Book of abstracts

10th NuGOweek

NUTRIGENOMICS & more

9 - 12 September 2013, Freising–Weihenstephan, Germany

Nutrigenomics & More

– NuGOweek 2013 –

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NuGOweek 2013

Freising-Weihenstephan, Germany
9-12 September 2013

A joint symposium of NuGO and the German Nutrition Society

Proceedings publication and Abstract Submission System (OASES) by

Wageningen Academic
Publishers

First published, 2013

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The Netherlands, 2013

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Wageningen Academic Publishers
P.O. Box 220
6700 AE Wageningen
The Netherlands
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Welcome

NuGOweek 2013 is a joint activity of the "NuGO family" and the German Nutrition Society and is held at the Technische Universität München (TUM) in the campus Weihenstephan in Freising.

Nutrigenomics as a surrogate term covers the genetics but also the genome responses of an organism to changes in its nutritional environment. 10 years after completion of the *Human Genome Project*, NuGOweek asks numerous distinguished experts for a critical assessment of the achievements of Nutrigenetics research in relation to normal human variability as for example in taste perception but also in relation to obesity or diseases such as diabetes or cancer. In addition, advances in technologies that allow a more comprehensive molecular analysis of the genome responses at the levels of the epigenome, transcriptome, proteome and metabolome are presented as tools for identifying markers of exposure or of biological response. NuGOweek also serves as a platform for exchange of information on on-going collaborative EU-funded research projects funded but also on future activities under the umbrella of Horizon 2020 and other European initiatives. Finally, NuGOweek 2013 will help to create and promote the nutrition researchers cohort (NRC) as a worldwide group of nutrition experts dedicated to serve as their own study subject for comprehensive phenotyping and lifestyle recording.

On behalf of the local organisers and TUM, the German Nutrition Society and the NuGO team we would like to welcome you here in Bavaria for the 10th edition of the NuGOweek. I would like to thank our sponsors and all people that helped to organise NuGOweek in 2013. We are looking forward to have a couple of great days with excellent science, vital discussions and a good spirit. We will do our best to make your stay with us and the NuGO family enjoyable.

Hannelore Daniel

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Archaic genomes and human evolution

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Fossil, archaeological, and genetic evidence are united in supporting a recent African origin of modern humans and dispersal out of Africa within the past 60,000-80,000 years. However, given that archaic humans (such as Neandertals) preceded the exodus of modern humans out of Africa by several hundred thousand years, the question then arises as to the nature of the interactions between modern and archaic humans. In particular, did archaic humans contribute any genes to modern humans, or is all of our ancestry derived from the recent origin in Africa? This question proved remarkably difficult to answer, until genome sequences from archaic humans recently became available. These archaic genome sequences have provided a number of important insights into the history of our own species, and I shall present the latest of these, including the potential adaptive value of genes that we might have received from interbreeding with archaic humans outside of Africa.

Molecular evolution of taste perception

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Taste responses enable animals to evaluate the quality of potential foods before they are even consumed, positioning them to have strong, direct effects on fitness. Patterns of diversity in genes encoding taste receptors harbor signatures of these ancient pressures. Human populations exhibit high levels of diversity in genes encoding bitter taste receptors (TAS2Rs), including high levels of differentiation consistent with the effects of adaptation to local environments during the dispersal of humans out of Africa ~50,000 years ago. In contrast, genes encoding sweet and umami receptors (TAS1Rs) exhibit lower levels of diversity and population differentiation, consistent with greater selective constraint. Comparisons across the primate order reveal that diversity levels are lowest in gene regions encoding domains critical to receptor function, such as transmembrane regions, and higher in regions potentially relevant to agonist response, such as external loops, further supporting the hypothesis that receptor-ligand interactions are key drivers of taste receptor evolution. Remarkably, taste receptors also show high rates of duplication and loss pointing to substantial flexibility in the evolution of taste perception systems. Thus, patterns of genetic and phenotypic diversity in taste responses relevant to diet and nutrition today represent a legacy of dynamic processes occurring throughout our evolutionary history.

From receptors to hedonism

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Each of the basic taste qualities plays a specific role in the evaluation of food. Bitterness is often associated with the presence of potentially harmful substances. Its valence is generally negative which leads to rejection in order to minimize the risk of food intoxication. Amazingly, the same compounds that we perceive as bitter act as repellents in numerous animal species including, mammals, other vertebrates, insects, and worms. On the other hand it is known that numerous bitter substances are healthy, at least over a certain concentration range. Thus, ingestion of such compounds would be rather beneficial. Indeed, consistent with such possible positive health effects we consume many bitter compounds with our food and beverages. Moreover, because such foods even give us pleasure, we may assume that the associated bitterness is of positive valence. Not only humans accept some bitter compounds, also animals use specific natural bitter compounds to cure themselves from infections and other diseases or use them in chemical defence or attack strategies. Thus, the chemosensory systems are apparently able to distinguish between various types of bitter compounds raising the question whether this distinction is based on intrinsic properties of the gustatory system or on the mere association of postingestive consequences with the taste of food. My laboratory takes an integrative approach to elucidate how bitter compounds are detected, bitter receptor cells are organized and wired to the peripheral and central nervous system, and how signal input into this system drives ingestive behaviour. To this end, we combine human psychophysics and genetics with receptor expression assays, genetically modified mice, neurophysiological recordings, molecular biological and cell biological methods as well as behavioural experiments. Our findings provide a conceptual framework for the reception of the innumerable bitter compounds. They also demonstrate an unexpected complex organization of the oral sensor cells for the recognition of bitter compounds and of the peripheral and central neurons that process bitterness. The system is characterized by multiple parallel extensively overlapping but functionally distinct pathways. This design minimizes the risk of a fatal block of bitter perception through the known presence of potent bitter blockers in food or by desensitization mechanisms during eating bitter food items. The functionally distinct pathways of bitter detection and processing could also function as different input channels into neural circuits regulating appetitive and repulsive behaviours.

Genetics and food preferences: perception and liking

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Genetic variation in sensory genes determine what can be perceived, for instance, there are some common variants in bitter receptor genes that reduce or eliminate the ability to perceive the bitterness of some chemicals found in vegetables, but person-to-person differences in perception do not automatically predict food preferences, which are determined in whole or in part by development, experience and culture or genetic variation in genes unrelated to flavor perception. To better understand food preferences, our laboratory studies human twins and their response to common taste qualities like sweet, salty and sour compounds, as well as the response to more complex stimuli, such as vegetables; we also study a subset of people over years, to track changes in their ratings over time. These studies indicate that perception and liking have a heritable component which can be explained in some cases by single gene effects whereas in other cases, environmental effects are more potent. We have also learned that liking is a more stable trait than ratings of taste intensity when subjects are studied over years rather than days. In addition to classic twin studies aimed at understanding the genetic architecture of these traits, we are also investigating how gene expression and epigenetics may affect preference and liking for bitter stimuli. These studies suggest that bitter receptor gene expression may explain up to 40% of person-to-person differences in perception and liking for simple bitter stimuli as well as vegetables.

A machinery for gustatory fat sensing in humans?

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The ability to select energy-dense, high quality food is crucial for development, health and survival. Dietary fat is one of our most important energy-providing macronutrient and contains essential fatty acids and vitamins, leading to its pivotal functions in brain development, prevention of inflammatory, coronary and psychiatric diseases. However, concurrently the incidence of obesity continues to escalate, and with it, its related diseases including diabetes, metabolic syndrome, cardiovascular and neurodegenerative diseases. The positive correlation between obesity and high dietary fat intake has provided a strong incentive to understand the consumption of high fat-food. Traditionally, the sensory perception of dietary fat has been characterized in terms of textural, olfactory and post-ingestive effects. In the last decade, evidence from molecular and behavioral research has begun to challenge traditional perspectives by introducing the concept of gustatory chemoreception of fat. Rodent models with different oral fatty acid-sensitivity showed a differential regulation of fat intake and body weight. Also oral hypo- or hypersensitivity to fatty acids in humans causes differences in the evaluation of fat content, energy consumption and BMI. In rodents, various lines of evidence suggest that fatty acids liberated from triglycerides by lingual lipases are the predominant stimuli of gustatory fat detection. Presumably, fatty acids mediate their effect via special receptors present in taste cells. In humans, however convincing data on the mechanisms underlying gustatory fat sensing is limited. In order to understand human gustatory fat sensing, we are investigating the molecular mechanisms of oral fat detection. Human subjects are able to taste predominantly medium and long-chain fatty acids. Therefore, we characterized by functional calcium imaging experiments the putative taste receptors for fatty acids, GPR40 and GPR120. We found that the agonist spectra and activation patterns of these receptors matched human sensory data. Further, we demonstrate by RT-PCR analyses, in situ hybridizations and immunohistochemistry that GPR120 is indeed expressed in human taste papillae, yet the exact cell type and the possible function of GPR120 still needs to be determined. Fatty acids in food are mainly present as triglycerides which fail to activate GPR120. Thus, for fat to be perceived, fatty acids must be enzymatically liberated from triglycerides even though we did not find any expression of lingual lipase in human taste tissue. However, we observed expression of the secretory lipases K, M and N in Von Ebner's salivary glands which are located underneath the taste epithelium and release their secretions in the troughs of vallate and foliate papillae. Together, our results provide initial evidence for the existence of fatty acid sensors and fatty acid liberating enzymes in human taste tissue which may contribute to gustatory fat sensing. In the future, this knowledge could offer an opportunity to reduce fat in food without taste impairments and to develop intervention strategies in order to limit calorie intake for combatting obesity and its associated diseases.

Mechansims of fructose on lipid and carbohydrate metabolism

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The prevalence of obesity and type 2 diabetes is increasing worldwide. The rising amount of dietary sugars have been identified as contributing factors to the epidemic of metabolic diseases. Along with the increase in total energy consumption, the intake of dietary fructose has considerably increased. Since our diet rarely encounters fructose as single nutrient, dietary intake results from the co-exposure with glucose from digestive hydrolysis of sucrose (table sugar) or by the use of industrial blends containing fructose as sweeteners. High-fructose consumption has already been shown to contribute to high blood levels of triglycerides, high blood pressure, dyslipidemia, impaired glucose tolerance and the development of type 2 diabetes. In our study we focused on the impact of a high-sugar, in particular a high-fructose diet on gene expression patterns in lipid and sugar metabolism using *Caenorhabditis elegans* as a model system. Feeding studies on *C. elegans* showed that the concentration and the ratio of fed sugars (glucose:fructose) strongly affect gene expression in lipid and sugar metabolism and lead to diminished insulin signaling. Increased mRNA levels of pod-2, the coding gene for acetyl-CoA carboxylase that catalysis the first and rate limiting step in de novo lipogenesis were found in nematodes upon high-fructose feeding. We also found altered gene expression patterns in the Insulin-like signaling cascade with increased mRNA levels of daf-2, a receptor tyrosine kinase that is the *C. elegans* insulin/IGF receptor ortholog. First results of our gene expression study in *C. elegans* show that high-fructose feeding induces lipogenesis and alters insulin sensitivity and signaling.

FOOD4ME: consumer opinion of genetic testing for personalised nutrition

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Personalised nutrition is concerned with providing individualised diets, taking into consideration an individual's phenotype and genotype. Previous research has shown that European and Australian consumers have positive opinions towards nutrigenomics, however, concerns surrounding on-line delivery, data protection and trust in the provider have arose. The aim of this study was to probe factors determining uptake and compliance with personalised nutrition based on genetic data. Focus group discussants (n=68) were recruited in the United Kingdom and Ireland as part of the Food4Me Project. Two focus groups were held in each of four centres and segregated by age, one comprised of those aged 18-65 years and the other 30-65 years. Using a standardised interview schedule developed by Food4Me, discussion was prompted using the following scenario, 'Imagine that you want to change your diet to improve your health. You therefore go online and find a website', followed by 'Imagine the service also sends you a home kit to collect information regarding your genetic information, using a cotton bud to collect saliva from the inside of your cheek'. Discussions (n=8) were recorded, transcribed verbatim and data content analysed. Data were interpreted with reference to Social Cognitive Theory. Discussants did not appear convinced that genetic testing could offer outcome expectations over and above that of consuming a healthy diet. Genetic testing for dietary intervention was considered appropriate only where there were consequences of life threatening conditions. Concerns were conveyed over efficacy to correctly use the home test kit and interpret results. Discussants also perceived that recommendations would require motivation to be successful. Outcome expectations related to genetic testing, consequences associated with perceived propensity for serious disease and self-efficacy to take samples and apply results of genetic tests may affect uptake of and compliance with nutrigenomic intervention. The implications are that consumers require a goal or incentive to be willing to undergo genetic testing. Promotion of efficacy in those undertaking nutrigenomic interventions, particularly in their ability to complete the tasks involved in acquiring genetic samples and complying with recommendations is required. Those with low self-efficacy and motivation may benefit from more tailored advice or interventions promoting self-efficacy. This work is supported by the EU funded 7th Framework Food4Me Project. Food4Me is the acronym of the project: 'Personalised nutrition: an integrated analysis of opportunities and challenges' (Contract no. KBBE.2010.2.3-02, Project no. 265494), http://www.food4me.org.

Genetic variability in human bitter taste perception

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Taste is an inherent characteristic of food organoleptic properties, and therefore appears to play a major role in food preferences, behavioural attractions/aversions and food consumption patterns. Unlike other basic taste qualities, bitter taste perception is often synonymous with displeasure, consistent with its role as a warning sensor against potentially harmful substances. Paradoxically, its perception differs across individuals. Different factors determine these phenotypic differences including genetic diversity, environmental influences, as well as confounding factors, e.g. age, gender and experience. The most famous example of variability concerns the bitterness of PTC and thioamides, where the perceptual differences in the population are directly linked to genetic polymorphisms in one of the ~25 members of the bitter taste receptor (TAS2R) gene family. Our present work extends the analyses of genetically determined variation in bitter perception to all TAS2R genes. Genetic diversity at the 25 functional TAS2R loci and linkage structure in the corresponding genomic region were examined in a sample of the European population. Whereas copy number polymorphisms and gross rearrangements are rare, numerous coding single nucleotide polymorphisms were seen in nearly all receptor genes, confirming previous observations. However, values of linkage disequilibrium were high in several distinct TAS2R loci, demonstrating that numerous TAS2R alleles are associated in a non-random fashion. In this way long-range haplotypes exist, which limit to some extent the genetic diversity. Using the subjects' genetic data and *in vitro* cell-based assays we can predict potential subjects' sensitivities to bitter compounds and food. We will use inter-individual variation in bitter perception as tool to elucidate the establishment of food preferences, behavioural attractions/aversions and food consumption patterns.

An acute bout of exercise increases plasma and PBMC gene expression of inflammatory markers

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Physical inactivity is associated with an increased risk for premature morbidity, and regular exercise has been shown to prevent chronic diseases. Low-grade chronic inflammation is suggested to be a key factor in the pathogenesis of chronic diseases, and long-term effect of physical activity reduces the levels of inflammatory markers. In response to exercise however, the level of circulating IL-6 increases, and declines in the post-exercise period. Previous studies have shown that peripheral blood mononuclear cells (PBMCs) may contribute to the increase in circulating levels of cytokines after exercise, however this remains to be elucidated. Consumption of a carbohydrate-protein sports drink has been shown to lower post-exercise muscle damage compared to intake of carbohydrate sports drink alone. The aim of the present study was to investigate: (1) the effect of a standardized and controlled bout of exercise on circulating levels of inflammatory markers and PBMC gene expression; and (2) to study the effect of intake of carbohydrate rich versus carbohydrate -protein rich sports drinks on circulating levels of inflammatory markers and PBMC gene expression in the recovery period after exercise. 11 healthy, non-smoking men 22-28 years old were enrolled in the study. The study was designed as a one-day, two-period double blinded randomized crossover study with an intermediate wash-out period of one week. The two test days were exercise intervention days where the participants performed the cycle test. In the recovery period the subjects received either a carbohydrate rich sports drink or a sports drink with carbohydrate and protein. VO_2 max was measured prior to the test days. Total RNA was isolated from PBMC samples. Custom designed TaqMan Low-Density array (LDA) cards were used for gene expression analysis. Inflammatory markers were analysed with ELISA. An acute bout of exercise significantly upregulated the gene expression of IL-1β, CXCL16 and IL-8 in PBMC. Concomitantly, circulating inflammatory markers, including IL-6, TNFα, IL-10 and CXCL16 were also significantly increased. Serum markers and genes were changed in the recovery period after training, but no significant difference between the intakes of the two different sports drinks was observed. In conclusion, PMBC may contribute to the increased inflammatory makers after an acute bout of exercise.

In search of the genetic basis of human obesity

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Over the last twenty years, the genetic analysis of obesity led to the identification of confirmed major genes. While such major genes have a clear influence on the development of the phenotype, they are however rare and thus of minor clinical importance. Polygenic effects concern a clearly larger number of affected subjects. The first truly validated polygenic variant with an influence on body weight pertains to the V103I polymorphism in the melanocortin-4 receptor gene. The I103 variant leads to a mean BMI reduction in the magnitude of 0.5 kg/m^2; approximately 4% of the German population is heterozygous. Genome wide association studies (GWAS) have made it possible to detect several more polygenes underlying obesity. The FTO (fat mass and obesity associated) gene was found to be associated with type 2 diabetes mellitus as based on a GWAS; statistical adjustment for BMI revealed that this association actually reflects an association with obesity. This finding has subsequently been replicated in large independent study groups. In the first genome wide association study for obesity, which was based on 487 obese children and adolescents and 442 lean adult controls a FTO SNP was significantly associated with obesity after correction for multiple testing. The mean BMI increase is 1.2 kg per allele. A variant downstream of the melanocortin-4 receptor gene was also identified as having an influence on body weight; the mean effect of a single allele corresponds to +0.22 kg/m^2. Several recent GWAS meta-analyses have led to the detection of several more SNPs involved in body weight regulation in children and adults. In total, genetic variation at more than 40 loci has been found to be associated with obesity. Functional studies have been initiated to detect the relevant mechanisms *in vitro* and *in vivo*. Specific copy number variants have been identified more frequently in obese patients than in controls. In conclusion, the advent of GWAS has led to the detection of several SNPs associated with obesity; effect sizes in terms of grams per allele are small; the BMI variance explained by these associations is in the magnitude of 2% and thus substantially lower than predicted as based on current heritability estimates for body weight in the magnitude of 0.5 to 0.7.

From a sequence variation towards a mechanism

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Forward genetics faces the challenge to identify the genomic sequence variation responsible for phenotypic variation. Candidate gene approaches and genome wide association studies have provided a large number of genomic loci harboring sequence variations which may be markers for risk assessment or even causally linked to the etiology of complex disease. A majority of these sequence variations are either found in non-coding regions of the genome with no assigned function, or within putative gene regulatory regions. As exemplified for UCP3, a PPAR responsive gene implicated in the development of metabolic disease, the published studies addressing the association of sequence variations in the human UCP3 gene with metabolic disease are inconsistent. We devised a successful strategy to identify the function of a novel sequence variation in the first intron of the UCP3 gene. The UCP3 gene is expressed in skeletal muscle, heart and brown adipose tissue, and regulated in response to high fat feeding, food restriction and cold exposure. PPAR response elements have been identified in the proximal promoter and in the first intron. A naturally occurring sequence variation (IVS1+1505G>A) originally identified in the first intron of the hamster UCP3 gene results in a loss of transactivation by the PPARγ ligand rosiglitazone. This sequence variation is located in juxtaposition of a previously identified PPAR response element. Our analysis revealed that IVS1+1505G>A disrupts the functionality of a binding site for the transcription factors SP1/3 which act as gatekeepers for PPAR activity on the UCP3 gene. Further deletion studies demonstrate that this intronic region containing PPAR and SP response elements harbors a further DNA binding site for MyoD/NF1. Taken together our data establish the presence of a novel intronic enhancer in the UCP3 gene which is conserved across mammalian species. In this enhancer we seek to identify functionally relevant sequence variations as these may exhibit stronger associations with metabolic disorders than the variants studied previously.

TFAP2B rs987237 and dietary protein/carbohydrate interacted to modify weight maintenance

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TFAP2B rs987237 is associated with obesity and has shown interaction with dietary fat-to-carbohydrate ratio on weight loss. We investigated interactions between rs987237 and protein-to-carbohydrate ratio or glycemic index (GI) in relation to weight maintenance after weight loss. We used linear regression analyses and additive genetic models to investigate main and diet interaction effects of TFAP2B rs987237 in relation to weight maintenance in the DIOGENES study. DIOGENES includes 742 obese individuals from 8 European countries who lost ≥8% of their initial body weight during an 8-weeks low calorie diet and were randomized to one of five ad libitum diets with a fixed energy percentage from fat, and either low protein/low GI, low protein/high GI, high protein/low GI, high protein/high GI, or a control diet for a 6-month weight maintenance period. In total, 468 completers were genotyped for rs987237. High-protein diets were beneficial for weight maintenance in the AA genotype group (67% of participants), but in the AG and GG groups no differences were observed for low or high protein diet. On the high protein diet, carriers of the obesity risk allele (G-allele) regained 1.84 kg (95% CI: 0.02; 3.67, P=0.047) more body weight per risk allele, compared to individuals on a low protein diet. TFAP2B rs987237 and dietary protein/carbohydrate interacted to modify weight maintenance. This association was different from our previously reported association with fat-to-carbohydrate-ratio interaction for weight loss. Thus, TFAP2B-macronutrient interactions might diverge depending on nutritional state.

Transcriptional regulatory network underlying adipose tissue adaptation to high-fat diet

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Adipose tissue health is influenced by an interplay of different biological processes. Network analysis provides a tool to help understand these complex mechanisms and their role in cellular adaptation to a challenge. We investigated the long-term adaptation mechanisms of adipose tissue to high fat diet (HFD) based on data from our study of male C57BL/6JRccHsd mice fed semisynthetic low fat diet (10 energy % fat) or HFD (40 energy % fat). In this study, epididymal white adipose tissue (eWAT) after 5 days and after 12 weeks was collected and corresponding transcriptomics data was measured. By applying network analysis on the early (5 days) and late (12 weeks) transcriptional response to HFD in adipose tissue, we here elucidated processes underlying adaptation, their regulation by transcription factors and their relation to changes in physiological parameters. This was done by creating multi-layer networks that link enriched transcription factors, biological processes, and changes to physiological parameters. This allowed us to investigate the dynamic changes in these networks over short and long term adaptation to HFD. Our network analysis showed that the largest part of regulated processes was up-regulated after 5 days of HFD. These processes were involved in immune response, stress response, signaling, cell remodeling, and transcription regulation. The few down-regulated processes included mitochondrial functioning and energy metabolism. In contrast to 5 days, processes involved in transcription and regulation were down regulated at 12 weeks. Moreover, mitochondrial functioning was further down-regulated. At 5 days, the three layered regulatory network did not show any correlation between processes and physiology, indicating an early adaptation at molecular level that did not yet reflect effects in physiology. However, at 12 weeks, eWAT weight and energy intake negatively correlated with cellular respiration, which was down-regulated. Overall, the differences between the network structure for the 5 days and 12 weeks transcriptional response indicated a transition from an adaptation state at 5 days to a more steady state after 12 weeks. Using network analysis, we were able to highlight specific transcriptional regulatory mechanisms underlying the dynamics of cell adaption to HFD at different time points and link these mechanisms to physiology. This research has received funding from the European Union's Seventh Framework Program FP7 2007-2013 under grant agreement nº 244995 (BIOCLAIMS Project).

Susceptibility to adult obesity is modulated by early maternal leu-supplementation in offspring

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The main aim of this study has been to assess the impact of maternal leucine supplementation during lactation, particularly on the susceptibility to obesity on offspring in adulthood. Pregnant Wistar rats were fed standard chow diet (3% kcal-fat). At day 1 after delivery, dams were distributed in two groups (n=6): control group (C), maintained under the same diet and, Leucine-treated group (Leu) which had free access to chow diet supplemented with 2% of L-leucine. At day 21 of lactation, one female and one male from of each litter were sacrificed, tissues were collected and stored at -80C for ulterior analysis. At weaning (day 21), Leucine supplementation was removed, and the rest of offspring was fed with the standard chow diet until the age of 6-months. Then, animals were fed a High-Carbohydrate (HC: 70% Kcal-carbohydrate and 10% Kcal-fat) or a High-Fat diet (HF: 45% kcal-fat and 35% Kcal-carbohydrate) until the age of 9-months. Then, animals were sacrificed, tissues rapidly removed and stored for gene expression analysis by RT-PCR. Males and females were analysed and eight groups were followed: HC-C and HC-L (HC fed animals coming from control (C) and Leucine (L) supplemented dams) and HF-C and HF-L (HF fed animals coming from C and L supplemented dams). Univariate analysis of variance (ANOVA) to assess the effects of early treatment (L, C), diet (HC, HF) and sex (males, females) was performed, significance was set at $P<0.05$. We observed a higher susceptibility to obesity, particularly in Leu female offspring, when HF diet was offered: HF-L females shown a 62% higher body weight (BW) gain than the respective controls (HF-C); this was also accompanied by a higher % of body fat/BW; whereas no differences were observed within males associated with the early Leu treatment. Neuropeptide hypothalamic gene expression was found altered in adult female animals. Of particular interest was the ratio of expression MCR4/NPY which was similar in all male groups whereas in Leu-females the ratio was higher than in the respective controls (2.6-fold and 3.4 fold under HF and HC diets, respectively), indicating a higher orexigenic potential in Leu females. Then, characterization of offspring at weaning allowed tracking early metabolic adaptations in offspring (before onset of obesity). Maternal leucine supplementation during lactation was associated with hypothalamic adaptations in the expression of neuropeptides in offspring. Particularly Leu-females had an increase in the expression of the NPY/POMC ratio; this was associated with a decrease in the innervation of NPY fibers from arquate nucleus (ARC) towards to the paraventricular (PNV). This was accompanied by a reduction in the expression of key energy metabolism genes in adipocytes, Leu females showed a decrease expression in adipose tissue of leptin (25%), adiponectin (60%) and UCP2 (40%). In conclusion, we have shown a significant impact of early-maternal leucine supplementation in offspring. Leu predisposes female offspring to higher susceptibility to adult obesity, whereas this is not the case in male offspring by a mechanism involving early hypothalamic adaptations.

