active compound. The effects of ECG on acylated ghrelin were abolished by the bitter blocker and the PLCb-2 inhibitor. On the other hand, GA, oligomeric proyanidins and GSPE induced an inhibitory effect on ghrelin secretion that, in the case of procyanidins, increased with its structure complexity. A reverse agonism on TAS2R14 and TAS2R39 was excluded to play a role in the GSPE-mediated inhibition. Rats treated with GSPE decreased its ghrelin circulating levels and mRNA expression in stomach, in agreement with decreased food intake and body weight gain. In conclusion, grape seed phenolic compounds distinctly modulate ghrelin secretion. In first place, monomeric flavanols stimulate ghrelin secretion in a ghrelinoma cell line by a bitter sensing mechanism. On the other hand, GA and proanthocyanidins are inhibitors of ghrelin secretion by mechanisms not involving bitter sensing. The inhibitory effects of GSPE on ghrelin secretion were confirmed in rats, and were accompanied by an effective reduction of food intake and body weight gain. Thus, GA and proanthocyanidins could be effective agents against overweight-related health problems.

Standardized LC-MS/MS assay for the analysis of microbiome-modulated bile acid composition

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Over the last decade, researchers have discovered many important functions of bile acids. Biochemically, bile acids are the end-products of the cholesterol metabolism and play an important role in the regulation of lipid digestion. Bile acids are known as endogenous markers for regulating various metabolism processes and as mediators for gut microbiome status. Gut microbiota is involved in the biotransformation of bile acids through their deconjugation, dehydroxylation, and reconjugation, thereby altering bile acid composition and modulating FXR and TGR5 signaling. These gut microbiota-induced changes of bile acid composition could, in turn, influence bile acid receptor-mediated effects on glucose and lipid metabolism. An accurate quantitative determination of individual bile acids is, therefore, very important. We have developed and validated the worldwide first kit, based on (U)HPLC-MS/MS technology, which enables the standardized quantification of individual bile acids from only 10 µl of either human plasma or human serum (16 bile acids) or mouse plasma samples (19 bile acids). Other biologically relevant matrices (e.g. mouse liver homogenate, human urine, human faeces) have also been tested with the kit. The bile acid panel consists of cholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid, muricholic acids and their glycine as well as taurine conjugates. 10 µl sample and 10 µl internal standard mixture were transferred onto the filter spot in a 96-well BIOCRATES Kit plate. Bile acids were extracted with 100 µl methanol. The extract was filtered through the plate by gentle centrifugation. The analysis was carried out using (U)HPLC-ESI-MSMS in negative mode. 7-points calibration curves were used for quantitation. A very simple and robust assay procedure was developed, which is based on extraction from filter spots placed in a 96-well plate. Only 3 steps were needed to complete the sample preparation. Seven calibrator levels and three quality control levels are also part of the kit. This new assay in kit format was validated for different LC-MS/MS platforms from Waters, AB Sciex and Thermo Scientific™. Overall, LLOQ values between 0.01 and 0.03 µM were achieved for all targeted bile acids. Based on the measurements of a large number of plasma samples (n=155) from healthy adults (>18 years old), the reference concentration ranges for individual bile acids were established. The Biocrates® Bile Acids Kit measures a wide range of bile acids, including secondary bile acids. As microbiome-synthesized secondary bile acids play important roles as signaling molecules in cellular signaling processes, the Biocrates® Bile Acid Kit is a valuable tool to address questions concerning bile acid-mediated signaling in nutrition-related diseases, drug metabolism, cardiovascular diseases, and diabetes.

Calcium and phosphate intake may influence bone remodeling in hemodialysis patients