Vitamin D protects against diet-induced obesity and metabolic disorders by enhancing fat oxidation

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Obesity and associated disorders, such as low-grade inflammation or insulin resistance, have been associated by many epidemiological and observational studies with low circulating concentrations of 25 hydroxy-vitamin D. However, the causality between these observations is not yet established. If the role of the vitamin D receptor has already been investigated through the use of VDR-/- mice or transgenic mice overexpressing the human VDR in adipose tissue, the impact of vitamin D3 supplementation has never been studied. In the present study, we investigated the effects of vitamin D3 supplementation in a diet-induced obesity mouse model (high fat diet providing 45% of total energy as fat). The supplementation was conducted for 10 weeks with 20000 UI of vitamin D3 per kg of food We showed that the vitamin D3 supplementation limited weight gain induced by high-fat diet (measured using ^{1}H-magnetic resonance (MR) spectra), which paralleled with an improvement of glucose homeostasis, notably fasting insulinemia glycemia and HOMA index. Leukocyte infiltration was also reduced in adipose tissue of supplemented mice (quantified by cytometry), resulting in a lower inflammatory status in adipose tissue as revealed through the expression level of several interleukins and cytokines. The limitation of weight gain was further explained by an increase of lipid oxidation, measured by indirect calorimetry, due to an up-regulation of genes involved in lipid oxidation and mitochondrial metabolism, notably in muscles and brown adipose tissue, leading to increased energy expenditure. Altogether, these data show that vitamin D3 supplementation regulates energy expenditure, and suggest that vitamin D3 supplementation may represent a nutritional strategy in the treatment of obesity and associated metabolic disorders.

Adipocyte biology as a driver for weight regain after weight loss in overweight/obese subjects

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Adipocytes regulate total body energy metabolism by the reversible storage of energy and by the secretion of adipokines. Preadipocyte differentiation is characterized by an enormous increase in cell volume due to the growing fat droplet. In parallel, the adipocyte metabolism and adipokine profile change and a basal lamina develops as a protective shield and important survival factor. In the obese, adipocytes display hypertrophy linked to a further modification of the adipokine profile, which is possibly involved in type II diabetes and cardiovascular disorders. Several factors may self-limit adipocyte expansion, some of which may affect basal lamina formation. Overgrowth leads to intracellular hypoxia. Since oxygen is crucial for the modification of collagens, hypoxia may block the growth of the basal lamina. Further, since insulin is the key promoter of collagen maturation, insulin resistance may assist in blocking cell expansion. Thirdly, variation of metabolic response may prevent cell growth. In preadipocytes PPARγ stimulates fat storage, but in mature adipocytes it may induce beta-oxidation. Losing 5% of the body weight reduces the risk for complications of overweight/obesity. This 10-15% reduction in adipocyte volume leads to normalization of the adipokine profile. We have performed discovery and targeted proteomics to study the glucose and fatty acid metabolism during weight loss in the adipose tissue. *In vitro* data show that going from feeding to starvation, molecular pathways do not absolutely revert. Surprisingly, not the fatty acid but the glucose metabolism seems to be the gatekeeper of adipocyte volume reduction *in vivo*. Our recent personalized analysis of the omics data point at protein translation as an important regulator of adipocyte metabolism during weight loss. After weight loss on a low-calorie diet 50% of subjects regain their weight within 1-2 years, which mainly seems accounted for by adipocyte-refilling instead of preadipocyte differentiation. We have proposed a model to explain this prominent refilling process in a biological way. Calorie restriction may hamper reconstructing the basal lamina to accommodate the shrinking adipocyte. This leads to traction forces between the cell and the surrounding extracellular matrix. Such cellular stress may result in aberrant adipokine secretion including leptin, which shows a disproportionate decrease after weight loss. This probably redirects the eating behavior of the host to an increased energy intake allowing the refilling of adipocytes. Here I will present data to support the occurrence of cellular stress in adipose tissue during weight loss with influence on the risk for weight regain.

Genetic susceptibility for obesity and diet intakes: association and interaction analyses in MDCS

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Gene-environment interactions need to be studied to better understand obesity. We aimed at determining whether genetic susceptibility for obesity associates with diet intake levels and if diet intakes modify the genetic susceptibility. In 29,480 subjects of the population-based Malmö Diet and Cancer Study (MDCS) we first assessed association between 16 in genome wide association studies identified obesity-related single-nucleotide polymorphisms (SNPs) with BMI and associated traits. We then conducted association analyses between a genetic risk score (GRS) comprising of 13 by us replicated SNPs, and the individual SNPs, and relative dietary intakes of fat, carbohydrates, protein, fiber and total energy intake, as well as interaction analyses on BMI and associated traits among 26107 non-diabetic MDCS participants. GRS associated strongly with increased BMI ($P=3.6\times10^{-34}$), fat mass ($P=6.3\times10^{-28}$) and fat-free mass ($P=1.3\times10^{-24}$). Higher GRS associated with lower total energy intake ($P=0.001$) and higher intake of fiber ($P=2.3\times10^{-4}$). No significant interactions were observed between GRS and the studied dietary intakes on BMI or related traits. Of the individual SNPs, after correcting for multiple comparisons, NEGR1 rs2815752 associated with diet intakes and BDNF rs4923461 showed interaction with protein intake on BMI. In conclusion, our study does not provide evidence for a major role for macronutrient-, fiber- or total energy intake levels in modifying genetic susceptibility for obesity measured as GRS. However, our data suggests that the number of risk alleles as well as some of the individual obesity loci have a role in regulation of food and energy intake, and that some individual loci interact with diet.

Opposite effects of daidzein and genistein supplementation on adipose tissue gene expression

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The isoflavones daidzein, genistein and glycitein are mainly present in soy foods and related products like isoflavone supplements. Isoflavone supplements are consumed by peri- and postmenopausal women because of their advocated effects on menopausal symptoms. During menopause, body composition of many women changes and fat accumulates around the central abdomen. A possible cause is the decline in estradiol during menopause and isoflavones, resembling the molecular structure of estradiol, might attenuate these effects. How isoflavones can regulate these potential changes in adipose tissue distribution remains to be elucidated. Our objective was to study the molecular effects of consumption of two different isoflavone supplements on adipose tissue in postmenopausal women. Therefore, a randomized double blind placebo-controlled cross-over intervention trial with supplementation periods of eight weeks was performed in which whole genome gene expression in adipose tissue of postmenopausal women was measured. Both supplements, D and G, provided ±100 mg isoflavones a day (aglycone equivalents); supplement D, rich in daidzein, contained 56% daidzein and 16% genistein; supplement G contained more genistein than supplement D, i.e. 49% daidzein and 41% genistein. Whole genome arrays (Affymetrix) were performed in adipose tissue of 24 women in the supplement D group and 31 women in the supplement G group and were collected after eight weeks of isoflavone as well as placebo supplementation. Mean plasma concentrations were 0.44 and 1.19 μM genistein and 1.40 and 1.15 μM daidzein after eight weeks of supplement D and G intake, respectively. After intake of supplement D, expression of 1,245 genes was statistically significantly changed in adipose tissue compared to placebo and after supplement G intake expression of 528 genes was changed. Gene set enrichment analysis showed that the same gene sets were changed after both supplements, these included fatty acid synthesis, triglyceride synthesis, PPAR target genes and glucose metabolism. Interestingly, these gene sets were downregulated after supplement D and upregulated after supplement G. Supplement-specific effects included downregulation of gene sets related to oxidative phosphorylation after supplement D and upregulation of gene sets related to estrogen signalling, cell cycle and protein processing after supplement G intake. In conclusion, intervention of eight weeks with two different isoflavone supplements showed supplement-specific effects on gene expression regulation in adipose tissue, including opposite effects on pathway level. These results imply that the composition of the isoflavone supplements, e.g. daidzein/genistein ratio, directs the effects in subcutaneous adipose tissue in postmenopausal women. This abstract contains preliminary results, the mechanisms behind the observed effects, the impact of the equol-producing phenotype and the relevance for adipose tissue health will be further explored.

Omics applications in metabolic syndrome research

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Genetics, metabolomes and metabolic syndrome

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The incidence of type 2 diabetes (T2D) is rising worldwide. Prediction of individual risk is essential for the development of personalized prevention strategies. The combination of genetics, transcriptomics and metabolomics provides powerful tools to predict individual risk through identification of biomarkers for the early detection of T2D. We quantified 140 metabolites for 4,297 fasting serum samples in the population-based KORA cohort at baseline and at seven years follow-up (overlap n=1010). Our study revealed significant metabolic variation in pre-diabetic individuals, which are distinct form known diabetes risk indicators, such as HbA1c levels, fasting glucose and insulin. We identified three metabolites (glycine, lysophosphatidylcholine (LPC) (18:2) and acetylcarnitine C2) with significantly altered levels in impaired glucose tolerance (IGT) individuals compared to those with normal glucose tolerance. Low levels of glycine and LPC (18:2) at baseline were shown to predict risk of IGT and T2D significantly. Moreover, these two metabolites were replicated in the EPIC-Potsdam cohort. Additionally, we replicated four out of the five branched-chain and aromatic amino acids previously reported predictors of T2D using nested/selected case-controls samples. We illustrated that a unique combination of methodology employed, prospective population-based and nested case-control, as well as cross-sectional studies, is essential for the reliable identification of IGT and T2D predictors. Furthermore, using metabolite–protein network analysis, seven T2D-related genes that are functionally associated with these three metabolites were identified. These findings support the pathophysiological relevance of the newly identified candidate (pre-)diabetes biomarkers.

Metabolite patterns associated with components of the metabolic syndrome in a cohort study

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The epidemics of overweight and diabetes are the current dominant factors in nutritional disease in Europe and in many other parts of the world. Risk factors associated with overweight and diabetes risk tend to cluster in many pre-diabetic subjects. This has led to several definitions of this state as a metabolic syndrome (MetS). According to these definitions MetS may include factors such as high fasting glucose levels, increased BMI or waist circumference, dyslipidemia, and elevated blood pressure. Direct or surrogate measures of these risk factors are available for most volunteers in the Danish Diet, Cancer and Health study, which is an associated branch of the EPIC study. Using liquid chromatography-time-of-flight mass spectrometry we have profiled 350 plasma samples from women, aged 50-64, from this study and performed analyses of metabolites associated with MetS factors. Multiple linear regression analysis with false discovery rate corrections were used to find metabolites associated with MetS factors, including BMI, waist circumference, total cholesterol, systolic blood pressure, and plasma glucose levels as well as a combined 'MetS index' of these factors. Each of these MetS factors had characteristic metabolite features in their profiles, but several metabolite features were associated with most of them and with the combined MetS index. Some of the most dominant features are higher levels of branched-chain amino acids and glucose and lower levels of lysophospholipids according to higher levels of the MetS score reflecting differences in energy metabolism and higher levels of plasma insulin. Specific and common metabolic features associated with the MetS risk factors will be presented.

Effects of dietary fibres on microbiota, SCFA levels and transcriptional profiles in murine colon

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A diet rich in fibre has been shown to be beneficial for gastrointestinal health. Dietary fibre escape digestion by mammalian enzymes and reach the large intestine where they are degraded by intestinal microbiota. This degradation results in the production of metabolites of which short chain fatty acids (SCFA) are the most abundant fermentation products. SCFA are taken up by the host and are thought to mediate most of the health benefits of dietary fibre. The aim of the study presented was to investigate how different dietary fibres influence intestinal SCFA concentrations, microbiota composition and transcriptional regulation. Five dietary fibres differing in carbohydrate composition (inulin, fructooligosaccharide, arabinoxylan, guar gum, resistant starch) and a control diet (corn starch) were chosen to study the effects on microbiota (MITChip), intestinal luminal SCFA concentrations and gene expression (microarray) in a murine model (C57BL/6). All dietary fibres, except resistant starch, increased colonic luminal SCFA concentrations. Gene expression profiling revealed that these fibre modulated the expression of genes involved in multiple metabolic pathways such as fatty acid oxidation, TCA cycle, and electron transport chain. In addition, the transcription factor PPAR was predicted as potential upstream regulator of these processes. Using multivariate data integration techniques (mixOmics), we found strong correlations between the abundance of bacteria belonging to the group of Clostridiales cluster XIVa and the regulation of gene involved in energy metabolism. Using comprehensive omics technology we showed that different dietary fibres induce unique, but overlapping gene expression signatures in response to fermentation that highly correlated with known microbial butyrate producer. In future we will look further into the role of PPAR as upstream regulator.

A proteomic approach to detect regulatory networks in high fat diet induced fatty liver

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Inbred mouse strains have been widely used to identify diet specific mechanisms explaining the deleterious consequences of chronic high dietary fat intake. In this context we analyzed proteomic profiles of mitochondria-enriched protein fractions in liver of C57BL/6J mice which gained fat upon 9 weeks of palm-oil based high fat feeding. Following differential centrifugation to enrich mitochondrial protein, pooled samples of high fat-fed mice and controls were digested by the FASP approach and peptides were fractionated by strong anion exchange. LC-MS/MS was performed on an Orbitrap Elite mass spectrometer coupled to an Eksigent nanoLC 1 D+ ultra system. Quantitative analysis was performed by Progenesis and the Mascot search engine. UniProt knowledgebase was used as identifier. Statistical analysis was achieved using 'R'. In total, 1527 proteins were identified of which 206 proteins were regulated in response to high fat diet. Based on gene ontology for cellular localization, 41% (86 proteins) were mitochondrial, 19% designated to the endoplasmic reticulum (ER), 9% ribosomal (excluding mitochondrial ribosomal proteins), 4% lysosomal and 3% peroxisomal and vesicular, respectively. Subcellular distribution of 10 proteins is unknown and remaining proteins are unspecifically attributed to the intracellular milieu. Ingenuity pathway analysis (IPA) as well as Genomatix Pathway System (GePS) were applied to unravel regulatory networks. The top 5 canonical pathways identified by IPA as upregulated were EIf2 signaling, glutaryl-CoA degradation, superpathway of methionine degradation, arginine biosynthesis and valine degradation, resulting in a higher score for protein biosynthesis and amino acid metabolism than lipid metabolism. Influence of high fat feeding on lipid metabolism was identified by the top upstream regulators SREBP1 (IPA) and PPARα (GePS), which were reflected in upregulated sterol biosynthesis and mitochondrial β-oxidative enzymes. Publication text mining (Genomatix) revealed several transcription factors associated with the observed proteome changes, including Calreticulin, XBP1, DDIT3 and ATF6. These TFs are all connected to protein processing and unfolded protein response in the ER. Noteworthy, mitochondrial energy metabolism, despite some regulated proteins, was not listed among the top scorers in both pathway analysis tools. Some implications for oxidative stress response were given by IPA referring to e.g. upregulated microsomal glutathione-S-transferase and aldo-keto reductase, both enzymes which regenerate oxidized macromolecules. Conversely, catalase and superoxide dismutase 1 were downregulated pointing towards less capacity in liver of high fat-fed mice to detoxify reactive oxygen species. Conclusively, proteome analysis of a mitochondria-enriched protein fraction provides insight far beyond bioenergetics, suggesting a complex regulatory network within lipid and protein metabolism and some hints towards ER stress in fatty liver. These data will further be validated on protein and transcript level.

The impact of B-vitamin deficiency on global gene expression in vascular smooth muscle cell

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B-vitamin deficiency, especially folate deficiency, is a confounding factor in cardiovascular disease. In mouse models withdrawal of B-vitamins increases the number of atherosclerotic plaques. Folate is critically involved in the one carbon group metabolism of cells. Altered cellular methyl group metabolism may affect (among other things) DNA methylation. Vascular smooth muscle cells derived from atherosclerotic plaques indeed display a decrease in global DNA methylation in comparison to smooth muscle cells derived from healthy vascular tissue. We have analysed how folate deficiency affects gene expression in the rat vascular smooth muscle cell line A7r5 using micro-array and RT-PCR. The genes affected by folate supply were assessed for changes in DNA methylation. The results suggest that folate deficiency promotes expression of smooth muscle cell differentiation as indicated by up-regulation of myosin, troponin and actin. Folate deficiency also attenuates cell proliferation and differentiation of the smooth muscle cells could be a consequence of reduced cell proliferation. However, other inhibitors of cell division like serum withdrawal or iron deficiency only partially mirror the changes in gene expression found in folate deficient cells. Many of the folate responsive genes contain CpG islands in their vicinity. Analysis of DNA methylation of these islands using COBRA and pyrosequencing did not show any significant changes of DNA methylation. In addition global DNA methylation was unaffected by folate supply. These results suggest that folate supply does not affect gene expression changes in vascular smooth muscle cells by altering DNA methylation.

The effects of acute and chronic exercise on PGC-1α, irisin and browning of adipose tissue in humans

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Repeated bouts of muscle contraction associated with physical activity, is a stimulus for physiological adaption and remodeling of muscles as well as other organs. One of the potential exercise-induced adaptions of skeletal muscle is increased expression of the transcriptional co-activator peroxisome proliferator-activated receptor γ, co-activator-1α (PGC-1α). Irisin is a PGC-1α dependent myokine with the potential to induce murine brown-fat-like development of white adipose tissue. In humans, the regulatory effect of training on muscle FNDC5 mRNA expression and subsequently irisin levels in plasma is more controversial. To investigate the effect of acute as well as chronic exercise in physically inactive prediabetic subjects and healthy controls, we collected muscle biopsies and plasma samples, before, immediately after and 2 hours post-exercise of 45 min ergometer cycling (70% VO_2 max) at baseline and after 12 weeks of combined endurance and strength training, 4 times weekly. We also harvested subcutaneous adipose tissue biopsies before and after the 12 weeks training intervention. We observed that 12 weeks of combined endurance- and strength-training increased PGC1A and FNDC5 mRNA levels in human skeletal muscle biopsies obtained from healthy controls as well as prediabetic subjects. Plasma concentration of irisin was increased acutely (~1.2-fold) just after exercise but not after 12 weeks of training. There was no significantly enhanced expression of browning genes in subcutaneous adipose tissue after 12 weeks of training. UCP1 mRNA did not correlate with FNDC5 expression in subcutaneous adipose tissue or skeletal muscle or irisin levels in plasma. Our data indicate that there is little or no effect of long-term training on plasma concentration of irisin, and no browning of subcutaneous white adipose tissue in humans in relation to extensive physical activity for 12 weeks.

The role of the GTPase ARFRP1 in lipid droplet turnover of adipose tissue

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ADP-ribosylation factor related protein 1 (ARFRP1) is an ubiquitously expressed monomeric GTPase of the ARF-family and known to be associated with trans-Golgi membranes in the activated GTP-bound state. There it regulates intracellular membrane and protein trafficking by coordinating the recruitment of ARL1 (ARF-like 1) and its effector golgin-245 which itself binds several Rab-GTPases. Based on our previous data showing that a fat-specific deletion of Arfrp1 resulted in a lipodystrophic phenotype due to a defective lipid droplet (LD) formation as well as an increased activation of lipolysis, it appears most likely that an interplay between trans-Golgi and LDs is essential for proper lipid storage capacity. In order to study the molecular mechanisms of ARFRP1 action on LD turnover we have generated an inducible fat-specific Arfrp1 knockout mouse (Arfrp1$^{ad\text{-}ER\text{-}/\text{-}}$) because prenatal deletion resulted in a reduced survival rate. After tamoxifen-mediated induction of fat-specific Arfrp1 deletion in week 5 body weight development and body composition were monitored weekly. Additionally, energy expenditure, the respiratory quotient as well as body temperature and plasma parameters were determined. In order to analyze fat distribution the mice were scanned by computed tomography. Starting from the age of 10 weeks Arfrp1$^{ad\text{-}ER\text{-}/\text{-}}$ mice exhibit a significantly lower body fat content and slightly elevated lean mass in comparison to their control littermates (Arfrp1$^{flox/flox}$). Furthermore, blood glucose measurements displayed higher levels in Arfrp1$^{ad\text{-}ER\text{-}/\text{-}}$ mice under fed and fasted conditions indicating an impaired glucose homeostasis. However, triglycerides in liver and muscle of Arfrp1$^{ad\text{-}ER\text{-}/\text{-}}$ mice were rather reduced so that insulin resistance is no consequence of ectopic fat accumulation. So far neither food intake nor energy expenditure, respiratory quotient or body temperature could account for the difference in fat mass between Arfrp1$^{ad\text{-}ER\text{-}/\text{-}}$ mice and their wildtype controls. The postnatal adipocyte-specific deletion of Arfrp1 followed by a reduction of adipose tissue depots points towards a defect in lipid storage capacity. Further investigations are needed to elucidate the detailed action of ARFRP1 on LD turnover.

Effect of exercise on fatty acid and glucose metabolism in cultured human myotubes

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Physical activity has an important place in prevention and treatment of type 2 diabetes. In this study we examined fuel handling in skeletal muscle cells (myotubes) isolated from subjects with normal and abnormal glucose metabolism before and after 12 weeks of extensive endurance and strength training. Skeletal muscle cells were isolated from m. vastus lateralis of male volunteers before and after an intervention period, including two endurance and two strength-training sessions of 90 min per week. Subjects with normal as well as abnormal glucose metabolism were included. The muscle cells were cultured and differentiated into multinucleated myotubes. Lipid and glucose metabolism were studied using radiolabeled 1-[^{14}C]oleic acid and D-[^{14}C(U)]glucose, respectively. In the pre-intervention experiments the cells were incubated with the PPARδ agonist GW501516 for the last 4 days of the differentiation period. Gene expression was studied using qPCR. Both groups of subjects showed an increased strength capacity (measured by three different strength tests), increased endurance capacity (measured by VO_2 max) and increased insulin sensitivity (measured by clamp GIR). The group with abnormal glucose metabolism had the largest increases, except for in two of the strength tests. Myotubes isolated after a training period of 12 weeks showed an increased oxidation of oleic acid as well as glucose compared to myotubes isolated before the 12 weeks training period. The total uptake of oleic acid in myotubes was also increased after the exercise intervention period. Preliminary data indicated that the expression of some genes important for lipid metabolism and mitochondrial function/biogenesis, such as CD36, PGC1α, Cyc1 and PPARδ, were increased in myotubes after the exercise intervention period. Exercise has a positive effect on glucose oxidation and fatty acid uptake and oxidation that appears to be conserved in human myotubes. The study and biobank is financed with research funding from Helse SørØst, and funding from The University of Oslo, Oslo University Hospital and Norwegian School of Sport Sciences.

Adipose tissue expandability and liver function during aging: sex-associated differences

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A failure in the adipose tissue expansion capacity has been proposed as an important factor involved in the origin of metabolic syndrome complications, the so called 'adipose tissue expandability hypothesis'. The liver plays a key role in the maintenance of nutrient homeostasis and enzymes involved in hepatic lipid partitioning are important in the maintenance of metabolic health. During aging, the functionality of liver and of other tissues is impaired, and this is linked with an increase in metabolic-related alterations. The aim of this study was to analyze differences in adipose tissue expandability between male and female rats and its relation with the age-linked alterations in the expression of genes involved in lipid metabolism in liver and with features of the metabolic syndrome. Male and female Wistar rats of different age groups (26 days and 3, 7 and 14 months old) were studied. Body weight, fat depot weights, triglycerides (TG) levels in liver, blood metabolites, and the expression of genes involved in lipid metabolism in liver were measured. In female rats, fat tissue weight increased gradually with age, whereas males reached a maximum weight at 7 months-old and showed no differences in the adiposity index from 7 to 14 months. Concomitantly, males from 7 to 14 months showed a significant increase in the HOMA index (from 3.98±1.85 to 12.2±4.6), a decrease in the levels of adiponectin (from 1678±165 to 931±165ng/ml) and an increase of TG content in liver (from 2.29±0.26 to 3.28±0.44 mg/g); however no significant changes were observed in females. Males also showed an age-associated increase in plasma TG levels, whereas levels remained unchanged in females. Leptin levels increased with age in both males and females, although the increase was more marked in males. Concerning liver gene expression, 14-month-old males displayed a significant decrease in mRNA levels of key enzymes involved in lipid metabolism, such as CPT1L, FASN and ACC, as well as of insulin receptor and AMPKα, compared with rats at the age of 7 months. Female rats did not show these differences with age. Moreover, mRNA levels of leptin receptor in liver decreased with age in males and were negatively correlated with plasma levels of leptin and with the adiposity index. In females, the expression of leptin receptor decreased from 26 days to 3 months of age, but levels remained unchanged from 3 months onwards. In conclusion, during aging (particularly from 7 to 14 months old), male animals showed a decrease in the mRNA levels of key genes involved in hepatic lipid metabolism, together with the impairment of metabolic parameters (insulin resistance, hypertriglyceremia and ectopic accumulation of TG in liver), whereas females were more protected from most of these age-associated alterations. These differences between sexes on the aging effects may be related to the different expansion capacity of the adipose tissue. The greater capacity of females to expand its adipose tissue, and hence to store the excess of fuel in this tissue, seems to protect them from the deleterious effects of aging compared to males.

Gene-dietary interactions and risk of colorectal cancer

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Intake of various dietary factors, most notably meat, fruits and vegetables and fiber, have been extensively investigated in relation to colorectal cancer risk. Overall the evidence suggests that consumption of red and processed meat modestly increases the risk of colorectal cancer; and fruits, vegetables and fiber decrease risk. These effects are however potentially modified by genetic factors. From candidate-gene studies and genome-wide association studies of colorectal cancer, we have identified key genetic variants in selected genes that modify risk of certain dietary factors including: MTHFR and folate intake and EIF3H/UTP23 and vegetable intake. We will present data from ongoing colorectal cancer studies using conventional and novel statistical methodologies for the detection of gene-dietary interactions. Our findings highlight that diet may modify the effect of genetic variants on diseases risk, which may have important implications for prevention.

Genes versus environment in cancer development

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A small proportion of total cancer is attributable to highly penetrant cancer predisposition genes, where individuals carrying a mutation in one of these genes have significantly higher risk of developing certain types of cancer over their lifetime, compared with the general population. In contrast, many sporadic cancers are associated with lifestyle factors. Dietary choices, weight control, physical activity, and moderation of alcohol intake are primary lifestyle strategies offered as cancer-prevention guidelines, and studies estimate that adherence to these guidelines may reduce cancer incidence by 20-30%. Further, observational studies support the combined association between lifestyle, genetics and cancer risk; individuals with particular common, less-penetrant genetic variants being at altered cancer risk in the face of certain exposures. Nonetheless, combined effects of genetics and diet on biochemical and physiologic processes underpinning cancer susceptibility in humans are not well understood. Controlled dietary interventions and observational studies in well-characterized populations are useful for testing complex relationships between genetics and diet in modulating cancer susceptibility biomarkers. Biotransformation enzymes [i.e. cytochromes P450 (CYP) and conjugating enzymes, e.g. glutathione S-transferases (GST), UDP-glucuronosyltransferases (UGT), and sulfotransferases (SULT)] catalyze metabolism of a range of compounds, including carcinogens and chemoprotective agents, and therefore play a critical role in carcinogenesis. Genetic variation resulting in differential expression or activity of these enzymes may influence response to a dietary exposure. For example, biologic response to well-cooked red meat on the basis of polymorphisms in UGT1A1, the gene product of which is responsible for heterocyclic amine and polycyclic aromatic hydrocarbon metabolism, provides insight into cancer susceptibility. Similarly, individuals who are GSTM1- and GSTT1-null compared to those who were GSTM1+/GSTT1+ have higher serum GST-alpha concentrations with cruciferous-vegetable feeding, suggesting differential response to a vegetable intervention on the basis of genotype. Circulating sex steroids are implicated in the etiology of breast, ovarian, endometrial cancers. UGT and SULT metabolize sex steroids to less active compounds. We evaluated the phenotypic effects of UGT and SULT polymorphisms on circulating sex steroids in 170 premenopausal women. Women with the UGT1A1(TA7/TA7) genotype had 25% lower mean estradiol concentrations compared to the wildtype (TA6/TA6) (P=0.02). Similar associations were observed between SULT1A1(R213/H213) and estrone (P=0.02) and UGT2B4(E458/E458) and dehydroepiandrosterone (P=0.03). Further, polymorphisms in SULT1A1 and the UGT1A locus were associated with altered percent breast density in these women. Characterizing, *in vivo*, the functional effects of particular genotypes in the context of different exposures is important to understanding cancer susceptibility and may help us to more precisely tailor cancer-prevention guidelines.

Epithelial deletion of mitochondrial HSP60 causes focal hyperproliferation of intestinal stem cells

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Heat shock protein (HSP) 60, a mitochondrial (mt) unfolded protein response (UPR)-associated chaperone, is implicated in the pathogenesis of chronic intestinal inflammation. This study investigates the role of HSP60 in intestinal epithelial cell (IEC) homeostasis using novel tissue-specific knockout mouse models. Generation of epithelial-specific Hsp60 knockout mice (Hsp60$^{flox/flox}$XVillinCre) antagonized embryonic development upon day eleven after conception and induced embryolethality. Postnatal induction of an epithelial specific Hsp60 knockout (Hsp60$^{flox/flox}$XVillinCreER^{T2}) caused a severe phenotype with rapid weight loss and mortality. Histological evaluation of intestinal gut sections revealed aberrations in villus-crypt architecture including focal crypt hyper-regeneration dominantly in the upper small intestine. While HSP60-negative crypt epithelial cells showed an abrogated expression of the proliferation marker KI67, hyper-regenerative crypts were positive for HSP60 and KI67. These crypt foci originate from few HSP60-positive cells which express the stem cell marker olfactomedin (OLFM) 4 and escaped from the genetic knock out in the crypt. Although a loss of HSP60 led to an abrogated KI67 expression in colonic epithelium as well, this did not cause overall tissue pathology. Absence of HSP60 both in jejunal and colonic epithelium induced hallmarks of the mtUPR in IEC, suggesting the presence of compensatory mechanisms upon loss of HSP60 at early phases of mtUPR. These include induction of mitochondrial chaperones like mtHSP70, proteases like the ATP dependent caseinolytic peptidase and transcription factors like C/EBP-homologous-protein. Mitochondria showed spherical deposits in the matrix and a decreased expression of functionality markers like the mitochondrial encoded cytochrome c oxidase (COX) I. Tissue-specific deletion of Hsp60 disrupts epithelial cell homeostasis both pre- and postnatally leading to crypt aberrations and compartmentalized pathologies in the small intestine. Activated unfolded protein responses in the intestinal epithelium appear to be not causative for the emergence of intestinal inflammation but create an environment that favors crypt hyperplasia with the possibility for neoplastic transformation and tumor development.

The effects of trans fatty acids on the miRNA expression profile in human colon cancer cells

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Trans fatty acids (TFA) are unsaturated fatty acids with at least one double-bond in trans configuration. They are generated during industrial oil hardening and refinement processes at high temperatures or are formed by bacterial metabolism in ruminant animals. While there is a clear correlation between the consumption of TFA and an increased risk of coronary heart disease, a potential cancer risk is still controversially discussed. MiRNAs are small, non-coding RNAs that regulate the gene expression on a post-transcriptional level. By regulating target mRNAs they can act as oncogenes or tumorsupressors and it is known that they are differentially expressed in a multitude of tumors. The aim of this project was to analyze potential fatty acid-induced changes of the miRNA expression profile in human colon cancer cells to investigate their potential role in colon carcinogenesis. We investigated 16 different C:18-Isomers with double bonds in trans and/or cis configuration. Caco-2 cells (human colorectal adenocarcinoma cells) were incubated for 24 h with different fatty acids. The RNA was isolated and transcribed to cDNA. Subsequently we analysed the expression of 84 cancer-associated miRNAs by qRT-PCR using the Human Cancer PathwayFinder miScript qRT-PCR Array (Qiagen). The Fold Change of miRNA expression of fatty acid-treated Caco2 cells in comparison to the miRNA expression levels of untreated cells was determined and analysed. Hsa-miR-184, hsa-miR-146a-5p, hsa-miR-9-5p and hsa-miR-10a-5p were significantly upregulated by several fatty acids whereas hsa-miR-32-5p and hsa-miR-15a-5p were significantly downregulated after fatty acid exposure. In particular hsa-miR-32-5p (miR-32) is an interesting subject for further investigations. We provide first evidence that fatty acid-induced downregulation of miR-32 correlates with an upregulation of mRNA expression of bim, a proapoptotic member of the Bcl-2 pathway. Thus, our data support the hypothesis that fatty acids have an impact on the induction of apoptosis via downregulation of miR-32. The fatty acid-induced effects, however, were not dependent on the presence of trans double bonds in the tested compounds as the homologous compounds with double bonds in cis configuration showed comparable effects.

Urolithins A and B regulate the expression of PSA at a transcription level in prostate cancer cells

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Prostate cancer is the second leading cause of death in men in the United States and the sixth cause of cancer-related death in men worldwide, therefore the identification of new agents that may prevent and/or regulate the progression of cancer cell growth and/or development is of paramount importance. Walnuts contain several nutrients and compounds such as vitamin E, omega-3 fatty acids, phytosterols and polyphenols, which have been shown to slow down cancer development and progression. Ellagitannins (ETs) are the most abundant polyphenols present in walnuts, and play an important role in their reported biological properties. After consumption, ETs hydrolyze to release ellagic acid (EA), which is converted by gut microflora to urolithin A (UA) and other derivatives, such as urolithin (UB). This study investigated the effects of UA and UB on the expression of Prostate – Specific Antigen (PSA), as well as the regulatory mechanism in a prostate cancer cell model using LNCaP (androgen responsive) and PC-3 (androgen independent) cell lines. Urolithins A and B decreased the transcriptional activation of PSA by both androgen dependent and independent mechanisms. The mRNA levels of both PSA and Androgen Receptor (AR) were down-regulated after treatment with UA, UB and a MIX (M) of both metabolites. PSA protein levels were also decreased in these conditions. Transient transfection of PC-3 cells with a luciferase construct of the PSA-promoter containing three AREs showed that UA, UB and M inhibited AR-mediated PSA expression at the transcription level. Our results suggest that Urolithins A and B attenuate the function of the AR by repressing its expression, causing a down-regulation of PSA mRNA and protein levels, which indicates the potential role of walnuts as a chemo-preventive and/or chemo-therapeutic agent for prostate cancer.

Network gene expression analysis of breast cancer and obesity with vitamin D intake

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DNA Microarrays 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. We analyzed the gene expression data associated to the breast cancer and relationship with obesity in a profile of microarray data with possible application to bioactive components like vitamin D. We found responsible genes of Obesity and intersection with breast cancer genes and we analyzed the possible interaction between specific genes for studying a network model of interaction. We have modelling the clustering of gene networks by applying the mathematical procedure Principal Component Analysis (PCA) to the gene expression data. As well, this network could help to control dietary variables associated with vitamin D intake to prevent risk of obesity and breast cancer and to obtain evidence of their interaction with certain human genome modules.

Tissue-specific overexpression of UPR-related C/EBP homologous protein impairs mucosal tissue repair

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Loss of epithelial cell homeostasis and apoptosis highly contribute to intestinal inflammation. While endoplasmic reticu¬lum and mitochondrial unfolded protein responses (UPR) have been implicated in chronic intestinal inflammation, functional correlation between UPR-related C/EBP homologous protein (CHOP) expression and CHOP-mediated programming towards inflammation-related disease susceptibility remains to be elucidated. In this study, we generated and characterized a novel transgenic mouse model expressing high levels of HA-tagged CHOP in intestinal epithelial cells (ChopIEC Tg/Tg). Transcriptional profiling of disease-free transgenic mice identified a set of CHOP-dependent target genes related to inflammatory and microbial defense program in the intestinal epithelium. Since ChopIEC Tg/Tg mice do not spontaneously develop an inflammatory phenotype, the effect of CHOP overexpression was characterized on intestinal homeostasis in response to infectious bacteria and Dextran Sodium Sulphate (DSS)-induced colitis, as well as mechanical injury. Under conditions of acute colonic inflammation, transgenic mice are not affected by Citrobacter rodentium infection, but reveal more severe inflammation and tissue injury in response to acute DSS-induced colitis. At the molecular level, transcriptional activation of transgenic CHOP was increased by site-specific phosphorylation leading to significantly increased expression of CHOP target genes. Delayed recovery from DSS-induced colitis and impaired closure of mechanically-induced mucosal wounds clearly indicate a role of CHOP in epithelial cell homeostasis in response to injury. In conclusion, transgenic epithelial cell-specific overexpression of CHOP aggravates intestinal inflammation associated with the loss of epithelial cell restitution as well as impaired tissue healing and repair, supporting a disease-conditioning role of UPR-related CHOP expression in the epithelium.

Genes versus environment in type 1 diabetes

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Type 1 diabetes(T1D) is an autoimmune disease. Both, genetic susceptibility and environmental factors contribute to its rising incidence. A proportion of estimable genetic risk can be quantified from family history information and the presence of HLA DR/DQ susceptible genotypes. Children with a family member with T1D have a T1D risk that exceeds 5%, as compared to 0.4% in the children without family history. Risk can be further stratified on the basis of which affected family member has T1D and the presence or absence of HLA DR/DQ susceptible genotype: T1D risk in a child who has a family member with T1D can be stratified 100-fold from 0.3% up to 30% depending on his or her HLA DR-DQ genotype. Importantly, T1D risk in the children without a family history of T1D can be stratified from around 0.01% to over 5%. Further improvement in genetic prediction can be obtained by combining family history, HLA, and minor T1D susceptibility gene loci. In our own study in offspring of people with T1D, the best predictive model was obtained by the sum of risk alleles for 8 genes (IFIH1, CTLA4, PTPN22, IL18RAP, SH2B3, KIAA0350, COBL, ERBB3) in children with HLADR/DQ susceptibility alleles. Environmental factors are also important. In addition to dietary factors such as gluten, and cow's milk proteins in the first year of life, and early perinatal determinants such as C-section, infections have been discussed as important environmental determinants in the pathogenesis of T1D. We analyzed infections and fever episodes during the first three years of life in children with familial risk of T1D and identified respiratory infections, especially in the first year of life, as a risk factor for the development of T1D. We also identified viruses from plasma samples by next generation sequencing but did not find viruses in plasma more frequently in children who developed autoimmunity and T1D than in children who remained healthy. Essential for studying genes and environment in type 1 diabetes are prospective cohorts which follow children at risk. The German BABYDIAB/BABYDIET study and the international TEDDY study follow over 10,000 children at increased T1D risk from birth to the age of 20 years. These studies contribute to the identification of genes and environment and its interaction in the pathogenesis of T1D.

The impact of genes and dietary components on the development of type 2 diabetes

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Type 2 diabetes mellitus is a complex disease characterized by beta-cell failure in the setting of insulin resistance. In early stages of the disease, pancreatic beta-cells adapt to insulin resistance by increasing mass and function. As nutrient excess persists, hyperglycemia and elevated free fatty acids negatively impact beta-cell function by impairing insulin secretion, decreasing insulin gene expression and ultimately causing apoptosis. Type 2 diabetes is also determined by multiple genes that we aim to identify by quantitative trait loci (QTL) mapping techniques. The New Zealand Obese (NZO) mouse that develops a polygenic disease pattern of obesity, insulin resistance, and type 2 diabetes was used to generate outcross populations with C57BL/6J (B6) and SJL mice for linkage studies. We located more than 15 QTL of which we successfully identified three responsible gene variations by positional cloning (Tbc1d1; Zfp69; Ifi202b). NZO mice exhibit a strong expression of interferon-inducible gene Ifi202 in adipose tissue, liver, muscle, and pancreatic islets, whereas B6 mice lack an Ifi202b expression in these tissues due to a microdeletion including the first exon and the 5'-flanking region. We identified 11β-hydroxysteroid dehydrogenase type 1 (11β-Hsd1) as a putative downstream target of Ifi202b that might participate in the development of obesity. Suppression of Ifi202b by siRNA in 3T3-L1 adipocytes resulted in a significant inhibition of 11β-Hsd1 expression, whereas an adenoviral-mediated overexpression of Ifi202b increased 11β-Hsd1 mRNA levels. NZO and B6 mice markedly differ in their diabetes prevalence. In both strains carbohydrate-restricted diets fully prevent beta-cell destruction in spite of an extreme insulin resistance. However, addition of carbohydrates induces glucolipotoxic conditions in NZO mice by inhibiting insulin/IGF1 signalling and inducing apoptosis in beta-cells, whereas beta cells of B6-ob/ob mice respond with an induction of proliferation, thereby preventing hyperglycemia. Transcriptome analysis of islets from NZO and B6-ob/ob mice kept on a carbohydrate-free diet for 15 weeks and a subsequent two-day treatment with or without carbohydrates identified several differentially expressed genes that are located in QTL and involved in the regulation of proliferation. In conclusion, the switch from an insulin resistant state to manifest type 2 diabetes markedly depends on the genetic predisposition, specifically on the disability to induce beta-cell proliferation in response to glucose.

Alternative routes to gene discovery in type 2 diabetes

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Until date, genome wide association studies (GWAS) have identified 65 common genetic variants associated with T2D or glucose/insulin level. Despite this apparent success, these variants explain only about 10% of the heritability of the disease emphasizing the need for novel approaches to identify susceptibility genes for T2D. One reason could be that the GWAS variants are usually 100s of generations old and the effect size must be small to allow the variant to escape purifying selection. On the other hand, the change in environment affects only the last 2-3 generations. Given that most people have inherited genetic susceptibility to T2D (average frequency of risk alleles for T2D is 54%) it may be more rewarding to search for variants that protect us from the change in the environment. Recently applying such a design (controls had despite clustering of risk factors escaped T2D, whereas cases had early-onset disease) to target sequencing of known T2D-associated loci we observed a very rare variant in the SLC30A8 gene protecting from T2D. We should also keep in mind that SNPs only provide a snapshot of one type of variants in the human genome but does not provide any functional explanation on how it would increase susceptibility to disease. We clearly need a more holistic systems genetics approach to identify novel T2D genes. It can be anticipated that many variants could exert their effect by influencing expression. One alternative approach is therefore to use genetic loci associated with expression traits in disease-relevant tissues and to relate gene expression to not only SNPs and disease risk but also to intermediate phenotypes. Although T2D is characterized by both impaired insulin secretion and action, most of these associated variants seem to influence insulin secretion. One of the obstacles in human diabetes research has been the inaccessibility of pancreatic islets. This hurdle has to some extent been circumvented by research using islets from cadaver donors intended for islet transplantation. Using a systems genetics approach in a large number of well phenotyped human islets we were able to identify 20 top ranking genes that could explain 24% of the variance in HbA1c in these individuals. Using an alternative systems biology approach we identified SFRP4 as a potential mediator of the deleterious effects of inflammation on insulin secretion and risk of T2D. SFRP4 concentrations in plasma also predicted future T2D with an OR of about 5. These data demonstrate the value of alternative approaches and systems medicine for the identification of novel genes involved in T2D pathogenesis.

Metabolite profiling in ob/ob and db/db mice to discriminate obesity- from diabetes-related changes

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Multiple studies in humans with obesity or type 2 diabetes have identified changes in the plasma metabolites for which the origin remains obscure. Type 2 diabetes often coincides with obesity and this state of 'diabesity' challenges research to separate diabetes-associated from obesity-associated effects. We used two mouse models for separating obesity- from diabetes-related changes in the plasma metabolome and profiled in addition a number of organs. Obese but rather diabetes-resistant ob/ob mice were compared with 20 weeks old obese and diabetes-prone db/db mice. Both mouse models have a defective leptin-signaling pathway but the different genetic backgrounds of the ob/ob and the db/db mouse (C57BL/6 and C57BL/Ks, respectively) determine their susceptibility to diabetes, due to hypertrophy or atrophy of the pancreas, respectively. For standardization, all mice were fed the same chemically-defined control diet. Metabolite profiles from plasma and tissues (liver, muscle, kidney) were quantified via LC-MS/MS using Biocrates Absolute IDQ p180 kit measuring 186 metabolites in 5 compound classes (acylcarnitines, amino acids, biogenic amines, hexoses and phospho- and sphingolipids). Furthermore, amino acid profiles were quantified via LC-MS/MS using aTraq labeling. Data analysis was performed using the software environment R. A hyperglycemia was only observed in the db/db mouse model and here metabolites typically associated with diabetes such as ketone bodies increased significantly. On the other hand specific plasma amino acids revealed similar changes in both db/db and ob/ob mice suggesting that these metabolites are more associated with obesity and insulin resistance (IR). Most significant in both models was the decrease in plasma hydroxyproline levels. The ob/ob mouse displayed in addition prominent increases in plasma ornithine and tryptophan levels which seem to be associated with hepatosteatosis. A substantial increase in liver and adipose tissue mass in both models could be observed, while at the same time, muscle mass was strongly reduced. For the liver, prominent decreases in both models were seen for glycine levels. Aromatic amino acids were specifically increased in livers of ob/ob mice. In contrast to humans with increases in branched chain amino acids in plasma in IR and diabetes, these amino acids were not significantly different in db/db and ob/ob mice compared to their controls. However, high cross-correlations between plasma and muscle metabolite concentrations were found. Kidney metabolite profiles also changed but correlations with plasma metabolites were rather low. In conclusion, metabolite profiling in these mouse models identified known but also new metabolites that change in obesity and diabetes in plasma and for the first time also in tissues. Our comparative approach opens the chance to separate the 'diabesity' metabolite profile into specific diabetes and obesity subsets and the tissue metabolite profiling provides a starting point for more mechanistic approaches to find the origin of the changes.

Type 2 diabetes variants in Wnt-associated genes interact with dietary fiber on type 2 diabetes risk

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TCF7L2 is a principal transcription factor in the canonical WNT signaling pathway. Short chain fatty acids, fermentation products of dietary fibers, are potent histone deacetylase inhibitors that can up-regulate WNT activity. We have previously reported that TCF7L2 rs7903146 interacts with dietary fiber intake on type 2 diabetes (T2D) incidence. Here we study whether other GWAS identified T2D associated single nucleotide polymorphisms (SNPs) in genes involved in the WNT pathway interact with dietary fiber on T2D incidence. We included 26,930 individuals without diabetes from the Malmö Diet and Cancer (MDC) population based cohort. Diet data was collected at baseline (1991-1996) using a modified diet history method (a food frequency questionnaire, a 7-day food diary, and an interview). 51 gene loci (58 SNPs) were analyzed for links to WNT-signaling using annotations from Gene Ontology, Kioto Encyclopedia of Genes and Genomes, Biocarta, Panther pathways and Literature integrated within the DAVID-ABR. Over a mean follow up period of 14 years 2,860 cases of T2D were recorded. COX proportional hazards was used to analyze association with T2D (adjusted for age and sex) and interaction between genotypes and quintiles of fiber intake (adjusting for age, sex, BMI, total energy intake, season, diet method version, physical activity, smoking, alcohol intake, and education). Seven genes (9 SNPs) were annotated as involved in WNT-signaling (upstream of TCF/LEF) including TCF7L2 (rs7903146 and rs12255372), HHEX (rs1111875), HNF1A (rs7957197), NOTCH2 (rs10923931), TLE4 (rs13292136), ZBED3 (rs4457053) and PPARG (rs1801282 and rs13081389). SNPs in TCF7L2, HHEX and HNF1A loci predicted future T2D [HR 95% CI: 1.32 (1.24-1.39), 1.23 (1.16-1.30), 1.07 (1.01-1.12), and 1.14 (1.07-1.22) per risk allele]. SNPs in TCF7L2, NOTCH2 and ZBED3 loci interacted significantly with fiber intake on T2D incidence ($P_{interaction}$=0.04, 0.006, 0.01, and 0.003, respectively). Higher fiber intake associated with lower T2D risk only among homozygotes for the non-risk alleles of TCF7L2 SNPs [n=13,482 and 13,522: HR 95% CI 0.95 (0.91-0.99) and 0.94 (0.90-0.98) per fiber intake quintile]. Higher fiber intake associated with lower T2D risk only among risk allele carriers of NOTCH2 rs10923931 [n=4,375 (GT) and 228 (TT): HR 95% CI: 0.90 (0.84-0.97) and 0.70 (0.50-0.99) per fiber intake quintile]. Higher fiber intake associated with lower T2D risk only among homozygotes for the risk allele of ZBED3 rs4457053 [n=1,643: HR 95% CI: 0.84 (0.75-0.94)]. HHEX rs1111875 had a tendency for interaction with fiber intake ($P_{interaction}$=0.11), but no interaction was observed for HNF1A, TLE4, and PPARG SNPs. None of the 44 loci not annotated to WNT signaling interacted with fiber intake. Our results indicate that several T2D susceptibility genes involved in WNT signaling interact with dietary fiber intake on T2D incidence. The putative mechanisms by which fiber intake could modify the WNT signaling by the susceptibility variants in T2D pathogenesis include effects on beta cell survival, adipogenesis and adipocyte proliferation, and/or GLP-1 production.

Identification of human exercise-induced myokines using secretome analysis

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Endurance exercise is associated with significant improvements in cardio-metabolic risk parameters in disease like diabetes and obesity, partly via mechanisms independent of weight reduction. A role for proteins secreted from skeletal muscle, the so called myokines, has been hypothesized. However, limited information is available about the myokines induced by acute endurance exercise. The aim is to identify novel endurance exercise-induced myokines in humans. Twelve male subjects engaged in one-legged cycling exercise at 50% Wmax for 1 hour. Muscle biopsies were taken immediately before and after the exercise bout. Whole genome gene expression analysis was performed, followed by selection for genes encoding secreted proteins. Changes in muscle mRNA expression of selected myokines were confirmed by qPCR and plasma levels of selected myokines were determined by multiplex assay and ELISA. Secretome analysis resulted in a list of 96 putative myokines, which was further reduced to 30 putative myokines by applying a fold-change cut-off of 1.5. Most putative myokines were altered in the exercising leg or in both exercising and non-exercising leg. The most highly induced gene encoding a secreted protein was ADAMTS1 (FC 4.9), plasma levels if which were induced 5-fold two hours post-exercise. Significant induction of mRNA and plasma levels by exercise was also observed for CX3CL1 and MCP-1 (CCL2). Known myokine IL-6 and putative myokines CYR61 and VEGFA were significantly induced at the mRNA level without a significant change in plasma concentration. mRNA expression and plasma concentrations of known myokines IL-8, IL-15, and BDNF were not altered by acute exercise. We identified several novel myokines that were induced by exercise at the gene expression level and in blood plasma and that may be involved in communication between skeletal muscle and other organs.