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The link between vascular calcification and increased mortality is now well established in hemodialysis (HD) patients. Besides the traditional predisposing factors (diabetes, hypercholesterolemia), inhibitors and promoters of calcium-phosphate and bone metabolism have been involved. Recent studies demonstrated, in experimental Chronic Kidney Disease (CKD) animal model, a positive association between sclerostin production and dietary phosphate intake. Sclerostin is a glycoprotein mainly expressed by osteocytes, involved in regulating bone formation by inhibiting Wnt–β-catenin signaling. Mounting evidence indicates that a disturbed Wnt-β-catenin signaling is implicated in the pathogenesis of the CKD-associated bone and mineral disorder (CKD-MBD). The present study investigated the relationship between sclerostin and dietary habits in 49 Caucasian HD patients (age 36-90, M/F 31/18, dialysis vintage 1-144 months). Nutrient intake was analyzed by 24 h dietary recall, and sclerostin serum levels were measured by ELISA. Mean calcium and phosphate intakes of HD patients were 705±584 and 1196±498 mg/day, respectively. As expected serum sclerostin (3356±2118 pg/ml) levels were increased in HD patients. A positive correlation was found between serum sclerostin levels and calcium intake (r=0.33, P=0.025), and between sclerostin and phosphate intake (r=0.34, P=0.021). Patients with higher sclerostin levels were older (P=0.003), and showed higher intake of protein (P=0.012), calcium (P=0.017) and phosphate (P=0.004). However, multiple linear regressions, testing sclerostin as dependent variable (dialysis vintage, age, body weight, serum calcium and phosphate, serum PTH, intake of calcium and phosphate as independent variables), demonstrated that dietary phosphate intake was the main significant determinant of sclerostin serum levels in HD patients (r=0.356, B=1.5, P=0.004). Analyzing only the patients treated with calcium carbonate or acetate (n=31), sclerostin serum level again correlated positively with calcium (r=0.433, P=0.017) and phosphate intake (r=0.377, P=0.04). In conclusion, the dietary phosphate and calcium intake may influence sclerostin serum levels and potentially affect bone remodeling or soft tissue calcification in HD patients.

Modelling gene expression network for Alzheimer disease based on DNA microarray data

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Gene expression by microarray data is a topic of Nutrigenomics to investigate complex gene networks and their relationship to nutrition. DNA Microarray are used to quantify and compare gene expression on a large scale and to explore a major subset or all genes of an organism. As a consequence, formal methods and computer tools for the modelling and simulation of gene networks are indispensable. The chronic disease such Alzheimer disease is a serious health problem worldwide and the high prevalent in Mexican people. We analyzed the gene expression data associated to the Alzheimer disease in a profile of microarray data with possible application to bioactive components like omega 3 and selenio. We propose a network of interacting genes (APOE, APP, PSEN1, MAPT and GPX1) for Alzheimer disease. We have modelling the clustering of gene networks by applying the mathematical procedure Principal Component Analysis to the gene expression data. As well, this network could help to control dietary variables associated with selenio and omega 3 intake to prevent risk of Alzheimer disease and to obtain evidence of their interaction with certain human genomes modules.

Polyprenol effects on blood biochemical parameters in statin myopathy model rats and humans

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PPs are natural long-chain isoprenoid alcohols, which play an important role as natural bio regulators and precursors of dolichols. PPs and dolichols are synthesised in a mevalonate-dependent pathway, similar to cholesterol. Statins – widely used cholesterol-lowering drugs, tend to halt the production not only of cholesterol but also of PPs and dolichols, which may lead to statin-induced myopathies. Aim of the study was to investigate the effects of PPs isolated from Picea abies L. spruce needles on rat and human blood biochemical parameters in the model of statin-induced myopathies. PPs were extracted from the Norway spruce (P. abies needles and isolated by column hromatography with purity >95%. Female Wistar rats weighing 230-245 g were treated with PPs at the doses of 1, 10 and 20 mg/kg and Atorvastatin 80 mg/kg for 16 days during which period various tests were performed (open field, grip, wire and rotarod). Assessment of cholesterol levels and creatine kinase (CK) activity in myopathic rats was done on day 17. Rats were anesthetised and blood cholesterol level and CK activity measurement was collected by cardiac puncture. The study was carried out at the Faculty of Medicine, University of Latvia. Human trial was an open-label, one-center prospective pilot study, which included five patients (2 women and 3 men) matching strict statin-induced myopathy criteria. The study was carried out at the Latvian Centre of Cardiology between March 2014 and January 2015. All patients received supplementation of PPs (4 mg/day) in combination with Coenzyme Q10 (100 mg/day) for 2 months. In rats cholesterol levels were unchanged by the administration of PPs, but the CK levels (administration of 20 mg/kg of PPs) were elevated by approx. 25%. Although at present this is difficult to pin down to a specific mechanism, we believe that PPs in higher doses elevate CK due to reinforcement of the intracellular transport and ATP production by promoting energy normalization process, worsened by Atorvastatin activity. In the human trial all determined biochemical parameters (K, KB, AlAT, AsAt, TSH, hs-CRO) during the recurring visits were statistically significantly unchanged, except for the CK, which was elevated by 9.2%. Present data suggest no major safety issues of PPs in rats and humans used during statin therapy. Therefore, PPs can be considered safe and potentially beneficial for relief of statin-induced myopathy. Financial support was given from Pharma and Chemistry Competence Centre of Latvia, Ltd. grant No. L-KC-11-0001 with the co-financing of the European Regional Development Fund. Project P29: 'The conifer isoprene alcohol biological activity studies in pathology models'.