Metabolomics analysis in backcross populations of the NZO mouse

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Certain trends in levels of metabolites have been previously associated with impaired metabolic function, such as increases in the branched-chain amino acids (BCAAs) and acylcarnitines. The New Zealand Obese (NZO) mouse, a model of the human metabolic syndrome, was used in the past to identify various quantitative trait loci (QTL). Through the strategy of positional cloning, these QTL have thus revealed genes relating to a particular phenotype or metabolic trait. We aim to apply this strategy, combining an analysis of blood metabolites from backcross (N2) mice with genotype data in order to identify genes that impact diabetes or other metabolic disorders. Two different mouse strains, C57BL/6J (diabetes resistant) or DBA/2J (diabetes susceptible), were mated to female NZO mice to produce two F1 hybrids. Male F1 mice were then backcrossed with female NZO to generate two separate populations of N2 mice. All mice in this study were placed on a high-fat diet (45% fat) at weaning. For the metabolomics analysis, tail blood from 15 males of each of the parental inbred strains and approximately 300 male N2 (10 weeks of age) was collected. Metabolites in blood samples from parental strains were analysed by liquid chromatography – mass spectrometry. The metabolites profiled included 28 amino acids, of which 13 were different between male NZO and C57BL/6J mice, whereas only 4 were different between NZO and DBA/2J. All 3 BCAAs (leucine, isoleucine and valine) were increased in NZO males as compared to their lean counterparts. Of the 35 acetylcarnitines analysed, 21 were significantly different in NZO compared to C57BL/6J while 17 were altered compared to DBA/2J. Increases in BCAAs are concomitant with both insulin resistance and glucose intolerance due to their effects on the regulatory protein mTOR, as well as on gluconeogenesis and other catabolic pathways. Widespread increases in acylcarnitines also suggest an impaired long chain fatty acid β oxidation in the diabetes-prone NZO mice. NZO mice have a well described hyperphagia, and their increase in food intake is likely to explain some of the differences in their metabolic profile compared to the lean strains. However, differences between the lean DBA/2J and C57BL/6J also indicate a genetic influence on metabolites. Analysis of the N2 population is ongoing, however we predict from the large variation in their phenotypes that QTL may be identified that correlate with levels of certain metabolites. The unique metabolomics profiles we obtained may provide further evidence for both the genetic influence on metabolite levels as well as relationship between these metabolites and mechanisms that relate to diabetes susceptibility.

Apelin is a myokine with potentially important metabolic effects

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Some of the beneficial metabolic effects of exercise may be mediated by secreted factors from skeletal muscle termed myokines. Apelin is a small peptide originally described as an adipokine. It is expressed in many peripheral tissues, including skeletal muscle. In muscle of insulin-resistant mice, injection of apelin increases fatty acid oxidation, mitochondrial biogenesis and glucose uptake. In addition, aerobic exercise increases plasma levels of apelin. The expression and function of apelin has so far not been well documented in humans In a human intervention study, 26 healthy and physically inactive men aged 40 to 65 y underwent combined strength and endurance training for 12 weeks including two endurance bicycle sessions (60 min) and two whole-body strength-training sessions (60 min) per week. Thirteen of the participants had normal glucose metabolism and normal weight, whereas 13 had prediabetes and were overweight. Before and after the 12 weeks exercise intervention the subjects underwent an acute 45 min exercise workload (70% of VO_2 max). Blood samples and biopsies from skeletal muscle (m. vastus lateralis) were collected before, directly after and 2 h after the acute and chronic exercise bouts. This approach enables us to study the effects of both acute and chronic exercise. To study the expression and secretion of apelin *in vitro*, primary human skeletal muscle cells were isolated and grown in culture and differentiated into multinuclear myotubes. These muscle cells exhibit similar properties to muscle fibers *in vivo*. We are planning to treat the cells with electrical pulse stimulation and pharmacological substances (such as caffeine, ionomycin and forskolin) to study the effect of muscle contraction in more detail. In the human intervention study, gene expression of apelin increased acutely in skeletal muscle measured directly after (fold increase: 1.5, $P<0.001$) and 2 h after (1.7 fold increase, $P<0.001$) the end of the acute 45 min exercise session. There was also a chronic upregulation after 12 weeks of exercise (2.3 fold increase, $P<0.001$). In plasma we observed a significant, acute increase in apelin concentration. It has been reported that caffeine increases the cellular concentration of cAMP and calcium release from intracellular stores – thereby mimicking some effects of muscle contraction. Our preliminary data indicate that apelin expression is induced >2-fold by incubating human skeletal muscle cells with 2.5-10 mM caffeine. The expression of apelin is regulated in skeletal muscle in response to acute and chronic exercise, and plasma concentration of apelin is increased immediately after acute exercise. We hypothesize that some of the beneficial metabolic effects of exercise are caused by secretion of apelin from skeletal muscle fibers.

The effect of the MyoGlu training intervention on lipid droplets in muscle by quantitative electron

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MyoGlu is a training intervention in Oslo where human volunteers, controls and prediabetics, exercise for 12 weeks. Muscle biopsies from m. vastus lateralis were obtained before and after the intervention. Muscle fiber samples were fixated, embedded in Durcupan and prepared for electron microscopy. A blinded quantification of lipid droplets (LD) in the subsarcolemmal (SS) and the intermyofibrillar (IMF) regions indicate that control as well as prediabetic participants respond to the training intervention, both concerning the numbers and diameters of LD. Preliminary results indicate that the LD of the SS region in the prediabetic subjects are more sensitive to training than in the healthy controls (n=4, full sample group n=18).

Characterization of diabetes traits in backcross populations in diabetes-prone and diabetes-resistan

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The New Zealand Obese (NZO) mouse has been utilized for many years as a model of the human metabolic syndrome. Outcross experiments with lean strains performed in the past have led to the discovery of various quantitative trait loci (QTL), wherein specific diabetes and obesity genes have been identified by positional cloning. Through analysis of additional traits, e.g. insulin sensitivity we aim to identify novel diabetogenic QTL. Two distinct backcross populations of the NZO with either diabetes resistant C57BL/6J or diabetes susceptible DBA/2J have been initiated. The phenotypes of males and females on high-fat diet (45% fat) from parental, F1 (intercross) and N2 (NZO. F1 backcross) were monitored in respect to body weight and blood glucose, as well as insulin sensitivity and body composition. At 16 (males) and 22 (females) weeks of age mice were sacrificed for multiple-organ collection. Systemic insulin sensitivity was determined at week 8 for males by measurements of blood glucose and insulin concentrations in plasma samples comparing 16 h-fasted with 2 h-postprandial state. Phenotypes of the N2 generation will be correlated with genotypes in order to produce a logarithm of odds (LOD) score and to map distinctive QTL relevant to the diabetic phenotype. As expected insulin sensitivity in NZO parental mice was markedly impaired in comparison to DBA and B6 mice. In both backcross populations the data show a broad variation in all monitored phenotypes and generally phenotypes were more pronounced in males than in females. Body weight and random blood glucose levels of male B6.N2 were higher compared to male DBA.N2 throughout the study. Females had lower blood glucose levels and were protected against hyperglycemia. The mean systemic insulin sensitivity was not different between both backcross populations. Postprandial blood glucose levels were moderately higher in B6.N2 than in DBA.N2, but lower than those of NZO mice (in mM B6.N2: 12.7; DBA.N2: 10.5; NZO: 27.5). Interestingly, postprandial insulin levels were clearly higher in both backcross populations in comparison to NZO parental strains (insulin in μg/l B6.N2: 2.9-73.6; DBA.N2: 1.1-51.7; NZO: 4.5±0.6), indicating compensatory mechanisms allowing a better regulation of glucose clearance. The broad variations in blood glucose and plasma insulin levels in both backcross populations indicate that we will identify QTL by linkage analysis reflecting diabetes-suppressor and diabetes-promoter genes. We expect to confirm former results, but also to identify novel diabetes and obesity QTL carrying genes relevant for the metabolic syndrome.

APO B insertion/deletion SNP and waist circumference in type 2 diabetics

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Apolipoprotien B insertion/deletion polymorphism plays a major role in development of chronic disease such as cardiovascular disease (CVD) and type 2 diabetes. This effect seems to be result of interaction between genetic and environmental factors including abdominal obesity. The aim of our study was to assess the effect of insertion/deletion polymorphism of apolipoprotien B gene on waist circumference (WC) in diabetic patients. This cross-sectional study was conducted on 700 diabetic patients (276 men, mean age 54.1±6.6 years and 423 women, means age 53.8±6.5 years) who were recruited from several health centers related to Tehran university of medical sciences. Analyses were conducted separately for men and women. Waist circumference was measured with a tape, with the subject standing. Genotyping was performed by using electrophoresis on polyacrylamide gel and polymerase chain reaction (PCR) technique. The possible association of insertion/deletion polymorphism of apolipoprotien B gene and WC were analyzed by using the SPSS version 18, at the alpha level of 0.05. Not women but men, homozygous for the insertion allele, were associated with higher waist circumference (P=.023) than men homozygous for the deletion allele and heterozygous for Apo B ins/del polymorphism, controlled for covariates. Logistic regression analyses of this polymorphism, adjusted for age, BMI, physical activity, smoking status and alcohol consumption disclosed the ins/ins genotype to be associated with abdominal obesity in men at an odds ratio of 2.33 (95% CI=1.33-4.8; P=0.02). Based on the results of the present study, Ins/Ins genotype of Apo B gene is a risk factor for abdominal obesity and high waist circumference in men with type 2 diabetes.

Effect of 12 weeks training in inactive men; overweight prediabetics and normalweight controls.

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Physical activity is important for prevention and treatment of type 2 diabetes. In this study we examined the effect of training on selected parameters of metabolic health. Overall aim was to identify signal molecules relevant to the effect training has on insulin resistance/type 2 diabetes. Healthy and physically inactive men (40-65 year) were recruited into two groups; overweight with abnormal glucose metabolism or controls with normal weight and normal glucose response. Abnormal glucose metabolism was defined as impaired fasting glucose ≥5.6 mmol/l and/or impaired glucose tolerance (2 h S-glucose ≥7.8 mmol/l). Both groups (n=26) underwent combined strength and endurance training for 12 weeks, including two endurance bicycle sessions (60 min) and two whole-body strength training sessions (60 min) per week. Before and after the intervention we monitored insulin sensitivity by euglycemic hyperinsulinemic clamp (glucose infusion rate), body composition by bioimpedance and magnetic resonance imaging/spectroscopy, maximum strength, endurance by maximum O_2 consumption (VO_2 max), as well as blood and tissue samples in connection with an acute 45 min endurance session with 70% load of VO_2 max. Both groups benefited from the training showing improved endurance, strength, and insulin sensitivity. VO_2 max increased 12.9% in both groups; 5.7 (4.1) and 4.8 (2.8) mg/ml/kg in the controls and prediabetic subjects, respectively. Overall maximum strength increased 11-15%. Insulin sensitivity increased by 2.7 (2.0) mg/kg/min in the control group and 1.2 (1.2) mg/kg/min in the prediabetic group, 36% and 30% respectively. The prediabetic group decreased in body weight (3.9%), waist circumference (3.3%) and fat% as measured by bioimpedance (9.5%). Plasma levels of selected signal molecules (myokines/adipokines) were measured following acute exercise as well as chronic training. Plasma concentration of IL-6 increased during acute exercise, but the baseline level and acute response was not changed as a result of chronic training. Leptin levels were higher in prediabetics, were unchanged during acute exercise, but decreased significantly following 12 weeks training in both groups. The reduction following chronic training was larger in prediabetics than in controls. Levels of high molecular weight adiponectin were lower in prediabetics and were decreased following chronic training in both groups. An acute increase in HMW adiponectin was seen only after chronic training. Parameters of metabolic health were improved following 12 weeks training. Levels of tissue-specific circulating signal molecules are influenced following acute exercise and chronic training. ClinicalTrials.gov Identifier: NCT01803568.

APO B insertion/deletion SNP and body mass index in patients with type 2 diabetes

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Apolipoprotien B (ApoB) is the principal protein component of LDL, VLDL and chylomicrons. Numerous polymorphisms of the ApoB gene have been described. Particularly, the insertion/deletion polymorphism, associated with obesity, diabetes and coronary heart disease (CHD). No such study in the Iranian population and also subjects with type 2 diabetes has been performed. The present study was carried out to investigate the relation between ins/del polymorphism of ApoB gen and BMI in diabetic patients. In this study, 700 patients with type 2 diabetes were enrolled from referral centers for diabetic patients from Tehran/Iran. We assessed BMI. The ins/del polymorphism was determined by electrophoresis on polyacrylamide gel after PCR amplification. The relative frequency of the ins & del alleles were 82 and 18%, respectively. There was no significant association between genotype and BMI in any models including recessive, dominant and co-dominant after adjustment for age, BMI, physical activity and smoking in multivariate regression model. The frequency constitution differed from the Korean, U.S white and French but resembled the Japanese. No relationship was found between ApoB ins/del polymorphism and general obesity. It seems that further studies are needed for conclusive findings.

The fasting mouse mimics the changes in the plasma metabolome of human diabetic patients

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Various metabolomics studies in humans have identified plasma metabolites as biomarkers for the development of insulin resistance and non-insulin dependent type 2 diabetes. In search of the origin of these changes in the metabolome we screened a variety of mouse models for similar changes in plasma metabolites. Only the type 1 diabetes model induced by streptozotocin treatment came close to the human condition with the characteristic changes. Since the streptozotocin-treated mouse is highly catabolic we assessed whether a fasting-induced catabolic state in C57BL/6 mice displays as well the diabetic plasma fingerprint of human patients. We profiled plasma, liver and skeletal muscle of C57BL/6 mice in response to fasting using a combination of metabolomics techniques (LC-MS/MS, GC-MS, NMR). Metabolite changes were submitted to metabolic network analysis for assessing the interrelationships of the different metabolite subgroups and the inter-organ coordination in the catabolic condition. Fasting caused changes in the plasma metabolome characterized by increased levels of numerous long-chain acylcarnitines and acetyl-carnitine, but also of the branched-chain amino acids, alpha-aminobutyric acid (ABA) as well as various ketone bodies including 2-OH-butyrate. These metabolites have been previously identified as markers in human IR or diabetes. Changes in branched-chain amino acids and ABA correlated positively with the changes of plasma acylcarnitines and inversely to free carnitine levels suggesting a coherent metabolic interrelationship. Amongst the organs, skeletal muscle showed changes in amino acid status very similar to those found in plasma, while in liver all amino acids were reduced by fasting, except for ABA and beta-aminoisobutyric acid displaying increased concentrations. In addition, hepatic concentrations of free fatty acids were increased. Our findings demonstrate that fasting in mice causes a plasma metabolite profile that mimics the changes reported in humans with insulin resistance or type 2 diabetes. This suggests that the prime cause of these changes in humans is – as expected – the impaired insulin action and in particular the reduced anti-catabolic effect on muscle protein breakdown, adipose tissue lipolysis and hepatic ketoneogenesis. As a next step a model based on mass balance analysis will be defined that simulates the changes in plasma and organ metabolite levels.

Metabolic hormones modulate the expression of IGF1 system members in mouse primary hepatocytes

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IGF1 is a polypeptide secreted mainly from the liver and exerts its effects by binding to its receptor in muscle, bone and adipose tissues, where it induces various mechanisms including proliferation, differentiation, protein and glycogen synthesis as well as inhibition of apoptosis. Its activity is determined by the presence of different kinds of IGF1 binding proteins such as IGFBP1, IGFBP2 and IGFBP3. They exert inhibitory as well as stimulatory influences on IGF1s ability to phosphorylate its receptor which has been shown to be involved in the development of many age and nutrition related diseases such as Diabetes mellitus, hepatic steatosis, cancer and cardiovascular disease. Growth hormone (GH) is the main mediator of IGF1 secretion from the liver but other hormones are also involved. We investigated whether there is any effect of metabolic hormones on IGF1 bioactivity. Mouse primary hepatocytes were isolated and treated with insulin or glucagon in the presence or absence of growth hormone, the main mediator of IGF1 secretion, and the transcription of IGF1 system members was measured using quantitative real-time polymerase chain reaction (RT-PCR). We found that glucagon, an important metabolic hormone secreted during fasting, can modulate the IGF1 system in mouse primary hepatocytes by affecting the transcription of IGF1 as well as the transcription of IGFBP3, the most predominant IGFBP in the blood and this effect seems to be dose-dependent. Insulin can also affect IGF1 bioactivity by modulating the transcription of IGFBP1, IGFBP2 and IGFBP3. First data indicated that Glucagon may modulate IGF1 bioactivity by affecting the transcription of some IGF1 system members, therefore the next step will be the measurement of IGF1 bioactivity *in vitro* as well *in vivo* under stimulation of these metabolic hormones.

Gene expression analysis of obesity and type 2 diabetes microarray data involving probiotic intake

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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 Microarrays 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. We identified gene expression data associated to the Obesity and type 2 Diabetes Mellitus (T2DM) in a profile of microarray data with possible application to bioactive components like lactobacillus probiotic intake. We found responsible genes of Obesity and intersection with T2DM genes and analyze the possible interaction between specific genes for studying a network model of interaction. We have modelling the clustering of gene networks by applying the mathematical procedure Principal Component Analysis (PCA) to the gene expression data. Were identified and compared across databases specific expression microarray and gene polymorphisms LEP obesity, LEPR, FTO and PPARγ2 and its relationship with Lactobacillus gasseri probiotic. We obtained a model of interaction between them from microarray systems integration and gene expression. This study analyzed the genes associated with obesity and type 2 Diabetes Mellitus and its relationship with Lactobacillus gasseri probiotic, which has been associated with reduction of adipose tissue. This network could help to control dietary variables associated with lactobacillus probiotic intake to prevent risk of T2DM and obesity to obtain evidence of their interaction with certain human genome modules.

L-glutamine metabolism and its anticatabolic effects in skeletal muscle

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L-glutamine is the most abundant amino acid in plasma. It is mainly produced in skeletal muscle from glutamate and ammonia, a reaction mediated by the glutamine synthetase. By its role as nitrogen carrier L-glutamine is a central metabolite in the interorgan ammonia turn-over and in protein synthesis but it also regulates gene expression in various cell types. In better understanding the role of L-glutamine in skeletal muscle with regard to overall amino acid metabolism and protein synthesis we used *in vivo* and *in vitro* approaches. Experiments were performed in wild type mice using a prolonged fasting model (24 hours) followed by analysis of expression of the protein ubiquitin ligase (Murf-1) and proteins involved in protein synthesis pathways such as phospho-Akt, phospho-GSK-3β, phospho-4E-BP1, phospho-S6 and phospho-eIF2α. *In vitro* studies employed the murine skeletal muscle cell line C2C12 exposed to different levels of L-glutamine. After distinct exposure times, changes in intracellular and extracellular amino acid profiles were analyzed by LC/MS/MS after aTRAQ labelling. To define the role of the glutamine synthetase in maintaining intracellular glutamine levels, C2C12 cells were also exposed to the synthetase inhibitor MSO (L-Methionine-S-Sulfoximine). *In vivo* experiments revealed a decreased mass in tibialis anterior, extensor digitorium longus (EDL) and soleus muscle when mice were fasted. However, when animals were supplemented with L-glutamine prior to fasting, the decline in muscle mass could be prevented. Fasting caused also a reduction in the levels of phospho-AKT and phospho-S6 and this decline could also be prevented by glutamine supplementation. While fasting also reduced phospho-4E-BP1 levels, no effects on phospho-GSK-3β and phospho-eIF2α protein density were found. In C2C12 cells, the addition of MSO in the absence of extra glutamine led to a reduction of the total protein, DNA and protein/g DNA while supplementation of glutamine could prevent this decline. As little as 2 mM of extra glutamine was sufficient to reduce Murf1 levels in cells suggesting that this low concentration can already reduce protein break-down significantly. Amino acid analysis in the culture medium and in C2C12 cells revealed that increasing extracellular glutamine supply is followed by a concentration dependent increase of the release of Glu, Ala, Asn, Pro and Orn from the cells. This indicates an increased metabolism of glutamine via glutamate and glutamic-semialdehyde followed by transamination to ornithine and via Δ1-pyrroline-5-carboxylate to proline. In addition, a large proportion of glutamine seems to be released as alanine after transamination. Since cellular glutamine efflux promotes uptake of essential amino acids including leucine by the LAT1 amino acid transporter (SLC1A5) that in turn feeds leucine to mTORC1 for initiation of protein translation.

Dietary n-3 polyunsaturated fatty acids, lipid oxidation and a peroxisomal metabolite signature

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Fish oil, that is rich in n-3 polyunsaturated fatty acids (n-3 PUFA), has been reported to prevent glucose intolerance and dyslipidemia and these effects have been associated with metabolic alterations such as increased fatty acid (FA) oxidation, mainly in the liver. N-3 PUFA are natural agonists of nuclear receptors of the PPAR family including PPARα in liver. Upon activation, PPARα induces the expression of large number of genes involved in metabolism and causes proliferation of peroxisomes. These organelles perform β-oxidation and α-oxidation of FA and play also a central role in oxidation of dicarboxylic FA which are generated through ω-oxidation. Alpha-oxidation was described in the 1960s as the pathway responsible for the degradation of branched-chain FA allowing the shortening of the acyl-CoA chain by just one carbon and not by two as in the β-oxidation. Later it was demonstrated that straight chain FA can also be oxidyzed via this mechanism in peroxisomes. Omega-oxidation does not shorten the FA acyl chain but oxidizes the terminal methyl group of the acyl chain to generate a dicarboxylic FA. Thus, α- and ω-oxidation can generate a unique pattern of intermediates of peroxisomal FA oxidation, namely dicarboxylic and odd-chain acyl-CoAs, or a combination of both. Carnitine is necessary for the import of FA into the mitochondria but it also acts as a buffer for the coenzyme A (CoA) pool inside the cell. The acylcarnitine pool mirrors in composition the acyl-CoA pool. Based on LC-MS/MS analysis of hepatic acylcarnitines in mice fed a hyperlipidic diet containing fish oil, we observed increased concentrations of odd chain and dicarboxylic medium chain acylcarnitines, suggesting increased peroxisomal oxidation of FA. Moreover, with palmitic acid as a substrate, liver homogenates from mice treated with a synthetic PPARα agonist (WY-14,643), were shown to produce more α- and ω-oxidation products under conditions of high PPARα activity. In summary, n-3 PUFA supplementation, similarly to WY-14,643 treatment, increases FA utilisation via α-, β- and ω-oxidation and produces a distinct metabolite signature in liver and plasma. Whether these unique metabolites contribute to the protective effects of fish oil in lipid metabolism is currently not known.

Nutrigenomics applications to omega-3-PUFA in pregnancy and obesity

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Experiments *in vitro* and animal studies have shown that long-chain polyunsaturated fatty acids (LCPUFA) and their metabolites can modulate adipogenesis. n6-LCPUFA promote adipogenesis whereas n3-LCPUFA can counteract this process. There is some evidence that the composition of fatty acids in the diet during pregnancy may play a role in determining the risk of the offspring of becoming overweight. According to the fetal origins of adult disease hypothesis, unbalanced nutrition during intrauterine development may contribute to a later risk of cardiovascular disease, type-2 diabetes, insulin resistance, and obesity. This suggests that low maternal dietary n6/n3-LCPUFA ratio during pregnancy and lactation may have the potential to diminish offspring adipose tissue growth during development and/or might have programming effects on adipogenesis at later stages. The fetal environment is largely generated by maternal factors, both genomic and environmental, and by placental function. However, the regulation of placental gene expression and nutrient-transport and nutrient-sensing signaling pathways regulating cell metabolism and growth upon altered maternal nutrient levels and growth factor signaling are not well understood. Consequently, we have performed the human dietary n3-LCPUFA intervention study INFAT (INFAT=Impact of Nutritional Fatty Acids during Pregnancy and Lactation on Early Adipose Tissue Development) to assess whether a reduced n6/n3-LCPUFA ratio in the diet of pregnant women/breastfeeding mothers may represent a new primary prevention strategy of childhood obesity. The intervention group (n=104) received 1.2 g n3-LCPUFA (docosahexaenoic acid and eicosapentaeonic acid) as fish oil capsules per day. The control group (n=104) received information on a healthy diet only. The primary outcome of the study was infant fat mass (birth until 1 year) estimated by skinfold thickness measurements and abdominal sonography. Moreover, we hypothesized that the n3-LCPUFA intervention may also have an impact on placental gene expression and epigenetic mechanisms, providing potential programming effects on offspring metabolism, physiology and growth. Placenta tissues of the INFAT-study were analysed by mRNA and microRNA expression profiling followed by pathway and gene ontology analyses. In addition, putative microRNA-mRNA interactions were explored and selected microRNAs and mRNAs were validated. Our nutrigenomic approach for the placenta reveals gender–specific gene expression and differential mRNA and miRNA expression, as well as potentially affected signalling pathways upon n3-LCPUFA intervention. Correlations of placental gene expression with offspring adipose tissue growth will be discussed.

Genetic determinants of endogenous arachidonic acid synthesis and prostaglandin E2 production

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Prostaglandin E_2 (PGE_2) is an eicosanoid derived from arachidonic acid (20:4n-6) which is increased in colorectal adenomas and cancers. Erythrocyte phospholipid membrane concentrations of arachidonic acid are associated with genetic variants in fatty acid desaturase (FADS). It is unknown whether genetic variation in FADS or elongation of long chain fatty acids (ELOVL), two enzymes involved in the conversion of linoleic acid (18:2n-6) to arachidonic acid, are associated with prostaglandin E_2 (PGE_2) production. We measured erythrocyte phospholipid membrane concentrations of arachidonic acid and urinary prostaglandin E_2 metabolite (PGEM) in a subset of 513 adenoma cases and 224 polyp-free controls enrolled in the Tennessee Colorectal Polyp Study. The Tennessee Colorectal Polyp Study is a large colonoscopy-based case-control study designed to determine genetic and environmental factors associated with colorectal polyp risk. Subjects underwent a standardize telephone interview to obtain demographic and lifestyle information and donated a blood and urine sample. Erythrocyte phospholipid membrane concentrations were measured using gas chromatography. Urinary PGEM was measured using liquid chromatography/tandem mass spectrometry. We genotyped two SNPs, rs174537 in FADS1 and rs2236212 in ELOVL2, previously reported as associated with phospholipid levels in GWAS using Sequenon iPlex assays. Demographic characteristics of the participants included a mean age of 58.4±7.13 years, 28% female sex (207/739) and 51% (375/735) reporting current use of non-steroidal anti-inflammatory drugs. Erythrocyte phospholipid membrane concentrations of arachidonic acid decreased with increasing copies of the minor allele for the rs174537 genotype (G/G=16.3±3.25 [n=336], G/T=15.7±3.81 [n=312], T/T=14.8±2.70 [n=79], means adjusted for age, gender, NSAID use, or adenoma status, p-value=<0.001). We found no significant differences in urinary PGEM levels by rs174537 genotype (G/G=2.38±0.78, G/T=2.43±0.81, T/T=2.40±0.80, log-transformed means adjusted for age, gender, NSAID use, or adenoma status, p-value=0.67). Erythrocyte phospholipid membrane concentrations of arachidonic acid increased with increasing copies of the minor allele for the rs2236212 genotype (G/G=15.6±3.79 [n=253], C/G=15.9±3.28 [n=369], C/C=16.4±3.48 [n=106], p-value=0.02). Urinary PGEM levels decreased by rs2236212 genotype (G/G=2.49±0.76, C/G=2.36±0.84, C/C=2.34±0.73, p-value=0.02). Genetic variants in both rs174537 and rs2236212 were associated with different erythrocyte phospholipid concentrations of arachidonic acid. We did not see a difference in urinary PGEM levels based on rs174537 genotype in FADS1 however the rs2236212 genotype in ELOVL2 did impact urinary PGEM levels with polymorphisms associated with higher arachidonic acid levels being also associated with lower urinary PGEM levels.

Nutrigenomic effect of omega-3 fatty acids in the aorta of LDLR-/- mice

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The omega 3 PUFA docosahexaenoic acid (DHA) exerts potent anti-atherosclerotic action. However, the mechanisms responsible for this effect are not completely elucidated. With regard to the complexity of the action of DHA, the present work aimed to determine its impact on the overall gene (transcriptomics) and protein expression (proteomics) in the aorta of atherosclerotic-prone mice. LDLR-/- male mice were fed for 20 weeks a diet enriched with lard (10%, w/w) and cholesterol (0.045%, w/w) in parallel with daily oral gavages with either oleic acid rich oil (Control group) or a mixture of oils providing 2% of energy as DHA (DHA group). DHA intake reduced the systolic blood pressure (-16 mm Hg, $P<0.01$), plasma cholesterol (-28%, $P<0.001$) and triacylglycerol (-37%, $P<0.01$) concentrations, and the extent of atherosclerosis (-35%, $P<0.001$). The transcriptomic analysis of aorta discloses that DHA supplementation was associated with anti-inflammatory and immunomodulatory effects, namely down-regulation of the expression of adhesion molecules (e.g. ICAM-2, fold change (FC)=-1.34, $P<0.01$), pro-inflammatory cytokines (e.g. CCL5, FC=-1.49, $P<0.01$) or of genes of the major histocompatibility complex (e.g. HLA-DRB1, FC=-1.68, $P<0.01$). Transcription factors analysis reveals the inhibition of the NFkappaB pathway and the activation of PPARgamma. The results from the proteomic analysis revealed modulation of glucose (e.g. up-regulation of glycolysis) and lipid metabolism (e.g. increase of b-oxidation) together with an enhancement of antioxidant defenses (e.g. up-regulation of superoxide dismutase 1 and heat shock protein 75 kDa). PUFA being highly susceptible to lipid peroxidation, protein modification by lipid peroxidation aldehyde (namely 4-hydroxynonenal (4-HNE) protein adducts) was investigated. DHA supplementation did not enhance these lipid peroxidation alterations of proteins. However, it is interesting to note that the proteomic analysis allowed the identification of new 4-HNE targets proteins at the vascular level. Most of them are mitochondrial and involved in pathways of energy production. In conclusion, our results indicate that the anti-atherogenic effect afforded by DHA is associated with changes in the abundance of transcripts and proteins related to lipid and glucose metabolism and modulation of inflammation and oxidative stress pathways. Future research should be strengthened in order to determine the impact of LC-n-3PUFA on these specific pathways.

Fish oil supplementation and transcriptome analyses in PBMCs

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Fish oil supplementation has been shown to alter gene expression of mononuclear cells both *in vitro* and *in vivo*. However, little is known about the total transcriptomic profile in healthy subjects after intake of fish oil compared to a control group. To examine the gene expression profile in peripheral blood mononuclear cells (PBMCs) in healthy subjects after intake of fish oil for seven weeks using whole-genome transcriptomic analysis. In a seven week double-blinded randomized controlled parallel-group study, healthy subjects received 8 g/d of fish oil (1.6 g/d EPA+DHA) (n=17) or 8 g/d of high oleic sunflower oil (n=19). This paper presents microarray analyses on RNA isolated from PBMC at baseline and after seven weeks of intervention. Cell cycle, DNA packaging and chromosome organization are biological processes found to be up-regulated after intake of fish oil compared to high oleic sunflower oil using a moderated t-test (Limma). In addition, gene set enrichment analysis identified several enriched gene sets after intake of fish oil. These gene sets were related to pathways involved in cell cycle, ER stress and apoptosis. Genes with common motives for 35 known transcription factors including E2F, P53, and ATF4 were up-regulated at a transcriptional level after intake of fish oil. We have shown that intake of fish oil for seven weeks modulates gene expression in PBMCs of healthy subjects. The increased expression of genes related to cell cycle, ER stress and apoptosis suggest that intake of fish oil may modulate basic cellular processes involved in normal cell function and lymphocyte activation.

The interactive impact of APOE genotype and fish oil fatty acids on adipose tissue inflammation

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The APOE4 genotype has been linked to cardio-metabolic and adipose tissue dysfunction and a pro-inflammatory status. Macrophages are important mediators of adipose tissue inflammation and adipocyte dysfunction in obesity, that in turn increases the risk for developing diseases, such as diabetes and cardiovascular disease (CVD). The fish oil fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have well established anti-inflammatory effects and beneficial effects on adipose tissue function. However, the interactive impact of APOE genotype and fish oil fatty acids on adipose tissue inflammation is unknown. To investigate the impact of APOE genotype and EPA+DHA on the adipose tissue macrophage (ATM) infiltration and phenotype in human APOE3 and APOE4 targeted replacement (TR) mice using diet-induced obesity (DIO) model. Forty 13-14 week old male APOE3 and APOE4 TR mice were fed ad libitum a high-fat diet (45 kcal% fat) without (HFD) or supplemented with 30 g EPA+DHA/kg diet (HFD+FO) for 8 weeks (n=10 per genotype*diet group). Bodyweight and food intake were monitored every second day. The epididymal adipose tissues were dissected using sterile techniques and snap-frozen for mRNA analysis or processed within 5 hours for the characterisation of the ATMs using flow cytometry. The fresh tissues were minced and digested with Collagenase Type II (1 mg/ml) in Krebs-Ringer Buffer (4% BSA, 10nM glucose) for 30 min, filtered through 40 µm mesh. The stromal vascular fraction (SVF) was resuspended in PBS (1% BSA, 10nM glucose), incubated with antibodies against CD11b, CD11c and CD206 which represent cell surface makers for total-, pro-inflammatory (M1) and anti-inflammatory (M2) macrophages, respectively. Initial analysis showed an impact of APOE genotype and EPA+DHA on body weight gain, with the impact on adipose tissue inflammation (cytokine gene expression analysis and ATM number and status) yet to be established.

Metabolomics and gene expression analyses of the Il10 gene-deficient mouse model of IBD

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The interleukin-10 gene-deficient ($Il10^{-/-}$) mouse develops intestinal inflammation which shares several features of human inflammatory bowel disease, specifically Crohn's Disease (CD). We have used this model extensively to better characterise the development of colonic inflammation, and to investigate foods or food compounds with the potential to prevent or reduce this inflammation. One food compound which has shown anti-inflammatory activity in this model is the omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA). This was used in the current study, which focused on the identification of relevant mRNAs and metabolites for use as non-invasive biomarkers of inflammation in human studies. In this study, 50 male $Il10^{-/-}$ mice and 50 male wild-type (C57BL/6J) mice were allocated to the following treatments at five weeks of age (n=10 animals of each genotype per treatment): (1) control group, AIN-76A diet, euthanized at 11 weeks of age; (2) early inflammation oleic acid (OA), AIN-76A + 3.7% OA (as a control fatty acid to match the composition of the EPA diet), euthanized at 8 weeks; (3) early inflammation EPA, AIN-76A + 3.7% EPA, euthanized at 8 weeks; (4) established inflammation OA, AIN-76A + 3.7% OA, euthanized at 11 weeks; (5) established inflammation EPA, AIN-76A + 3.7% EPA, euthanized at 11 weeks. Body weight and food intake were assessed twice weekly, and spot urine samples were collected at 6, 8, 9 and 11 weeks of age to identify potential non-invasive metabolite biomarkers of inflammation, and to establish the effect of feeding a diet enriched with EPA on these metabolites. Urine metabolomics profiles were analysed using LC-MS. Animals were euthanized at the times specified, and intestinal tissue samples taken for histological (formalin-fixed) and gene expression analysis. Blood was also collected for purification of peripheral blood mononuclear cells (PBMCs). Inflammation was determined in intestinal tissue using a histological injury score (HIS), and RNA was extracted from colon and PBMCs, and gene expression analysed using Agilent SurePrint G3 Mouse GE 8x60K microarrays. There was no effect of mouse strain or diet on food intake or body weight. $Il10^{-/-}$ mice showed increased inflammation (as determined by HIS) in the colon and caecum compared with C57 mice, regardless of diet. Both EPA and OA diets resulted in different urine metabolite profiles compared to the AIN-76A diet, with clearer differentiation of the EPA diet from the AIN-76A diet than was the case for the OA diet. Further analyses, including gene expression data from colon and PBMCs, and the relationships between inflammation and gene and metabolite data, will be described. The potential of specific metabolite and gene expression biomarkers for use in future human studies will be discussed.

Systems biology applied: the effects of salmon in a mouse model of inflammatory bowel disease

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Diet is known to play a major role in the symptoms of inflammatory bowel disease (IBD). In a self-assessment survey among IBD patients, eating salmon was frequently perceived to alleviate symptoms. Salmon, rich in long-chain omega-3 polyunsaturated fatty acids (n-3 PUFA), is also an excellent source of other nutrients that may synergistically enhance the anti-inflammatory effects of n-3 PUFA. It was hypothesised that a diet supplemented with salmon reduces colon inflammation by altering the expression levels of immune-related genes and proteins. The interleukin-10 gene-deficient ($Il10^{-/-}$) mouse, a recognised model of IBD, was used to determine the dose-dependent effects of dietary salmon on colon inflammation. Five-week-old male $Il10^{-/-}$ (n=8 per diet) and control (C57BL/6J; n=8 per diet) mice were randomly assigned to an unmodified AIN-76A diet, or one of six AIN-76A-based diets, either enriched with freeze-dried salmon fillets (15%, 30% or 45% (w/w)) or modified to match the macronutrient composition of each salmon diet. After seven weeks, colon tissue was collected to histologically assess the degree of inflammation. The underlying molecular changes by which salmon-enriched diets affect colon inflammation in $Il10^{-/-}$ mice were assessed by measuring changes in gene expression (microarray), protein expression (2D-DIGE and LC-MS), and urinary metabolite profiles (LC-MS). Inflammation was observed in colon tissue of $Il10^{-/-}$ mice fed each of the control diets ($P<0.05$ vs. C57BL/6J mice), which was linked to reduced expression levels of metabolism-related genes and enhanced expression of immune-related genes. Only $Il10^{-/-}$ mice on the 30% salmon diet showed reduced colon inflammation compared to $Il10^{-/-}$ mice on the matched control diet ($P<0.05$), characterised by reduced numbers of lymphocyte infiltrates. Transcriptomic analysis of colon tissue identified 266 genes that were differentially expressed in response to 30% salmon (fold-change ≥1.5, $P<0.005$). Functional analysis revealed decreased expression levels of genes associated with the homeostasis, stimulation and development of lymphocytes, which correlated with histological analysis. Proteomic analysis resulted in a preliminary list of 16 spots, each corresponding to a differentially expressed protein (fold-change ≥1.5, $P<0.05$). Principal component analysis of metabolite profiles revealed distinct clusters in the urinary metabolome separated by strain and diet. Identification of metabolites corresponding to the discriminating ions is in progress. In summary, a diet supplemented with 30% freeze-dried salmon fillets reduced colon inflammation in $Il10^{-/-}$ mice, potentially via alteration of genes linked to the regulation of inflammatory cells. Further studies are underway to understand the time-effect of salmon intervention on development of colon inflammation in $Il10^{-/-}$ mice. All authors are part of Nutrigenomics New Zealand (www.nutrigenomics.org.nz).

Impact of dietary n-3 LCPUFA supplementation on adipose tissues, intestine and spleen in mice

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In studies emphasizing anti-obesogenic and anti-inflammatory effects of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA), diets with very high fat content, not well-defined fat quality, and extreme n-6/n-3 PUFA ratios have been applied frequently. Additionally, comparative analyses of visceral adipose tissues (VAT) were neglected. Considering the link of visceral obesity to insulin resistance or inflammatory bowel diseases, we hypothesized that VAT, especially mesenteric adipose tissue (MAT), may exhibit differential responsiveness to diets through modulation of metabolic and inflammatory processes. Here, we aimed to assess dietary n-3 LCPUFA effects on MAT and epididymal adipose tissue (EAT) and on MAT-adjacent liver and intestine in diet-induced obese mice fed defined soybean/palm oil-based diets. High-fat (HF) and n-3 LCPUFA-enriched high-fat diet (HF/n-3) contained moderately high fat with unbalanced and balanced n-6/n-3 PUFA ratios, respectively. Body composition/organ analyses, glucose tolerance test, measurements of insulin, lipids, mRNA and protein expression, and immunohistochemistry were applied. Compared with HF, HF/n-3 mice showed reduced fat mass, smaller adipocytes in MAT than EAT, improved insulin level, and lower hepatic triacylglycerol and plasma non-esterified fatty acids levels, consistent with liver and brown fat gene expression. Gene expression arrays pointed to immune cell activation in MAT. For the intestine, expression changes for genes operating in endothelial cell activation, oxidative stress response and nitric oxide production were observed. Validations demonstrated simultaneously upregulated pro- and anti-inflammatory cytokines and M1/M2-macrophage markers in VAT and reduced CD4/CD8α expression in MAT and spleen. Our data revealed differential responsiveness to diets for VAT through preferentially metabolic alterations in MAT and inflammatory processes in EAT. n-3 LCPUFA effects were pro- and anti-inflammatory and disclose T cell-immunosuppressive potential. SW and TL, supported by the Deutsche Forschungsgemeinschaft (DFG)-Graduiertenkolleg 1482 at Technische Universität München, Freising-Weihenstephan, Germany.

Metabolic and inflammatory synergies – insights from the genetic and molecular perspectives

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Dietary fats and obesity represent important metabolic stressors that interact with inflammatory pathways to augment the risk of insulin resistance the key metabolic perturbation in the Metabolic Syndrome (MetS) and Type II Diabetes Mellitus (T2DM). This presentation will focus on recent work published as part of LIPGENE (www.ucd.ie/LIPGENE) a large pan European study that investigated the effect of modifying dietary fat composition and inflammatory genes on the risk of the MetS. A case-control approach showed clear links between inflammatory genes and saturated fatty acids (SFA) in the development of insulin resistance and MetS risk. Furthermore a dietary intervention study investigated the metabolic effects of substituting dietary SFA by replacement with monounsaturated fatty acids or as part of a low-fat diet in subjects with the MetS. However the efficacy of the intervention was highly variable within the cohort. This work provides clear examples wherein dietary fat composition interacts with different inflammatory genes to modulated risk factors associated with the MetS. Overall this research suggests that a personalised approach based on an individual's metabolic phenotype, which may include inflammatory status, may be more effective in providing more successful prevention strategies and treatments related to obesity and diabetes. From the mechanistic perspective, recent work showing the differential effects of dietary fatty acids on NLRP3 inflammasome activation and subsequent processing of pro- to active IL-1β will be explored in relation to obesity-induced insulin resistance. This work provides deeper mechanistic understanding of how different fatty acids can act as more or less aggressive metabolic stressors to promote / retard the development of diabetes despite obesity. The grand challenge lies in translating this information into effective treatments and understanding relative efficacy according to several components of the metabolic phenotype which may be related to dietary fat composition and/or obesity related risk factors including inflammatory phenotypes.

Gene-nutrient interactions in the context of insulin sensitivity and metabolic syndrome

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Metabolic syndrome (MetS) represents a combination of cardiometabolic risk determinants, including central obesity, insulin resistance, hypertension, and dyslipidaemia. This cluster of risk factors increases the risk of type 2 diabetes (T2D) and cardiovascular disease. The prevalence of MetS is growing, affecting almost a quarter of the global adult population, correlating with the global epidemic of obesity and T2D. Unfortunately, this rise has been observed not only in the Western world, but also in developing countries. The pathogenesis of MetS is complex and not completely understood, but the interaction of over-nutrition, lack of physical activity, and genetic factors are known to contribute to its development. Although pharmacological interventions are available for minimizing or delaying the comorbidities associated with MetS, initial management for the vast majority of the affected population remains focused on lifestyle modification, consisting of sustainable changes in dietary habits and physical activity. Thus, lifestyle modification has generally been accepted as a cornerstone of treatment for MetS, with the expectation that an appropriate. intake of energy and nutrients will improve its control and reduce the risk of complications. The number of studies investigating gene–nutrient interactions related to MetS continues to grow, and has potential for reducing the risk of disease at the level of the individual genotype. Previous evidence suggests that some people are genetically predisposed to insulin resistance, a possible underlying mechanism for these metabolic disturbances. In this context, nutrigenetics has emerged as a multidisciplinary field focusing on studying the interactions between nutritional and genetic factors and health outcomes. Thus, it would be expected that general dietary recommendations may not be beneficial for all individuals. Due to the complex nature of gene–environment interactions, however, dietary therapy in combination with MetS may require a "personalized" nutrition approach. Although results have not always been consistent, gene variants that affect primary insulin action, and particularly their interaction with the environment, are important modulators of glucose metabolism and insulin resistance syndrome. The purpose of this review is to present some evidence of studies that have already demonstrated the significance of gene–nutrient interactions (adiponectin gene, Calpain-10, glucokinase regulatory protein, transcription factor 7-like 2, leptin receptor, scavenger receptor class B type I etc.) that influence insulin resistance in subjects with metabolic syndrome.

CAV1/CAV2 SNPs associate with triglyceride traits and interact with glycemic load and saturated fat

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Caveolins 1 and 2 are structural proteins found in caveolae, the main function of which is general endocytosis. CAV1 has a role in triglyceride (TG) homeostasis as CAV1 knockout mice show elevated TG levels. Here, we looked at the effect of SNPs at the CAV1/CAV2 locus on fasted TG levels, TG absorption and TG clearance (both postprandial to a high-fat challenge) and the interaction of these SNPs with macronutrients and subclasses on TG levels in 820 subjects (GOLDN population, whites living in the USA). An LD block of 98 SNPs within the CAV1/CAV2 region has a direct effect on fasted TG in this population (P=0.0005), and this effect is stronger in the people with high beef and pork consumption (P-interaction=0.04). The same LD block delays TG clearance in people with a high glycemic load (GL) and high saturated fat (SF) intake in their habitual diet (P-GL=0.001, p-SF=0.017). After treatment with a PPARα agonist (fenofibrate) the main effect of the LD block with associations to TG measures was lower and the dietary interactions disappeared. In conclusion, a LD block at the CAV1/CAV2 locus has an effect on fasted TG levels and these SNPs interact with an unhealthy dietary pattern on TG clearance. We believe that a lowering of CAV1 expression by disruption of a transcription factor binding site facilitates this association. In this regard, several SNPs in this LD block map upstream of CAV1 and fall in predicted binding sites for transcription factors, including CEBPB and SMARCC1. Furthermore, TG levels are elevated in Cav1 but not Cav2 knockout mice, suggesting more influence of CAV1 on TG levels. Replication of these results is in progress.

MTHFR genotype affects lipid metabolism in IHD patients introduced to an antioxidant-rich diet

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Increased plasma homocysteine (hyperhomocysteinemia) is an independent risk factor for cardiovascular diseases (CVDs), which are a leading cause of death worldwide. Hyperhomocysteinemia is associated with increased oxidative stress status through the induced production of reactive oxygen species. Dietary antioxidants might have beneficial effects on CVD by attenuating oxidative stress. The aim of this study is to evaluate the impact of a six-week intervention, based on an antioxidant-rich diet, on serum homocysteine levels and lipid metabolism biomarkers among Polish ischemic heart-disease (IHD) patients. Since homocysteine metabolism is determined by nutritional and genetic influences, we also intend to test the effects of C677T MTHFR on the measured parameters. A group of 49 volunteers (27 women, 22 men) with diagnosed IHD, who have previously suffered myocardial infarction, were recruited at a cardiac intensive care unit. They were introduced to an antioxidant-rich diet based on natural products (e.g. olive oil, tomato juice, spices, nuts, and fruit or vegetable crisps. Lipid metabolism biomarkers were determined using biochemical analysis. Serum homocysteine concentrations were measured using high-performance liquid chromatography (HPLC). MTHFR genotyping was performed using the PCR-RFLP method. Following the dietary intervention, a significant decrease in serum homocysteine concentrations was observed in women only ($P<0.05$), but there was no genotype effect on this parameter. In men, the MTHFR genotype affected the initial blood total cholesterol ($P<0.05$) and LDL cholesterol levels ($P<0.05$) with much more favorable values in the CC homozygotes. The MTHFR genotype also influenced the effect of the diet. We observed an 18% decrease in total cholesterol ($P<0.05$) and a 20% decrease in LDL cholesterol ($P<0.05$) concentrations following the dietary intervention, but only in the T-allele carriers. In women, the MTHFR genotype affected the blood HDL levels both before and after the antioxidant-rich diet ($P<0.05$ for both associations), and these were higher in the CC homozygotes. The C677T MTHFR genotype may affect lipid metabolism in IHD patients and also may determine the effects of an antioxidant-rich diet.

Gene-diet interactions: important relations in the development of personalized nutrition

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Gene-nutrient interactions are seen as one of the parameters that can be used in personalized nutrition. However, solid evidence for these relations are not always available and the currently available knowledge is not structured. For that reason, one of the goals within the EU project Food4me (focusing on strategies in personalized nutrition) is to collect and structure the available data regarding gene-diet interactions. An expert network (Global Personalized Knowledge Network (GPKN) has therefore been established that will create and maintain the academic knowledge and information related to personalized nutrition with the focus on gene-nutrient interactions. The maintenance of this information will be accomplished through the Food4me knowledge database (FKD), which is currently filled with relevant information on interactions between health outcome-genetic variations, biomarkers-genetic variations etc. This information can then be assessed by the GPKN and can lead to established nutritional recommendations for specific alleles. For specific genes the current status of the information in the database will be summarized in nutrition gene cards. Currently, a list of top ten genes (BCMO1, APOA2, FTO, ADRB2, SOD2, APOE, APOA5, FADS1, MTHFR and TCL7F2) has been selected by GPKN for the creation of the initial Nutrition gene cards. We will present the data of one of the nutrition gene cards and show the evidence needed for solid gene-nutrition relations.

Differential response to marginal selenium deficiency in liver of C57BL6/J and Pept1-/- mice

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The essential trace element selenium (Se) exerts its main functions after incorporation into selenoproteins such as glutathione peroxidases or deiodinases. In mammals, the liver plays a crucial role in selenium homeostasis and its distribution to peripheral organs. Moreover, hepatic deiodinase participates in the control of thyroid hormone levels. Se is obtained from anorganic or organic sources. The latter are mainly Se-containing amino acids or peptides. Most interestingly, we observed that the intestinal peptide transporter Pept1 was up-regulated in Se-deficiency suggesting that it may contribute to Se-absorption. To further assess whether Pept1 participates in whole body Se-homeostasis, we fed C57BL6/J control mice and Pept1$^{-/-}$ either a selenium adequate or deficient diet starting after weaning and profiled liver tissues by non-targeted GC-MS. The selenium adequate diet met the mouse recommendations of 0.15 mg Se/kg diet with selenomethionine as the main selenium source. The selenium-deficient diet contained about half of the amount with 0.086 mg Se/kg. After 7 weeks of feeding, changes in the selenium status were assessed by the decline of total glutathione peroxidase activity in liver and duodenal homogenates. The activity of total glutathione peroxidase decreased in all animals to the same degree suggesting that PEPT1 is not affecting the absorption of selenium from the dietary sources. However, metabolite profiling of liver tissues after methanol:chloroform extraction, phase separation and derivatisation using MSTFA revealed changes due to selenium deficiency and depending on the genetic background. Metabolite detection and quantification performed with the software TargetSearch indicated prominent changes in carbon-1-metabolism. In the transsulfuration pathway with homocysteine levels were increased in selenium deficient animals. This could originate from a decreased cystathione beta lyase (CBS) abundance as observed by western blot analysis of liver tissues. In addition, spermidine, glutamate, cysteine-sulfinic acid as well as hypotaurine showed strain-specific changes in selenium deficiency. Hypotaurine levels were decreased in Pept1$^{-/-}$ mice compared to controls regardless of intervention. Effect of selenium deficiency in Pept1$^{-/-}$ mice caused only a slight elevation whereas wildtype animals showed ~3-fold elevation of hepatic hypotaurine levels. Hypotaurine, a derivative of cysteine, is considered to be hepatoprotective against ROS. Whether the decreased levels of hypotaurine in Pept1$^{-/-}$ mice compared to C57BL6/J is correlated with strain specific differences in oxidative damage of liver tissue remains to be shown.