Nutrimetabolomic strategies using NMR for foods, supplements & dietary patterns intervention studies

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Nutrimetabolomics is the 'omics' science that studies the effect of diet through the metabolome. The aim of this work was to present different nutrimetabolomics strategies to study nutritional biomarkers related to food consumption (biomarkers of intake) and to improve the understanding of their impact on human health (biomarkers of effect), from distinct metabolomic approaches using Nuclear Magnetic Resonance after intake of different food (wine), supplements (probiotic supplements), and dietary patterns (Mediterranean diet). Firstly, we evaluated the differential effect of wine, dealcoholized wine and gin on urinary metabolome. Metabolites from food metabolome (tartrate, ethanol and mannitol), endogenous (3-methyl-2-oxovalerate) and gut microbiota-derived metabolites (hippurate and 4-hydroxyphenylacetate) were identified. Concerning food matrix effect, a potential interaction between alcohol and biomarkers related to metabolites of the intestinal microbiota was observed. The study of food exposure biomarkers in an interventional study concluded that a model combining two wine biomarkers (tartrate+ethyl glucuronide) has greater predictive power (area under the curve [AUC]: 90.7%) than the individual markers alone, also achieving reproducible results in epidemiological data (AUC: 92.4%); with a high sensibility (≥84%) and reproducibility (>90%) in both populations. To study the dietary intervention effect on the metabolic phenotype (metabotype), high cardiovascular risk individuals were stratified based on their clinical parameters. The effect of wine polyphenols intake in the metabolic phenotype for the two most discriminant clusters (obese-diabetics vs healthy with risk) was evaluated, and a differential excretion was observed for the gut microbiota metabolite 4-hydroxyphenylacetate, exhibiting an alteration after wine consumption in gut microbiota metabolism in obese-diabetic cluster. In the dietary pattern evaluation, metabolic footprinting after 3-years follow-up with Mediterranean diet supplemented with virgin olive oil/nuts vs a low-fat diet was tested. Several endogenous metabolites from energy metabolism, food metabolome and those derived from gut microbiota were distinguished. Also, a dietary pattern association with certain metabolites was also observed, detecting that Mediterranean diets were associated with a high consumption of fruits and vegetables and a low-fat diet with red meat intake. Finally, a probiotic supplement was administered to assess the impact on health in mastitis breastfeeding women. The reduction of inflammation (medical test) and voluntary desertion of anti-inflammatory pharmacological drugs (ibuprofen and acetaminophen) was revealed in the urinary metabolome. Further, an increase of gut microbiota-derived metabolites (trimethylamine-N-oxide and hippurate) and creatine was also observed. These results may allow targeted analysis of interconnected pathways subsequently integrated into metabolic networks, which could provide a better understanding and interpretation of the overall health and diet status of individuals.

Zinc Transporter expression pattern related to zinc nutrition exposure in human dental pulp

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Zinc is an abundant micronutrient and has essential roles in human growth and development. Zinc is obtained from the diet and the distribution throughout the body and within cells is regulated by saturable, carrier-mediated transport proteins, known as ZnT's and ZIPs. Zinc deposition within deciduous teeth has been reported however the precise mechanism by which the zinc is transported from the circulatory system of the pulp, to the tooth hard tissue remains unclear. The objective of this study therefore, is to analyse the expression of zinc transporters at the mRNA level, in dental pulp and in long term studies, elucidate whether their expression is related to dietary zinc intake. Aims: to explore the level of expression of zinc transporters at the mRNA level in dental pulp, from both rats and humans teeth. Rat pulp tissue were removed from extracted incisor teeth and the RNA was isolated using TriZol (Ambion) RNA isolation. The RNA from rat intestine was isolated from the same animals, to be used as a control tissue. Zinc transporter expression at the mRNA levels was analysed using an RT-qPCR based method. In human studies, deciduous teeth were collected from Child Dental Clinic of Indonesia University Dental Hospital in Indonesia. Food frequency questionnaires were also obtained. RNA from dental pulp of the human deciduous teeth were isolated and zinc transporter expression at the RNA level measured using RT-qPCR. Analysis of zinc transporters in the rat pulp revealed a differential level of expression, with high levels of ZIP2 being expressed relative to ZnT1 in the rat intestine, whereas low levels of ZnT2 and ZIP4 were observed relative to ZnT1 in the rat intestine. We are currently analysing the pattern of expression of zinc transporters in human pulp RNA. For the first time, we have shown a differential level of expression of zinc transporters in dental pulp, indicating homeostatic control of zinc uptake into dental tissue, which may be regulated by dietary zinc intake.

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