Dissociation of metabolic and disease-conditioning effects of high fat diet in murine IBD

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Obesity has been associated with a more severe disease course in inflammatory bowel disease (IBD) and epidemiological data recently identified dietary fats but not obesity as risk factor for the development of IBD. The present work aimed to assess the impact of a high fat diet (HFD) on intestinal pathogenesis in different mouse models susceptible for ileal or cecal/colonic inflammation, with focus on interrelations of promoted pathogenesis and metabolic alterations during HFD feeding. $IL10^{-/-}$ mice and $TNF^{\Delta ARE/WT}$, models for colitis and Crohn's Disease-like ileitis respectively were fed HFD (palm oil based, 48%kJ from fat) compared to control diet (12%kJ from fat) for different durations. HFD aggravated ileal as well as colonic inflammation in early phase in $TNF^{\Delta ARE/WT}$ mice as well as in female $IL10^{-/-}$ mice, but did not result in significant overweight, glucose intolerance or increased adipose tissue inflammation in these mice. The distal ileum exhibited markedly reduced levels of Occludin in both susceptibility models. Under HFD, $TNF^{\Delta ARE/WT}$ and the corresponding wildtype mice showed enhanced endotoxin levels in hepatic portal blood, as well as increased expression of inflammation-related activation markers in the ileal epithelial cells, along with recruitment of $CD11c^+$ dendritic cells via CCL20, and Th17 biased lymphocyte infiltration into the ileal lamina propria. Some of the HFD-associated hallmarks found in these IBD susceptibility models were further confirmed to be independent of obesity in SWR/J mice, an obesity-resistant mouse model. Accordingly, *in vitro* studies were used to highlight that dendritic cell recruitment and Th17-commitment are possibly driven by luminal factors associated to HFD rather than the pathophysiological state of obesity. In summary, HFD feeding – independently of obesity and its associated pathologies – accelerated the onset of intestinal inflammation in the IBD-susceptible mouse models $IL10^{-/-}$ and $TNF^{\Delta ARE/WT}$ through mechanisms that involve increased intestinal permeability and altered luminal factors.

Resistance to diet-induced obesity conferred by dietary bile acid supplementation in mice is strain

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Bile acids (BA) not only aid in the absorption of lipophilic nutrients but also function as signaling molecules in a number of peripheral target tissues. BA activate thermogenic processes in brown adipose tissue and induce resistance to diet-induced obesity (DIO) in C57BL/6J mice. In our study, we aimed to assess the relevance of brown adipocytes within white adipose tissue depots in this context. In an animal experiment, we employed C57BL/6J and 129Sv/Ev mice, as they possess a different propensity to recruit brite adipocytes. Mice were assigned to one of four different diet groups: low fat (LFD) or high fat (HFD) diet either with or without 0.5% cholic acid supplementation. Energy assimilation, body mass development as well as body composition were determined. Inguinal white adipose tissue (iWAT) was screened for brite adipocyte recruitment by preparing histological sections as well as conducting quantitative PCR measurements of brown adipocyte markers. Moreover, plasma BA concentration was measured by HPLC-MS/MS. Mice of both strains displayed HFD-induced obesity. Cholic acid supplementation of LFD did not alter the body mass trajectory. As expected, feeding the HFD supplemented with cholic acid rendered C57BL/6J mice resistant to DIO. Surprisingly, cholic acid supplementation did not attenuate DIO in HFD fed 129Sv/Ev mice. Since energy assimilation was not affected by dietary BA, cholic acid must exert its protective effect in C57BL/6J mice by increasing energy expenditure. This suggestion was underlined by iWAT analyses: cholic acid supplementation caused brite adipocyte recruitment exclusively in HFD fed C57BL/6J mice, indicated by the morphological appearance of multilocular cells as well as increased brown adipocyte marker expression levels. Moreover, HPLC measurements revealed a considerable increase of plasma BA concentration in HFD containing cholic acid fed C57BL/6J, but not 129Sv/Ev mice. We identified 129Sv/Ev as a mouse strain resistant to the effects of dietary cholic acid supplementation. In future, the comparison of these animal models may serve to describe the underlying mechanisms of resistance to diet-induced obesity conferred by bile acids.

High fat diet induced effects and their reversibility in three inbred mouse strains

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Diet-induced obesity (DIO) generated by high-fat diet feeding of mice leads to adipose tissue expansion and impaired glucose tolerance. In three inbred mouse strains with different susceptibility to DIO we analyzed changes in glucose homeostasis and energy expenditure during high fat feeding and in response to caloric restriction. Male AKR/J, SWR/J and C57BL/6J mice first received high-fat diet (HFD, 48 kJ% fat) or control diet (CD, 12 kJ% fat) for 12 weeks, and then either refed CD for 12 weeks or pair-fed with HFD. Body mass, body composition, energy intake, energy expenditure and oral glucose tolerance were measured regularly. Energy expenditure was increased in HFD fed mice of all strains, but in AKR/J and B/6J this tachymetabolic response was not sufficient to defend their body mass. AKR/J mice increased body mass, mostly fat mass, more than B/6J mice whereas SWR/J mice were almost resistant against DIO. Acutely, HFD feeding for 1.5 days increased energy intake and reduced glucose tolerance in all three strains. During prolonged HFD feeding, DIO prone AKR/J and B/6J mice, but not DIO resistant SWR/J mice, maintained impaired glucose tolerance. Refeeding CD or pair-feeding HFD of obese AKR/J mice normalized glucose tolerance rapidly after one week. These results demonstrate that impairment of glucose sensitivity is a quick physiological response to HFD independent of DIO susceptibility. Impaired glucose tolerance is only maintained in DIO mice suggesting an important contribution of anabolic metabolism of adipocytes. Generating a catabolic state of adipocytes by caloric restriction normalizes glucose tolerance rapidly. All DIO effects seem to be reversible. This work was supported by the Bundesministerium für Bildung und Forschung to Martin Klingenspor (FKZ 0315674).

Variation in the BCMO1 gene and circulating levels of carotenoids in different ethnic groups

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Vitamin A is essential for normal growth and development, immune system, vision, and other functions in the human body. Because humans are unable to synthesize vitamin A de novo, they must consume diets with preformed vitamin A or provitamin A carotenoids. β-Carotene, the most abundant provitamin A carotenoid in the diet, is converted to retinol by β-carotene 15,15'-monoxygenase (BCMO1). However, β-carotene absorption and conversion into retinol is extremely variable among individuals, with proportions of low responders to dietary β-carotene as high as 45%. Genetic variability in β-carotene metabolism may provide an explanation for the molecular basis of the poor responder phenotype within the population. The aim of this study was to investigate the effects of the SNPs A379V:rs7501331 and R267S:rs12934922 from the BCMO1 gene on β-carotene conversion efficiency in two Brazilian ethnic groups: 26 African ancestry and 24 European ancestry were analyzed. Genotyping of polymorphic variants was performed by real-time polymerase chain reaction. Analysis of retinol esters and carotenes were performed using a Dionex HPLC system and the statistical analyses were performed using SPSS 17.0. Allele frequencies for the A and T allele of rs12934922, for African and European ancestry, were 53.10 and 54.55% and 26.90 and 45.45%, respectively. Likewise the allele frequencies for the C and T allele of rs7501331 were 86.55 and 63.05% and 13.45 and 36.95%, for African and European ancestry, respectively. The current study indicated that β-carotene and retinol concentrations were significantly positively correlated with genotype CC of rs7501331 in European ancestry ($r=0.752$; $P=0.031$), whereas they were significantly negatively correlated with rare genotype TT ($r=-1.00$; $P=<0.01$). Thus we can hypothesize that subjects with the allele T, specifically the genotype TT, are low/poor responders to dietary β-caroteno. Because the frequency of genotype TT was lower in both group, we need finish all the analysis to have a better results. In this study we are analyzing 100 African ancestry, 100 European ancestry and 100 Japonese ancestry that were already collected and are being genotyping. For European ancestry we were able to found a decrease tendency for β-caroteno and retinol conversion in genotype rs7501331, whereas this is more pronounced for African ancestry retinol levels in genotype rs12934922. The frequency of a rare genotype (in this case, TT) in the BCMO1 gene caused a dramatic decrease in the enzyme activity. We showed that two common nonsynonymous SNPs also exists in the Brazilian population and they are present in the two ethnic groups (African and European), at high frequencies, changing the β-carotene and retinol concentrations, demonstrating the importance for appropriate vitamin A recommendations for different ethnic groups. In conclusion, this study demonstrates that genetic variability should be taken into account in future recommendations for provitamin A supplementations in Brazilian population. Sponsored by FAPESP, CNPq and CAPES.

Cohousing of mice with different susceptibility to obesity influences energy balance

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Both host and environmental factors contribute to the increasing prevalence of obesity in Westernized countries. Recent studies on germfree and re-colonized mice suggest a role for the intestinal bacteria on host energy metabolism. However, investigations focusing on the interplay between gut bacteria and host energy metabolism of genetically obese mice and mice that are resistant to diet-induced obesity are lacking. We therefore investigate the role of the gut microbiota: (1) in inbred mouse strains either prone or resistant to diet-induced obesity (AKR/J > SWR/J); and (2) in a mouse model for genetic obesity (Mc4rW16X). In these settings we aim to identify: (1) the influence of the microbial community on DIO resistance; and (2) associations between the microbiota-derived metabolic profiles in the gut and the propensity for obesity. To assess the possible causal role of gut bacteria in mice prone (AKR/J) or resistant (SWR/J) to diet induced obesity a microbiota transfer experiment was conducted. To favor inter-strain microbiota transfer four cohorts of conventional AKR/J and SWR/J were swapped between cages twice weekly for 14 weeks. After 4 weeks of HFD feeding fat mass of AKR/J exposed to SWR/J microbiota decreased, whereas fat mass of SWR/J tended to increase by exposure to AKR/J microbiota. Cecal metabolite profiles were assessed in genetically obese Mc4rW16X mutant and wildtype mice at the age of 22 weeks. Mice were fed either low fat control (CD) or chow diet. Cecal mass was affected by diet and genotype with lower mass in CD fed mice and increased mass in the genetically obese Mc4rW16X mutant mice. Cecal contents were then analyzed by ICR-FT/MS. Hierarchical clustering revealed metabolite groups dependent on diet, genotype and cohousing. Diet, genotype as well as interaction effects were found for primary as well as secondary bile acid profiles confirming a role of gut bacteria in bile acid metabolism. Further identification of bacterial metabolites may help to find novel mechanisms involved in the interplay between microbiota and host energy metabolism. Analysis of gut microbial composition will also be necessary to confirm microbial contribution to the phenotypic outcomes.

The PNPLA3 I148M interacts with obesity status and dietary intakes on fasting triglyceride levels

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The Ile148Met (rs738409, G-allele) in the patatin-like phospholipase-domain-containing protein 3 gene (PNPLA3), also called adiponutrin, associates strongly with liver fat content and may lead to both loss-of-function (hydrolysis) and gain-of-function (CoA-dependent lysophosphatidic acid-acyltransferase; LPAAT) defects. PNPLA3 is up-regulated by dietary carbohydrates (CHO) and interactions between rs738409 and dietary CHO, sugar and ω-6:ω-3 PUFA ratio on hepatic fat accumulation have been reported. In addition, previous studies have observed the G-allele in paradoxical association with lower fasting triglyceride levels among obese but not among normal weight individuals. We examined interaction between rs738409 and obesity status, and between rs738409 and dietary intakes of CHO, sucrose and ω-6:ω-3 PUFA ratio, on fasting triglyceride levels. From the Malmo Diet and Cancer Study Cardiovascular Cohort (MDCS-CC) 4827 individuals without diabetes aged 58±6 years, 3343 with BMI≤27 kg/m^2 and 1481 with BMI>27 kg/m^2, were included in cross-sectional analyses. Dietary data was collected by a modified diet history method. Obesity status modified the association between rs738409 and fasting triglyceride levels ($P_{interaction}$=0.006). G-allele associated with lower triglycerides only among overweight individuals (P=0.005). Interactions on triglyceride levels were observed between rs738409 and sucrose among normal weight ($P_{interaction}$=0.02) and ω-6:ω-3 PUFA ratio among overweight individuals ($P_{interaction}$=0.03). G-allele associated with lower triglycerides among overweight in the lowest tertiles of CHO, sucrose and ω-6:ω-3 PUFA ratio (P=0.007, P=0.03, P=0.0004) and with higher triglycerides among normal weight individuals in the highest tertile of sucrose (P=0.02). All the results remained similar if BMI of >25 kg/m^2 was used to define overweight. We conclude that obesity status and dietary sucrose and ω-6:ω-3 PUFA ratio modify the association between rs738409 and fasting triglyceride levels. Our results indicate that overweight may uncover the defective hydrolysis function of PNPLA3-148Met, while high dietary sucrose and ω-6:ω-3 PUFA ratio may reverse this into an increased LPAAT activity, and that dietary intervention and weight loss may be effective in attenuating the effect of PNPLA3-148Met. Our study further highlights that fasting triglyceride levels is a bad marker for liver fat content among overweight individuals due to the strong modifying effect of dietary sucrose and ω-6:ω-3 PUFA ratio on PNPLA3 Ile148Met.

Health eating index as a tool for measuring quality of diets of metabolic groups

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In the process of analyzing gene-nutrient interactions subjects' diet history is a crucial task. To look for metabolic groups after multiple micronutrients supplementation in children and adolescents and to correlate with their global quality of diet. One 24-hour recall was assessed prior intervention period in 139 subjects from 9 to 13 years old. The subjects undergo public schools program on the outskirts of Ribeirão Preto (São Paulo, Brazil). The study design is based on measuring metabolites related to lipids and glycaemia at baseline, after six weeks of a micronutrient daily supplement, and following 6 weeks without the supplement. The revised Health Eating Index (HEI), 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 added together and the total rate classifies the quality of the diet. Score<51 points = inadequate diet; between 51 and 80 points = diet needs modification; ≥80 points = diet is considered healthy. Preliminary Two reverse metabolic groups were found based on cholesterol, LDL, triglycerides, and glycaemia levels. Cluster 1, n=116 subjects, had a better metabolites profile when compared to Cluster 2, n=23 subjects. ANOVA P<0.01 for all parameters. ANCOVA analyses, comparing variables between the two clusters and adjusting for age, pubertal status, and gender showed no differences in any food component between the two clusters. Total HEI indicates inadequate diets (Cluster 1: 62.2±7.5; Cluster 2: 62.2±8.0; P=0.96). The differences in the two metabolic groups cannot be explained by diferences in diets. Differences in genotype may explain why they have responded in distinct ways.
This study has been sponsored by Nestlé Institute of Health Science.

β-sitosterol and campesterol as biomarkers in predicting diabetes in the Finnish Diabetes Prevention

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Cholesterol metabolism is known to be altered in type 2 diabetes and prediabetes. In searching new biomarkers predicting the risk of diabetes we examined serum cholesterol synthesis precursors and plant sterols (β-sitosterol and campesterol) during the 4 years in the Finnish Diabetes prevention Study (DPS). Altogether there were 399 individuals (125 men and 274 women) that had β-sitosterol, campesterol and cholesterol synthesis precursors measured, aged 55.5±7.2 years and BMI 31.1±4.6 kg/m^2. From these, 198 were in the original DPS intervention and 201 in the control group: (1) we examined the β-sitosterol and campesterol serum concentrations in relation to the risk of diabetes; (2) we studied associations with insulin sensitivity and insulin secretion based on an oral glucose tolerance test; and (3) we tested if the genetic variation of ABCG8 gene (rs4299376, rs6544713, and rs6756629) in 372 individuals (185 in the intervention and 187 in the control group) would modify plant sterol concentrations in serum. Data from the 4 year-follow-up was averaged for analyses. Complete insulin secretion and sensitivity data was available for 291 participants (141 in the intervention and 150 in the control group). The mean plant sterol concentrations were higher in the intervention than in the control group during the follow-up (β-sitosterol: 9.1±3.8 vs. 8.1±3.1µmol/l, P=0.013; campesterol: 8.8±4.4 vs. 7.8±4.0 µmol/l, P=0.034), and the higher plant sterol concentrations associated with the lower risk of type 2 diabetes (RR (95% CI); β-sitosterol: 0.92 (0.87; 0.97) and campesterol 0.94 (0.90; 0.98), after adjustment for age, sex, and study group). Furthermore, higher serum plant sterol concentrations were significantly associated with better insulin sensitivity and insulin sensitivity-adjusted insulin secretion. Most of these associations, however, disappeared after adjustment for obesity. All three ABCG8 SNPs examined associated with β-sitosterol and campesterol concentrations throughout the 4-year follow-up (P<0.0001), but the strongest associations were found with rs6544173 using a dominant model: wild type (CC; n=242) vs. minor allele carriers (CT+TT; n=130) (β-sitosterol: $P=8.7\times10^{-6}$ and campesterol: $P=5.3\times10^{-6}$). Adjustment for BMI did not change the results ($P<1\times10^{-6}$). An interaction between study group and rs6544173 was observed (β-sitosterol: P=0.011 and campesterol: P=0.035). Only in carriers for the minor T allele the effect of study group affected sterol concentrations, which were higher in the intervention than in the control group (β-sitosterol: P=0.008 and campesterol: P=0.024 after adjusting for BMI in the models). None of the ABCG8 SNPs associated with diabetes risk. In conclusion, high plant sterol concentrations associate with low diabetes risk and with insulin sensitivity. Serum plant sterols are strongly genetically controlled and there is an interaction with lifestyle intervention.

A difference in transcriptional response to caloric restriction between old and young men

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Caloric restriction (CR) is considered to increase lifespan and to prevent various age-related diseases in different non-human organisms. So far, a limited number of studies on CR have been performed in humans. The results of these studies put CR as a beneficial tool to decrease risk factors in several age-related diseases, thus contributing to a healthy ageing process in humans. The molecular mechanisms orchestrating this effect of CR on ageing, however, remain unclear. The aim of our study was to elucidate the role of age on the transcriptional response to a 30% CR diet in immune cells. 10 healthy young men, aged 20-34, and 9 healthy older men, aged 64-85, were subjected to a two week weight maintenance diet, followed by three weeks of 30% energy restriction. Total RNA from peripheral blood mononuclear cells (PBMCs) was collected and isolated before and after the 30% CR diet and used to evaluate gene expression on human whole-genome microarrays. In the total group, CR resulted in an increased expression of 1123 genes that were among others involved in RNA processing and cell cycle. CR decreased the expression of 741 genes, that were mainly involved in immune response. A sub analysis by age revealed a change in expression of 1150 genes in young men, and a change in expression of 1191 genes in older men. Expression of 554 genes showed a different response between the young and older men upon CR. Gene set enrichment analysis revealed a downregulation of gene sets involved in immune response and cell cycle in young, while this downregulation was not present in older men. We therefore conclude that the transcriptional response to CR is age-dependent.

Genomes, promotors and diseases

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The establishment of next generation sequencing (NGS) technologies in labs worldwide has led to an ever increasing deluge of data pouring from sequencers every day. While the handling of the wet-lab part has become relatively straightforward and more and more convenient, data analysis remains tedious with many different bioinformatic tools often sacrificing ease-of-use for efficiency. However, turning NGS data into meaningful information and subsequently into relevant biomedical knowledge requires reliable workflows as well as high quality background information (like databases for promoters, transcription factors and literature). Integration of these parts into a user-friendly interpretative environment is provided by Genomatix solutions. During the presentation we will focus on real life examples for disease relevant analysis leading to clinical relevant results.

Metabolomics: enabling efficient nutrition and health research

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Metabolite profiling has established itself as a robust tool to learn from metabolic changes induced by stress, lifestyle, disease, medical- and nutritional challenges. With high sensitivity and appropriate quality control measures applied this 'omics' technology can be used effectively to detect even small deviations from individual homeostasis. Similar to the diagnosis of diabetes, where the risk interval of fasting glucose and HbA1C is only about 20% above normal plasma levels, we detect and interpret relevant metabolite changes as a result of nutritional intervention, disease state or therapeutic treatment. Metabolic changes best reflect individual physiology at a functional level integrating complex biological processes. Our exemplary nutrition case studies demonstrate applications of metabolite profiling for health research. We observed intriguing metabolomic changes in a 6 month dietary intervention trial with healthy overweight women and men, 55 completing a low fat, 56 a low carbohydrate diet arm. Metabolite profiles were associated with surrogate markers for metabolic disease risk. Insulin sensitivity correlated more closely with branched chain amino acids than with fasting glucose. Interestingly, dietary and plasma eicosapentaenoic acid correlated positively with HDL cholesterol and inversely with blood pressure. Metabolic effects of a singular glucose challenge have been described. We further examined the physiological response of cats and dogs to dietary glucose within a standard diet to study metabolically diverse models. As proof of principle new insights into adaptation processes were generated in the well-characterized metabolism of glucose utilization and deposition. Another type of adaptation was observed following the remission of diabetic-obese patients following bariatric surgery. Complete elucidation of the fundamental changes in diabetes remission still needs to be achieved, but metabolite pattern changes confirm the role of key metabolites in diabetes. Finally the metabolic characterization of subjects developing type 2-diabetes can be used to promote nutrition and lifestyle intervention strategies. We identified metabolomic changes which are associated with diabetes development several years prior to disease manifestation. Some of the metabolites such as glyoxylate, a small reactive aldehyde, may play a critical role in the generation of diabetes associated advanced glycation endproducts. Glyoxylate concentration can be affected by antidiabetic drugs *in vivo* and the aldehyde is associated with diabetes complications. This new finding may trigger innovative nutritional and lifestyle concepts targeting advanced glycation endproducts. Throughout our proprietary biomarker program as well as in service projects sample quality and validated method adaptation are the determining factors for robust and reproducible results. Our portfolio of case studies demonstrates that metabolite profiling can support the substantiation of health claims. Moreover, the identification of metabolite patterns characteristic for specific diseases can be exploited to tailor interventions that more effectively address nutritional needs.

The effects of selenium-enriched milk and meat on colon inflammation in a mouse model

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Compared to simple dietary supplements, selenium-enriched foods deliver this essential element in a variety of chemical forms that can affect metabolism in different and potentially beneficial ways. For example, selenium-enriched milk and meat fed to rodents increased GPX activity (a biomarker of selenium status) more than other sources. Furthermore, selenium-enriched milk was more protective than selenium yeast against colon tumorigenesis in a mouse model. It has been postulated that increased levels of selenium in meat may mitigate risks of colon cancer; however the role of selenium in intestinal inflammation is unknown. Crohn's Disease (CD), one of the human inflammatory bowel diseases, is characterised by chronic intestinal inflammation. While the exact cause of CD is still not established, there is clear evidence for a genetic component, with studies showing gene variants associated with the interleukin-10 signalling pathway. Il10 is an anti-inflammatory cytokine, and mice lacking a functional gene encoding this protein (IL10 gene-deficient ($Il10^{-/-}$) mice) develop intestinal inflammation which shares several features of human CD. We hypothesise that dietary supplementation with selenium via enriched milk and meat will decrease the level of inflammation observed in the intestine of $Il10^{-/-}$ knock-out mice. 50 male $Il10^{-/-}$ mice and 30 male wild-type (C57BL/6J) mice were allocated to the following treatments at five weeks of age: (1) control group, AIN-76A diet, sacrificed at 12 weeks of age; (2) early inflammation, AIN-76A +20% un-supplemented milk/lamb diet, sacrificed at 8 weeks of age; (3) early inflammation, selenium-milk and meat (20% incorporation; 0.2 ppm Se/d), sacrificed at 8 weeks of age; (4) established inflammation, AIN-76A +20% un-supplemented milk/lamb, sacrificed at 12 weeks of age; (5) established inflammation, Se-milk and meat (20% incorporation; 0.2 ppm Se/d), sacrificed at 12 weeks of age. Early and established inflammation time points were chosen to look for early markers of dietary intervention. Body weight and food intake were assessed twice weekly. At sacrifice, tissues were collected for chemical and histological analyses. There was no effect of mouse strain or selenium supplementation on food intake (c. 2.7 g/day). In C57BL/6J mice, selenium supplementation decreased body weight (20.7 vs. 22.1 g); however there was no effect of selenium supplementation in $Il10^{-/-}$ mice (c. 22.1 g). The effects of selenium supplementation on intestinal inflammation (as determined by histological injury score), liver selenium levels and urinary metabolite profiles will be presented.

Specific tocotrienols induce EndoR Stress and apoptosis in human cancer cells

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Tocotrienols (T3s) are very similar to Tocopherols (TOCs), only differing in the unsaturation of the phytyl chain. Even though T3s are frequently pooled together with TOCs within the family of 'Vitamin E molecules', they have been reported to have specific distinct functions and activities. In the recent past, utilizing a cDNA array based approach, we have demonstrated that a T3 Rich Fraction extracted from Palm oil (TRF) induces a significant reduction of cell proliferation both *in vitro* in cultured breast cancer cells and *in vivo*, in tumours induced by inoculating human breast cancer cells in athymic mice. More recently, a set of studies *in silico*, followed by *in vitro* binding experiments coupled with cell culture studies, suggested that the effects of specific T3s (γ and δT3 forms) on gene expression is, at least in part, mediated by the activation of estrogen receptor-β (ERβ) in cultured MDA-MB-231 and MCF-7 cells. The transcriptomic data-set obtained within these studies was interrogated by means of bio-informatic tools, and opened the avenue for investigating about the existence of an alternative pathway, activated by specific T3 forms leading to apoptosis, in tumour cells not expressing any of the two canonical forms of ERs, and mainly ascribable to the induction of a cellular stress at the level of the endoplasmic reticulum (EndoR). In the present study, starting from the interrogation of transcriptomic platforms, we demonstrated that treatment with specific forms of T3s is associated to apoptosis, to the activation of specific Ca-dependent signals, to the expression of IRE1-α and other and molecules involved in unfolded protein response (UPR), the core pathway to cope with EndoR stress in eukaryotic cells.

Nutrigenomic technologies to study the effect of a mediterranean-style diet on inflammation

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Previous research has shown that a diet, similar to a Mediterranean diet (i.e. high in long chain omega-3 fatty acids, olive oil, fruits, vegetables, nuts and whole grains and low in saturated fats and sugars) can help reduce inflammation in a susceptible population. Our primary aim was to compare traditional validated biomarkers of inflammation, C-reactive protein (CRP) levels and micronuclei numbers with the newer more sensitive ‘omic technologies. To prove proof of principle of our study, we initially selected 30 ‘healthy’ volunteers based on their existing diet. We provided them with food and recipes as well as advice regarding what and how they should eat for the 6 week duration of the study. We took samples before and after the diet intervention. We found that the diet significantly reduced the level of CRP and there was a trend for decreased micronuclei numbers, but this was not statistically significant. However we did see a significant change in gene expression, with expression both up- and down-regulated. As expected the transcriptomic data gave a more detailed response to pre and post intervention. As our aim is to be able to use this diet to treat IBD patients we looked at the expression of IBD susceptibility genes and found that TLR2, TLR4 and JAK2, which are commonly upregulated in IBD were significantly downregulated after the diet. Our data imply that small short-term studies using the new ‘omic technologies are now a viable alternative for human dietary intervention trials.

Can we trust untargeted metabolomics: results of the Metabo-Ring initiative

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The Metabo-Ring initiative brings together 5 Nuclear Magnetic Resonance (NMR) and 11 different Mass Spectrometers (MS), to assess the reliability of the untargeted metabolomic approach to obtain the same biological information. This was estimated by measuring the proportion of common spectral information extracted from the different MS and NMR platforms. Biological samples obtained from 2 different experimental tests were analyzed by partners using their own in-house protocols. Test #1 referred to a high biological contrast experiment, obtained by spiking the urines of 14 healthy adult volunteers with 33 metabolite standards. Test #2 concerned a low biological contrast experiment situation comparing the plasma of rats supplemented or not with vitamin D. Spectral information from each instrument was assembled into separate statistical blocks. Correlations between blocks (e.g. instruments) were examined using the RV coefficients (similar to the R2 coefficient of determination) and the structure of the common spectra by Common Components and Specific Weights Analysis. In addition, in test#1 an outlier individual was blindly introduced to evaluate its detection by the various platforms. Despite large differences in the number of spectral features produced by the instruments, the heterogeneity in the analytic conditions and the data post-processing, the spectral information both within (NMR and MS) and across methods (NMR vs MS) was highly converging (from 64% to 91% on average). No effect of the MS configuration (TOF, QTOF, Orbitrap) was noticed. An outlier individual was best detected and characterized by MS instruments. In conclusion, metabolomics profiling brings a consistent information within and across instruments of various technologies even without prior standardization.

Cross-study data integration to identify common and subgroup-specific gene expression responses

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The ability of a person to adapt to perturbations, or phenotypic flexibility, is a relatively new biomarker of health. Recently, we have performed several studies that examined phenotypic flexibility using oral lipid tolerance tests (OLTTs). Here, we selected 4 of these studies in which whole genome peripheral blood mononuclear cell (PBMC) gene expression changes were measured. In this study, we aim to identify the potential added value of cross-study data integration in nutrigenomics research and to increase our understanding of the biological processes underlying the response to OLTTs. In the 4 studies, OLTTs were performed using similar protocols, though differences in lipid content, number of subjects, subject characteristics, study location and microarray platforms were present. Preliminary analysis using separate pre-processing and statistical analysis of the 4 studies revealed that expression of only 14 genes was significantly altered by the OLTTs ($q<0.05$) in all studies. Furthermore, gene set enrichment analysis showed 5 of a total of 1188 gene sets were significant in all studies (FDR $q<0.25$). To increase statistical power, we will integrate the 4 PBMC gene expression data sets in an early stage of the data analysis pipeline and we will identify common response signatures as well as subgroup-specific response signatures. Additionally, pathway analysis will be performed on these signatures to determine common and subgroup-specific biological processes affected by OLTTs. Results of these analyses will be presented during the conference.

Plasma lipidomic profile in healthy subjects after fish oil supplementation

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While beneficial health effects of fish and fish oil consumption are well documented, the incorporation of omega-3 fatty acids in plasma lipid classes is not completely understood. To investigate the effect of fish oil supplementation on the plasma lipidomic profile. In a seven weeks double-blinded randomized controlled parallel-group study, healthy subjects received capsules containing 8 g/d of either fish oil (1.6 g/d EPA+DHA) (n=16) or high oleic sunflower oil (n=17). During the first three weeks of intervention, the subjects completed a fully controlled diet period. Lipidomic analyses were performed after three and seven weeks of intervention. In total, 568 lipids were detected and 260 identified, using Ultra Performance Liquid Chromatography (UPLC) coupled to electrospray ionization quadrupole time-of-flight mass spectrometry (QTOFMS). Both t-tests and Multi-Block Partial Least Square Regression (MBPLSR) analysis were performed for analysing differences between the intervention groups. Although BMI and serum triglycerides, total-, LDL- and HDL-cholesterol remained unchanged during the intervention period, the intervention groups were well separated by the lipidomic data after three and seven weeks of intervention. Several lipid classes such as phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine, sphingomyelin, phosphatidylserine, phosphatidylglycerol and triglycerides contributed strongly to this separation. Furthermore, selected phospholipids and triglycerides of long-chain polyunsaturated fatty acids were significantly increased after fish oil supplementation. In healthy subjects, fish oil supplementation alters lipid metabolism and increases the proportion of phospholipids and triglycerides containing long-chain polyunsaturated fatty acids. Whether such remodeling of plasma lipids contributes to the protective effects of fish oil needs to be further investigated.

Effects of the yerba mate (*Ilex paraguariensis*) on the expression of miRNAs associated with adipogen

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The investigation of the molecular regulatory mechanism of adipocyte differentiation is essential for understanding the physiological processes of adipogenesis, but it is also important for identifying new biomarkers and therapeutic targets for some metabolic diseases, such as obesity. The microRNAs (miRNAs) appear to play important roles in adipocyte differentiation. During adipogenesis, miRNAs can accelerate or inhibit adipocyte differentiation by acting on transcription factors, regulating signalling pathways related to adipogenesis, or blocking the mitotic clonal expansion stage, thus regulating adipocyte development. The research in the nutrition field has recently aroused considerable interest based on the potential of natural products to counteract obesity. Several studies have identified yerba mate (Ilex paraguariensis) as an excellent candidate. Thus, the aim of this study was to evaluate the *in vitro* effects of yerba mate on the expression of miRNAs associated with adipogenesis. The effects of yerba mate on miRNAs expression were evaluated in 3T3-L1 cells using the Mouse miRNome miScript miRNA PCR Array (V16.0) (Qiagen). The *in silico* network analysis was performed using MetaCore v6.13 to characterize the biological pathways connecting miRNA-mRNA pairs. The results from the miRNAomes indicated that yerba mate modulates significantly the expression of 23.6% (222/940) of the miRNAs analyzed. Among these, 84.2% (187/222) were up-regulated and 15.8% (35/222) down-regulated. The *in silico* analyzes indicate that the differentially expressed miRNAs possess important target genes involved in both fat cells differentiation and in cell-cycle control. Thus, the preliminary results indicate that yerba mate may inhibit adipogenesis through modulation of miRNA expression.

Food4Me: towards personalized nutrition

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This presentation will provide an overview of the Food4Me project, funded by the 7th EU Framework Programme (FP7). Food4Me is an acronym for Personalised Nutrition: An Integrated Analysis of Opportunities and Challenges. The project aims to explore the scientific, business and consumers aspects of personalised nutrition and to determine whether dietary advice based on nutrient, phenotypic and genetic information, could deliver consumer benefits. The Food4Me consortium is a multi-disciplinary team of 25 European partners and includes collaboration with key players in the area of personalised nutrition globally. Professor Mike Gibney of the Institute of Food and Health at University College Dublin is the lead coordinator of the project, which began in 2011 and will run until 2015. The concept of personalised nutrition emerged following the sequencing of the human genome in 2000. It was hoped that with the identification of gene-nutrient interactions, an individual's response and susceptibility to particular diets would be better understood and therefore appropriate dietary modifications could be made to optimise health and lower disease risk. Although research in the area of nutrigenomics has deepened and made significant advances, the translation of this knowledge to a sound public health service has not yet been reached. In addition, early attempts to produce a sustainable business model through personalised nutrition services have been unsuccessful leading to a sceptical vision for the future. Despite this, the potential of nutrigenomics in advancing public health awareness and delivery is too great to be dismissed without further exploration. Food4Me aims to explore all aspects and extend the current state-of-the-art in personalised nutrition by: (1) exploring the opportunities and challenges in establishing suitable business models for the delivery of personalised nutrition at all stages of the food chain, in collaboration with stakeholders (the food industry, the media, health insurers, patient groups, retailers, regulatory authorities, medical professionals and scientists); (2) developing new scientific tools for the exploration of dietary, phenotypic and genotypic data in the delivery of personalised nutrition; (3) assessing the validity of delivering a personalised nutrition service in a proof of principal research study, involving a large cohort of 1280 volunteers across 8 EU states; (4) assessing the attitudes of consumers in all regions of the EU to all aspects of personalised nutrition; (5) exploring the ethical and legal dimensions of personalised nutrition; and (6) developing best practice guidelines for communicating to consumers about personalised nutrition This presentation will touch on all aspects of the project, with an emphasis on progress to date. The business model work will be discussed in a separate presentation.

Exploring future opportunities and barriers for business model concepts in personalized nutrition

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Our societies have to find ways to release the pressure on health care budgets from the consequences of unhealthy life styles and dietary behavior. As the science on the genetic basis and metabolic dynamics that control the relation between food and health progresses, the difficulties of achieving the lasting dietary behavior change where needed still presents a daunting problem and is mainly due to the inherent individual dimension of food choice and the concept of health. Personalized nutrition is a new approach that may help to overcome this problem by matching dietary advise with health requirements at a more individual level. Food4me has explored the emerging personalized nutrition offerings in the market and the barriers and opportunities as currently perceived from a consumer, industrial as well as societal perspective. This resulted in the development of a personalized nutrition system model that describes the characteristics of a personalized nutrition concept and how it is influenced by its environment (societal, technical, commercial, political, legal, ethical, psychological). In order to use this to explore possible novel business model concepts, future scenarios were created to explore how value patterns around nutrition and health could shape future European societies by 2030. With two variables 'logic of health care systems' and 'concept of health' defining the scenario space, for scenarios have been created: 'Super Sister', 'My health My Home', 'Me Inc' and 'Nudging World'. They will serve in the next steps of the project as a rich background to design novel business model concepts and to evaluate barriers and opportunities.

Nutritional phenotype database

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Storing data in a structured and standardized way, facilitates the comparison of studies. We have used the system to compare challenges of different studies. The Phenotype Database (www.dbnp.org and test.dbnp.org) is a web-based application/database that is especially designed to store complex study designs including cross-over designs and challenges (but can store any biological study). It contains templates which makes it possible to customize the system in order to allow flexibility to capture all information available within a study. Comparisons between studies are facilitated by incorporation of ontologies. Different types of data including transcriptomics, clinical chemistry and proteomics can be stored in platform specific modules (for some data types analysis and processing pipelines are available). The Phenotype Database project can be downloaded (https://github.com/PhenotypeFoundation/GSCF), installed on your own server and developers can adjust the open-source software. Data can be shared via a nutritional instance of the system (studies.dbnp.org) and can be opened to the world, if wanted. The system is secured with username and password and is used in several European projects. By using the system to compare challenges of different studies we were able to answer questions that could not be answered by data from one single study only.

Using multiple source and triads methods to analyze vitamin B6, vitamin B12 and folate intake among

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The biggest challenge encountered in biology systems studies that associate diet with chronic diseases has been the inaccuracy of dietary information collected using food frequency questionnaires (FFQ), 24-hour recalls (24hR) and food record. This study evaluated the relative validity of a Food Frequency Questionnaire (FFQ), for adolescents at 10 to 18 years old in the city of Ribeirão Preto, São Paulo State. Three 24-hour recalls were carried out in non-consecutive days. The validity has been tested by means of comparison between the estimated values from the average of 3 24-hour recalls (reference method) and the values obtained by the FFQ. Blood samples were collected from 39 adolescents to assess serum levels of folic acid, vitamin B12 and homocystein. In this study, we applied the Multiple Source Method (MSM) to estimate the intra and inter-variability in 24hR and evaluate the validity of the FFQ. The comparison between the food ingestion estimated by the FFQ, the 24-hour recall and the biomarkers with actual intake, were made through a validation coefficient (p) of the triads method. The food frequency questionnaire (FFQ) and serum folate were better than 24hR to evaluate the folate intake (P=0.60; P=0.57; versus P=0.06). Vitamin B12 intake was better assessed by 24-hour recall (P=0.52) followed by FFQ (P=0.27) and by serum B12 levels (P=0.19). When we use the biomarker homocysteine to assess the vitamin B6 intake we observed high values, indicating that the homocysteine is a good marker to assess the vitamin B6 intake (P=0.93).

Biomarkers for phenotypic flexibility as evaluated in healthy and diabetic subjects

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'Optimal health' is not a fixed entity and can be defined as the ability of an organism to maintain or regain homeostasis in an ever changing environment, and especially in response to stressors. One of the methodologies to assess the capacity to adapt is the so-called high calorie challenge test. The current study was designed to identify the relevant biomarkers for the adaptive capacity as a health parameter after an oral high calorie challenge (OLTT) and oral glucose challenge (OGTT) in humans. Of all processes underlying phenotypic flexibility, 5 processes with high relevance to metabolic health were selected: glucose metabolism, lipid metabolism, amino acid metabolism, inflammation and oxidative stress. Ultimately, we aim to develop a standard test method that is generally applicable in dietary intervention studies. Two groups of male participants aged 30-70 years participated in this randomized cross-over study: a group of 20 healthy males and a group of 20 males with Type 2 Diabetes Mellitus (T2DM) who stopped medication temporarily. Healthy was defined based on medical history evaluation, physical examination, results of the pre-study laboratory tests and a normal body mass index (BMI) (20.0-25.9 kg/m^2). Main inclusion criteria for the T2DM-group were: (1) diagnosed as T2DM evidenced by a documented history and use prescription of at least one oral glucose-lowering drug; (2) BMI in the range of 25.1-34.9 kg/m^2. Both groups were given the OLTT or the OGTT on two different study days with a wash-out of at least 2 days in between. Furthermore, 10 healthy and 10 T2DM males underwent an indirect calorimetry measurement, aiming to establish the metabolic flexibility of subjects when the energy sources are switched between carbohydrate and lipids. The OLTT was a 500 ml drink of a mixture of 75 g glucose syrup, 20 g Protifar (Nutricia), 60 g palm oil and 64 g water. The OGTT was a 300 ml drink of 75 g dextrose in water. On study days, at t=0 (fasting) and 6 time-points (t=0.5, 1, 2, 4, 6 and 8 h) after challenge test, blood was sampled from each subject to measure the markers of glucose metabolism (including glucose, insulin, glucagon, C-peptide and fructosamine), lipid metabolism (including free fatty acids, triacylglycerol, cholesterol (HDL, LDL and total), inflammatory response (CRP, sAA, sICAM, sVCAM), adiponectin, leptin, GLP-1, GIP and glutathione ratio and sets of metabolites measured by metabolomics technology (including endogenous metabolites involved in urea cycle, endocrine responses, blood pressure regulation, ER stress, oxidative stress, glucose metabolism, ketone bodies, lipid and protein metabolism). Most metabolite concentrations returned to baseline values within 8 h after challenge. Preliminary results showed clear differences in responses between healthy subjects and T2DM subjects. Next to higher baseline values, plasma profiles of T2DM subjects were more responsive to the OLTT and showed slower return to baseline values as compared to the healthy subjects. Also, responses to OLTT and OGTT were different. Both in healthy and T2DM subjects glucose and insulin responses to the OLTT were suppressed and slower compared to the OGTT, whereas fasting values were comparable.

Time-resolved studies of postprandial metabolism: improvement by dietary standardization?

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Postprandial metabolite measurement can improve the identification of biological meaningful metabolic signatures. However, the high inter-individual variance found in the fasting state was shown to be extended in the postprandial state by time-resolved nutritional challenge tests. Here, we examined if dietary standardization reduces the inter-individual variation observed in postprandial metabolism. Six healthy male subjects were given a high-fat, high-carbohydrate (HFHC) meal at two independent study days and plasma samples were obtained in the fasting state and 1, 2, 4, 6 and 8 h after meal. In advance of study day 1 free food choice was permitted, whereas for day 2 food intake was standardized three days before the second identical meal. Samples were analyzed by targeted and untargeted mass spectrometric methods. To assess both differences of mean metabolite time-courses and the inter-individual variance, novel statistical approaches for the detection of paired time-resolved differences were applied. Comparing HFHC diet-induced metabolite levels with and without dietary standardization revealed a significant difference for isobutyrylcarnitine and for branched-chain amino acids. The inter-individual variance of all measured metabolites was not improved by dietary standardization. Assessing specific metabolite-categories a reduction of the inter-individual variance was observed solely for acyl-alkyl phosphatidylcholine metabolite levels. Thus, postprandial time-course measurement of most metabolite classes is feasible without lead-in periods of dietary control in healthy individuals, and may be useful for the identification of biomarkers. The here introduced novel statistical analysis is a powerful tool to assess differences in paired time-resolved observations while using the complete time scale and overcoming difficulties such as repeated measurements, missing values or non-synchronized time points.

A new strategy to analyze gene-nutrient interaction: looking for metabolic and proteomic groups

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Looking for metabolic and proteomic groups after nutrition intervention studies will help health professionals to translate science into practice and to prevent non-transmissible diseases. To look for metabolic and proteomic groups after multiple micronutrients 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 children and adolescents, using k-cluster technique. We are doing an intervention crossover study to examine how a child or an adolescent will respond to vitamins supplementation in terms of lipid and glucose profile. The subjects undergo public schools program on the outskirts of Ribeirão Preto (São Paulo, Brazil). The study design is based on measuring metabolites related to lipids and glycaemia at baseline, after six weeks of a micronutrient daily supplement, and following 6 weeks without the daily supplement. Comparing across the three time points will determine how an individual responds to changes in nutrient intakes. Two reverse metabolic groups were found based on cholesterol, LDL, triglycerides, and glycaemia levels. Cluster 1, n=116 subjects, had a better metabolites profile (cholesterol, LDL, triglycerides, and glycaemia levels) at baseline (M1) and after six weeks of micronutrient supplementation (M2) when compared to Cluster 2, n=23 subjects. Distance between final cluster centers =132.8; ANOVA $P<0.01$ for all parameters. 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. Cluster 1: Waist Circumference (cm) was 71.5±13 at M1 and 72.3±13 at M2; BMI (kg/m^2) was 19.6±4.5 at M1 and 19.6±4.5 at M2; Fat Free Mass (%) was 76.1±6.8 at M1 and 76.2±6.6 at M2; Fat Mass (%) was 23.9±6.7 at M1 and 23.7±6.5 at M2; C-Reactive Protein (mg/dl) was 0.06 (0.00 – 1.02) at M1 and 0.07 (0.00 – 3.25) at M2; and Lipid Intake (g) was 57.2±28.4 at M1. Cluster 2: Waist Circumference (cm) was 86.7±18.5 at M1 and 87.2±18.8 at M2; BMI (kg/m^2) was 25.3±6.87 at M1 and 25,3±6.96 at M2; Fat Free Mass (%) was 69.8±7.1 at M1 and 69.8±6.6 at M2; Fat Mass (%) was 30.1±7.1 at M1 and 30.1±6.6 at M2; C-Reactive Protein (mg/dl) was 0.18 (0.02 – 0.99) at M1 and 0.17 (0.01 – 2.77) at M2; and Lipid Intake (g) was 62.5±30.0 at M1. Between cluster 1 and cluster 2 the p value was 0.027 for lipid intake and less than 0.01 for the other parameters. This Project is sponsored by Nestle Institute of Health Science and FAPESP (number 2012/00783-2).

NuGO and EuroDISH: studying the need for food and health research infrastructures in Europe

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EuroDISH Europe increasingly experiences diet-related public health problems. High quality research is essential for improving nutrition, lifestyle, and thereby health. EuroDISH (www.eurodish.eu) is a 3 year FP7 programme with 15 partners including NuGO, focused on identifying existing food and health research infrastructures (RIs), their integration, and the need of new RIs that are relevant for innovations in mechanistic research and public health nutrition strategies across Europe. It provides recommendations on best practices to initiatives such as ESFRI, JPI-HDHL, and Horizon 2020. RIs include both 'hard' infrastructures, such as equipments, and 'soft' ones, such as data handling procedures. Case study Sharing and integrating (un)published data sets generated by different technological RIs are necessary to drive nutritional research, optimising study outcome. Besides exchange, data sharing requires standardisation and data normalisation. Ideally a data infrastructure would allow analysis and queries of results of different studies in combination. EuroDISH performs a case study on metabolic syndrome to investigate the usability and needed developments of the Nutritional Phenotype Data-sharing Infrastructure (dbNP, www.dbnp.org), which exploits existing RIs. dbNP allows description of studies, samples and assays using a template-based interface with standardised ontologies. It respects data ownership, but promotes data sharing. The core study-capture module points to raw and processed data and connects to data processing workflows for multiple technologies. dbNP currently contains a simple assay module and complex data processing modules, e.g. for metabolomics and metagenomics and is extended over time with new modules such as available at ArrayAnalysis.org, including quality control, pre-processing, statistics and pathway analysis, for the handling of 'omics' data types. Also the connection to other infrastructures (created within EU Projects such as ECRIN and ELIXIR) will be evaluated. Pathway, gene, and disease ontology enrichment analyses and storage of the results will allow comparison of studies based on comparable biological outcomes. The case study will reveal the usability of what is already in place, where improvements are needed, and where entirely new functionalities are required. The NuGO community The EuroDISH inventory for RIs in nutrition research will benefit from your input on what is required or still lacking. We would like to know your needs for RIs and whether they can become part of dbNP. Meanwhile dbNP is active: visit test.dbnp.org to explore!

Effects of menstrual cycle on activation of nuclear factor κB in peripheral blood mononuclear cells

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The nuclear factor κB (NF-κB) signaling pathway plays a central role in the regulation of genes involved in cell proliferation, apoptosis and angiogenesis. It is an important interface between inflammation and oxidative stress. The heterodimer p65-p50 is the most abundant transcription factor of the NF-κB family and expressed in many cell types, while c-Rel is exclusively found in lymphocytes. The purpose of this study of the FP7 EU project BIOCLAIMS was to investigate the activation of the p65, p50 and c-Rel-containing dimers in peripheral blood mononuclear cells (PBMC) in the course of the menstrual cycle. PBMC were collected from 27 women, aged 34.1±6.7 years, not using hormonal contraceptives, in the early proliferative ([mean±SD], day 5.8±0.96), late proliferative (day 11.9±1.9), mid secretory (day 19.7±2.4) and late secretory phases (day 25.4±2.0) of the menstrual cycle. Time points of investigation were scheduled based on basal body temperature curves over at least two menstrual cycles. Activation of p65, p50 and c-Rel subunits was determined in whole cell extracts using an ELISA-based assay (TransAM Active Motif), in which activated dimers bind in a specific manner to NF-κB consensus binding sites on immobilized nucleotides and can be detected by antibodies directed against either p65, p50 or c-Rel proteins. There was a significant difference (P=0.02) in the activation of p65 across the 4 time points of investigation. Activation was highest in the early proliferative phase (1.04±0.27 OD) and lowest in the mid secretory phase (0.90±0.17 OD). Activation of p50 and c-Rel did not change significantly. Age and length of menstruation were not associated with NF-κB activation. This is the first report on NF-κB activation in PBMC in different phases of the human menstrual cycle. So far, NF-κB activity has been determined only in endometrium. Increased endometrial NF-κB p65 activity has been reported to occur in the early proliferative phase which is in line with the findings in PBMC of the present study. Further studies are needed to elucidate the underlying mechanisms. However, significant changes in the activation of p65-containing NF-κB dimers across the menstrual cycle emphasize the need of taking into account the phase of menstrual cycle in the interpretation of data in NF-κB activation in pre-menopausal women. Supported by European Commission, FP7, contract 244995, and Austrian Government (bmwf).

Effects of renal function and antioxidant status on activation of nuclear factor κB in PBMC

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The nuclear factor κB (NF-κB) signaling pathway plays a central role in chronic inflammation and is an important interface between inflammation and oxidative stress. The heterodimer p65-p50 is the most abundant transcription factor of the NF-κB family. The purpose of this cross-sectional study of the FP7 EU project BIOCLAIMS was to investigate the activation of both the p65 and p50 dimers in individuals with normal and impaired renal function and possible associations with antioxidant status. Peripheral blood mononuclear cells (PBMC) were collected from 229 study subjects, 104 males and 125 females, aged 52.1±15.1 years, not taking statins or angiotensin-converting enzyme inhibitors (which have been shown to have an effect on NF-κB activation), and with estimated glomerular filtration rate (eGFR, MDRD) of 72±22.9 (19.3-123.4) ml/min/1.73 m^2. Activation of NF-κB dimers containing p65 and p50 subunits was determined in whole cell extracts using an ELISA-based assay (TransAM Active Motif), in which activated dimers bind in a specific manner to NF-κB consensus binding sites on immobilized nucleotides and can be detected by antibodies directed against either p50 or p65. Plasma antioxidants, including ascorbate, α- and γ-tocopherol, α- and β-carotene, lycopene, β-cryptoxanthin, and lutein/zeaxanthin, were determined by HPLC. Activation of p50-containing NF-κB dimers increased significantly with declining eGFR ($r=-0.215$, $P<0.001$), while activation of p65-containing NF-κB dimers did not change. Activation of p50-containing dimers decreased with increasing plasma ascorbate ($r=-0.167$, $P=0.012$) and lycopene concentrations ($r=-0.207$, $P=0.002$). Both ascorbate ($r=0.345$, $P<0.001$) and lycopene concentrations ($r=0.193$, $P=0.003$) decreased with declining eGFR. Plasma ascorbate and lycopene concentrations were 68±18.7 (9.76-121) μmol/l and 0.60±0.30 (0.06-1.68) μmol/l, respectively. These data indicate that impaired renal function has an impact on the activation of p50-containing NF-κB dimers, which could be explained by impaired vitamin C and lycopene status. Supported by European Commission, FP7, contract 244995, and Austrian Government.

The 'KarMeN' multi-platform approach: analytical advantages and potential biomarkers

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The analysis of the human metabolome holds many analytical challenges. Biological fluids like urine or plasma contain a large number of chemically diverse metabolites in a wide but dynamic concentration range. Therefore, no analytical technique can cover the whole spectrum of metabolites. Since all techniques have advantages and disadvantages, it is essential to use complementary analytical platforms in order to get the most comprehensive coverage of metabolites. For the chemical analysis of samples obtained from the KarMeN cohort (Karlsruhe Metabolomics and Nutrition) [see separate poster] we therefore chose three reliable analytical methods leading to a multi-platform-assay comprised of NMR, GCxGC-MS, and LC-MS, and combining targeted and untargeted approaches. NMR is initially used as a non-targeted fingerprinting technique. Urine or plasma samples are diluted with a phosphate buffer, and analysed on a 600 MHz NMR spectrometer. NMR can be used as a high-throughput technique, since sample preparation is simple and fast; it is an inherently quantitative technique, but its sensitivity is limited with a limit of detection of approximately 10 µM. GCxGC-MS, providing a much greater chromatographic resolution than conventional GC-MS, has developed into a very useful technique for the untargeted screening of many classes of primary metabolites. After intense in-house method and software development, the separation and semiquantitative detection of about 300 metabolites in each urine and plasma using a GCxGC quadrupole MS system became feasible. A quality control system based on frequent QC twin injections was established for analyte-specific correction of instrumental drift and enabled fold change calculations based on corrected signal intensities. The LC-MS platform is used for the targeted analysis and quantitation of bile acids, lipids, acylcarnitines, biogenic amines and amino acids. The analysis of the latter compounds was achieved by using the AbsoluteIDQ® p180 kit (Biocrates). Advantages of this method are its precision and high sensitivity which enables the detection of low-abundant metabolites. Some metabolites are detected with more than one technique, and this analytical overlap is suitable to confirm the validity of our results. Data from the multi-platform comparison and preliminary results of potential biomarkers based on plasma and urine samples from the first 75 volunteers of the KarMeN study will be presented.

Untargeted metabolomic fingerprinting of the Mediterranean dietary pattern

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Lifestyle plays an important role in the prevention and development of chronic diseases, with diet having a great impact. In this context, Mediterranean Diet (MedDiet) has been proposed as a healthy dietary pattern associated with lower risk of several diseases. Commonly used methods for estimating dietary exposure are related to measurement error, which can lead to bias in measuring the association between nutritional exposure and outcome, contributing to the inconsistent and even contradictory results observed in some epidemiological studies. For this reason, it is necessary to find new dietary biomarkers or biomarker patterns that represent food and nutrient exposure with more accuracy and precision. The aim of this study was to discover new biomarkers of MedDiet adherence (MedD-A) through a NMR-based untargeted metabolomic approach to provide a more accurate estimation of dietary exposure, and to generate new insights into the putative biological mechanisms underlying the health-related effects of this food pattern. MedDiet-A was defined according to the 14-point Mediterranean Diet Adherence Screener (MEDAS) in baseline data from the PREDIMED Study (www.predimed.org). This questionnaire was previously validated and specifically designed for this purpose. Subjects were stratified into the high MedD-A (≥10 points) or the low MedD-A (≤7 points). Fasting urine samples of 276 free-living subjects at high cardiovascular risk were analyzed by 1H-NMR 500-MHz (Varian). After inteligent bucketing (0.005 ppm) data was interquartile range filtered and row-wise normalized by sum. Mann-Whitney U test (SPSS-Statistics) was used to explore differences between both groups in an unadjusted model and after adjustment for potential confounders. Metabolites were identified using Chenomx-NMR-Suite-Profiler, Human Metabolome Database, Biological Magnetic Resonance Data Bank and the Madison Metabolomics Consortium Database. Subjects with high MedDiet-A were characterized by statistically significant higher levels of compounds related to food intake such as proline-betaine (citrus intake), 3-methyl-histidine (protein intake) and 4-hydroxyphenylacetate (gut-microbiota metabolism) as well as lower levels of glucose (endogenous metabolite). As conclusion, the urinary fingerprint of MedDiet dietary pattern adherence was defined by a heterogeneous and diverse mixture of biomarkers including specific-food biomarkers and others non-specific-food biomarkers. These results reinforce the capacity of nutrimetabolomics to explore the impact of dietary components and the ability to obtain new biomarkers combining epidemiological data and metabolomics.

Level of circulating miRNAs in patients before and after surgical removal of colorectal tumors

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MicroRNAs (miRNAs) are small, noncoding RNAs that function as regulators of many critical cellular processes, are known to be abnormally expressed in cancer, and play a role either by oncogenic or tumor-suppressor function. miRNA expression differs between normal and tumor tissue and varies among tissue types. Upregulated miRNAs in tumor tissue can also be detected in the blood circulation and are emerging as promising biomarkers. So far, little is known about how rapid changes, i.e. surgical removal of a colorectal tumor, will be reflected by changing miRNA level in the circulation. Thus, within this pilot study we aim to characterize the level of circulating miRNA prior to and after surgical removal of a colorectal tumor. The investigations are based on the ColoCare Study, an ongoing cohort study of colorectal cancer patients (stage I-IV). Newly-diagnosed colorectal cancer patients are recruited at the ColoCare study site in Heidelberg, Germany prior to surgery for tumor resection. For this pilot study, pre- and post-surgical blood (4.1 (2-9) days after surgery) from n=31 patients was examined. Plasma was obtained by centrifugation (2,500×*g*) and stored at -80 °C upon isolation of miRNA using the Qiagen miRNeasy Mini Kit. We selected 17 candidate miRNAs which have been previously noted to be altered in the circulation of colorectal cancer patients. miRNA levels (n=17) were measured by qRT-PCR using Taqman miRNA reverse transcription kits with miRNA-specific stem-loop primers and Taqman MicroRNA Assays. Data were normalized by subtracting the C_T value of the spike-in control from the target miRNA's C_T value and miRNA expression is presented as 2^-ΔCT transformed values. Statistical analysis was performed using log-transformed normalized C_T values. Study participants were on average 61 years old (35-79), 10 of the participants were diagnosed with colon and 21 with rectal cancer. Ten patients had later stage disease (III or IV). Comparing pre- and post-surgical miRNA levels, revealed a statistically significant decrease of 7 circulating miRNAs (miR-106a: P=0.002, miR-16: P=0.02, miR-18a: P=0.004, miR20a: P=0.01, miR223: P=0.04, miR-320: P=0.006, miR-92: P=0.0008). The remaining candidate miRNAs did not change significantly between the time points. Although the sample size of this pilot study is small, we observed a substantial decrease of 7 target miRNAs approximately one week after surgical tumor removal. Our results underscore the role of these miRNAs in colorectal carcinogenesis. As next steps, we will continue characterizing the changes in plasma level of the described miRNAs for subsequent post-surgical follow-up time points.

Network biology of systems flexibility

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Current definition of health implements a view of optimally functioning human physiology as the ability to adapt to one's environment. To achieve such optimal function, flexibility should be established and maintained at all levels of systems complexity: at the level of molecular regulation, in various physiological processes, in different organs, etc. To comprehend the organization of the system required for flexible response to perturbations – and therefore maintenance of health – we are using network biology as a mean for multi-level mapping of systems components and interactions between them. This enables understanding of the processes required for adaptive response in various organs and discovery of (combinations of) 'hotspot' nodes that need to be fine-tuned in order to achieve optimal flexibility of the system. A growing compendium of nutrigenomics and challenge test studies now allows for integrative analysis and reconstruction of 'reference networks' as a mean to capture dynamic relations between molecular entities, physiologically relevant marker processes and health benefit endpoints. In the context of the NutriTech project, we develop and use such reference networks in combination with the time series data derived from the challenge tests to address systems flexibility using network properties and characterize the network underlying the challenge response. This results in identification of molecules and processes involved the response to the challenge as well as characterization of the system (network topology, capacity) and key nodes (bottlenecks, regulators), which helps to understand and quantify differences in flexibility of relevant physiological processes and organs. To assess the change in flexibility following intervention, the networks generated from the challenge data before and after intervention are compared, revealing the system properties driving the change in flexibility. Static data that remains constant, such as genetics, can be integrated as an additional layer on these networks providing further insight into systems regulation. The key nodes in the networks are prioritized based on multiple scoring methods and used as a biomarker panel that reflects specific aspects of the systems flexibility. In summary, we develop and use networks-based methods to address systems flexibility using network properties as a readout parameter for the health effects of nutrients, for discovery and prioritization of mechanisms, biomarkers and intervention targets and as a guidance for improved, evidence-based dietary interventions.

The genetic and phenotypic determinants of flavonoid absorption and metabolism (The COB study)

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Diet related diseases, including cardiovascular disease, cancer and obesity, account for a large part of the disease burden in Europe. There is increasing evidence to suggest that flavonoids, a group of polyphenols found in plant foods, may contribute to the benefits of such foods in reducing risk of these chronic conditions. However, the absorption, and metabolism of flavonoids and their associated health benefits within the population seem highly heterogeneous, with the reasons for this variability currently unknown. The COB study (2013-2014) will examine the impact of genotype, amongst other factors including age, gender and intestinal microbiota, on the absorption, metabolism and elimination of flavonoids. This will involve an acute feeding trial of 220 healthy individuals (half male and female and half 18-30 year and 65-77 year) consuming a mixed flavonoid meal rich in flavan-3-ols, flavanones and anthocyanins (consisting of chocolate, orange and blackberries), after a 3 day polyphenol restricted diet. Flavonoid metabolites will be characterised in urine and plasma using HPLC and Mass Spectrometry. Gut microbiota will be assessed in faecal samples using pyrosequencing. Participants' DNA undergo exome sequencing and targeted genotyping for SNPs in genes associated with: (1) enzymatic pathways involved in flavonoid metabolism; (2) genes that may alter the intestinal microbial composition; and (3) those modulating gut physiology. It is predicted that the proposed work will greatly advance current knowledge regarding the determinants of flavonoid absorption and metabolism. By understanding the key genetic factors which modify these processes, nutritional genomics in the future may allow greater refinement of current recommended intakes of flavonoid rich foods, such as fruits and vegetables.

Intestinal stem cell derived organoids as a novel model for nutrigenomic research

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The intestinal epithelium is the first barrier for luminal compounds, and the fastest self-renewing mammalian tissue, making it highly susceptible for (patho)physiological challenges. Recently, the nutrition-microbiota-gut interaction has emerged as a possible player in onset and course of a broad spectrum of metabolic and inflammatory disorders. However, current models rely on the use of animal subjects or *in vitro* cultures not representative for the complex organization of the intestinal epithelium. Intestinal organoids, an *ex vivo* system initiated from intestinal stem cells, much better resemble the intestinal epithelium. Intestinal organoids form a self-renewing system consisting of all epithelial cell types including stem cells, progenitor cells and all functional cells of the intestinal epithelium, absorptive, hormone-secreting enteroendocrine, mucus-producing Goblet cells, and Paneth cells. The aim of our studies is to explore and optimize the application of organoids from different species, ranging from small rodents to pigs and humans. Furthermore, we aim to set up a generic and experimentally accessible screening platform that can be broadly applied, with an expanded repertoire of functional read outs. We show that exposure of organoids to both SCFA, as well as to members of the commensal microbiota, resulted in a distinct organoid transcriptional signature. In addition, we show that pig organoids are capable of GLP-1 release, as analyzed by ELISA. In conclusion, our preliminary data present an advanced screening tool to study host-gut microbe interactions by transcriptomic and functional analysis in different species, which we are currently extending towards the broader range of read-out possibilities such as metabolomics and proteomics. The possibility to study organoid structures derived from diseased or genetically modified host species and the broad applicability to different microorganisms and microbial components are the key features of this system.

Effects of time and initial concentrations on ascorbate losses in 24-h urine samples

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Vitamin C is a water-soluble vitamin that is readily excreted in urine. Determination of vitamin C status is based on ascorbate concentrations in plasma and urine. Urinary excretion of substances is known to vary across individual spot urine samples. Therefore, urine samples collected over 24 h are used to quantify urinary excretion of different substances. However, ascorbate is known to be unstable due to redox reactions. To study the effects of time, initial concentrations and urine volumes on ascorbate concentrations and ascorbate losses in individual spot urine samples collected over a 24 h period from healthy volunteers when the samples are processed fresh or kept at room temperature for up to 24 h prior to storage at -80 °C and analysis. Eight healthy individuals (3 females, 5 males), aged 25-34 years. Pre-analytical sample preparation for ascorbate analysis included immediate sample centrifugation and addition of metaphosphoric acid. Ascorbate concentrations were determined by HPLC with electrochemical detection. Statistical analysis included t-tests, linear regression analysis and multiple regression analysis. Initial ascorbate concentrations decreased with increasing volume of the spot urine samples ($r=0.403$, $P=0.004$). Ascorbate losses in the individual urine samples, expressed as percent of initial concentrations, increased significantly with time elapsed since void ($r=0.92$, $P=0.027$) and with initial concentrations ($r=0.801$, $P<0.001$). Multiple regression analysis revealed that percent losses increased significantly both with time elapsed since void and initial concentrations ($P<0.005$), while absolute losses increased significantly only with increasing initial ascorbate concentrations ($P<0.001$). There are significant ascorbate losses in urine samples kept at room temperature for up to 24 hours. The relative losses increase with time elapsed since void and initial concentrations. Because the initial concentrations are the major determinants for the absolute losses, losses cannot be predicted from the time elapsed since void. Therefore, quantification of urinary ascorbate excretion remains a challenge for future research. Supported by European Commission, FP7, contract 244995, and Austrian Government.

Kidney function, dietary intake and ascorbate concentrations in plasma, spot urine and 24-h urine.

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Vitamin C, a water-soluble vitamin, is readily excreted in urine. Vitamin C status is impaired in chronic renal failure, but data on the evolution of changes in slightly and mildly impaired renal function are lacking. The aims of this study were to investigate the effects of kidney function on ascorbate concentrations in plasma, spot urine and 24-h urine and the effects of dietary vitamin C intake on these concentrations in study subjects with normal or impaired renal function. Subjects: 40 subjects, non-smokers, aged 50-82 years, equal numbers of males and females, assigned to 4 groups based on estimated glomerular filtration rates (eGFR) of either <30, 30-60, 60-90 or >90 ml/min/1.73 m^2 according to MDRD and matched for age, were enrolled. Pre-analytical sample preparation included immediate sample centrifugation and addition of metaphosphoric acid to the plasma or urine obtained. Ascorbate concentrations were determined by HPLC with electrochemical detection. Dietary vitamin C intake was estimated from 5-day prospective food records using an Austrian food composition software (nut.s, Dato Denkwerkzeuge, Vienna). Statistical analysis included t-tests, linear regression and multiple regression analysis. Plasma ascorbate concentrations decreased significantly with declining GFR (r=0.389, P=0.013). Dietary vitamin C intake was lower in subjects with lower eGFR (r=0.388, P=0.013). Higher dietary vitamin C intake was associated with higher ascorbate concentrations in spot urine (r=0.429, P=0.006) and 24-h urine samples (r=0.473, P=0.002), but not in plasma. Ascorbate concentrations increased in both spot urine (r=0.456, P=0.003) and 24-h urine (r=0.635, P<0.001) samples with increasing plasma concentrations. There were no gender differences. In this pilot study impaired kidney function was associated with impaired vitamin C status and lower vitamin C intake. Higher dietary intake and higher plasma concentrations were associated with higher urinary ascorbate excretion. Supported by European Commission, FP7, contract 244995, and Austrian Government.

A new strategy to analyze gene-nutrient interaction in children and adolescents: preliminary results

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Although seemingly paradoxical, micronutrient deficiencies are linked to risk of being overweight or obese and to non-transmissible diseases, such as heart disease and diabetes. To describe metabolic and nutritional responses to vitamin and mineral 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 children and adolescents; correlate the response to genes involved in metabolism of the micronutrients with the metabolic groups; analyze the sequence of genes that interact with, are regulated by, or metabolize micronutrients; analyze energy and nutrient intake. An intervention crossover study is being done to examine how a child or an adolescent will respond to multiple micronutrients supplementation. The subjects undergo public schools program on the outskirts of Ribeirão Preto (São Paulo, Brazil). The study design is based on measuring metabolites and nutritional parameters at baseline, after six weeks of a micronutrient daily supplement, and following 6 weeks without the daily supplement. Comparing across the three time points we will determine how an individual responds to changes in nutrient intakes. Genomic analyses using 5 M feature arrays will be used to associate changes in metabolites levels to clusters of gene variants involved in nutrient metabolism. 139 subjects, 9-13 year-old, improved their anthropometric and body composition parameters after 6 weeks of supplementation. Height (cm) at baseline was 152.22±9.31, after intervention was 153.08±9.34 ($P<0.01$), BMI (kg/m2) was 20.56±5.39 before and 20.56±5.40 after intervention ($P=0.99$), Fat Free Mass (kg) increased from 35.43±9.48 to 36.00±9.65 ($P<0.01$). They also improved their metabolic profile regarding lipid status, with cholesterol (mg/dl) decreasing from 164.67±30.89 to 156.28±27.17 ($P<0.01$) and LDL (mg/dl) from 104.46±25.96 to 94.77±22.72 ($P<0.01$). Glycemia (g/dl) also decreased from 92.77±7.84 to 90.61±6.81($P<0.01$). Multiple micronutrients supplementation may improve nutritional and metabolic profile in 9 to 13 year-old subjects. This Project has been funding by Nestle Institute of Health Science and FAPESP (number 2012/20421-8).

The 'KarMeN'-cohort: a cross-sectional study of nutrition, life-style and metabolomics

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Metabolomics has become an important approach in nutrition and health research. It allows to analyse a wide range of small molecules present in a biological system. Major determinants of the composition of the human metabolome are not well defined yet including the impact of specific foods, of acute and long-term food consumption, of the level of physical activity, or of the genetic background. Therefore, the primary objective of this cross-sectional study is to assess the human metabolome in a well-defined healthy cohort and its major life-style-related determinants using a multi-method platform approach. The Karlsruhe Metabolomics and Nutrition (KarMeN) cohort recruits healthy female and male subjects (age >18 years, BMI ≤30) until July 2013 (approximately 300 study participants). Anthropometric parameters determined include height, weight, waist circumference, and body composition (DEXA). Further, blood pressure, arterial stiffness, and pulmonary function are measured. Clinical parameters include ECG, blood and urine clinical chemistry. A food frequency questionnaire and two 24h-recalls (EPIC-Soft) are used to assess food consumption, allowing to differentiate between acute and long-term impact of diet on the human metabolome. The level of physical activity is determined via a standardised questionnaire (IPAQ) and by a combined heart rate monitor/accelerometer (Actiheart®). Resting energy expenditure is measured by indirect calorimetry, while cardio-respiratory fitness is investigated by means of spiro-/ergometry and lactate diagnostics. All study participants are subjected to a standardised examination schedule. Fasted blood plasma, 24h urine, and three spot urines (fasted and non-fasted) are available for metabolomics measurements. Additionally, DNA is available to investigate selected SNPs, especially if interesting phenotypes are observed. Samples are stored at -190 °C until analysis (gas phase of liquid nitrogen). The multi-method platform consists of targeted LC-MS, non-targeted GCxGC-MS, and 1D-1H-NMR analysis [details see separate poster] allowing the detection of more than 400 metabolites. The well-characterised KarMeN cohort in combination with this multi-method metabolomics approach and biostatistical predictive modeling will help to unravel how different life-style factors including acute and long-term food consumption as well as physical activity/inactivity and fitness impact the human plasma and urine metabolome. New insights into these complex interrelationships will provide a substantiated basis to develop new and reliable biomarkers defining the nutritional as well as the health status of people.

Nutritional and metabolic assessment in pediatric patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic, autoimmune inflammatory disease characteristic. Cardiovascular disease, specifically from atherosclerosis, is a major cause of morbidity and mortality in SLE in developed countries. Given their lifelong exposure to atherogenic risk factors, children and adolescents with SLE are at particularly high risk of developing premature atherosclerosis and are therefore ideal candidates for primary prevention. Inflammatory biomarkers have been consistently associated with the presence of CVD in multiple studies from different populations. Recently, attention has focused on high-sensitivity C-reactive (hs-CRP) protein as a potentially important biomarker for CVD. In recent years, several clinical and epidemiological studies hypothesized that an increasing concentration of total plasma homocysteine could represent an additional independent risk factor for cardiovascular disease in SLE. Some studies show that the total homocysteine concentrations are higher in SLE patients when compared with healthy controls, with renal failure being the most frequent cause of hypermocysteinemia in SLE patients. Vitamins B12, B6 and folate are required for homocysteine metabolism. The aim of our study was to describe homocysteine, vitamin B12, folate, lipoproteins, TNF-alfa, hs-CRP concentrations and food intake in pediatric SLE patients and in their healthy controls and, with these results, create metabolic groups to do proteomic technique. Nineteen children with SLE and thirty-nine healthy controls matched for age and sex were included in the study. Clinical and nutritional evaluation (weigh, height, waist circumference and body mass index – BMI) were done. Plasma homocysteine, vitamin B12, folate, lipoproteins, TNF-alfa, and hs-CRP concentrations were analyzed. There were no significant differences regarding age (P=0.117), pubertal stage (breast P=0.158 and pubis P=0.578) and nutritional status (P=0.107) between groups. Body mass index, waist circumference and systolic blood pressure values were statistically higher in SLE group when compared to healthy ones, but height was statistically lower. Homocysteine, TNF- α, CRP and cyanocobalamin levels were higher in SLE subjects when compared to healthy controls but plasma folate, HDL-cholesterol were lower in SLE compared to control. There were no significant differences in energy, macronutrients, vitamin B12, folate and pyridoxine intake between groups. We concluded that our results may signalize possible future cardiovascular complications in SLE pediatric patients and possible associations between nutritional status and risk factors. This study is sponsored by FAPESP (number 2011/16141-7).

Proteomic analysis of effects induced by 3-MCPD and its dipalmitate in rat kidney

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3-monochloropropane-1,2-diol (3-MCPD) and its esters are formed during thermal treatment of fat-containing foodstuff in the presence of salt. Toxicological studies indicate a carcinogenic potential of 3-MCPD, pointing to kidney as the main target organ. A recent study demonstrated that these effects were similar, but milder after a diester treatment. Based on a repeated-dose 28-day oral toxicity study using male Wistar rats, a comparative proteomic approach was performed in order to understand the molecular mechanisms leading to toxicity of 3-MCPD and its esters. Rats were treated with equimolar doses of either 3-MCPD (10 mg/kg body weight) or 3-MCPD dipalmitate (53 mg/kg body weight). A low dose of 3-MCPD dipalmitate (13.3 mg/kg body weight) was also applied. The snap-frozen kidney samples were analysed by two-dimensional gel electrophoresis-mass spectrometry. Ingenuity Pathways Analysis was used for data mining. Network analysis revealed that in all treatment groups several proteins controlling cell survival and cell death were deregulated, including an increased expression of glutathione S-transferase P1, a well-known marker of preneoplastic or neoplastic tissue. Interestingly, a high upregulation of alcohol dehydrogenase, an enzyme which is postulated to be involved in the metabolic pathway of 3-MCPD to β-chlorolactaldehyde, was also observed. Moreover, several enzymes of the carbohydrate metabolism were downregulated, e.g. the glycolytic enzyme triosephosphate isomerase, which is known to be inhibited by β-chlorolactaldehyde. Overall, results indicate similar toxicity of 3-MCPD and its dipalmitate, demonstrating that a deregulation of the energy metabolism and an induction of renal necrosis may lead to toxicological effects in rat kidney.

Holistic modeling of the metabolic syndrome

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Metabolic syndrome is defined as the clustering of abdominal obesity, elevated blood pressure, impaired glucose tolerance, high triglycerides (TG), and low HDL-cholesterol and is presenting worldwide a rapidly growing health and economic problem. For personalized lifestyle/medical treatment advice, it is essential that diagnostic tools become available to probe the function of all involved organs/processes at the same time, and that computer modelling tools are available to integrate the results based on the interplay of multiple processes and mechanisms in various organs aiming to maintain homeostasis i.e. a true systems view. Our objective is to build such a model for type-2 diabetes, an important comorbidity of metabolic syndrome. This is a huge challenge since the model fed with diagnostic measurements should deliver a mechanistic insight in the physiological functioning of the body as a whole, across space and time scales, also involving psychosocial aspects, at a given time in the n=1 situation when a (pre)diabetic overweight person visits a physician. As a first step towards fully personalized quantitative diagnosis and prediction we developed a descriptive model based on causal loop diagrams at a very high aggregation level that from the beginning integrates mechanisms across all relevant domains including body weight dynamics, glucose/insulin dynamics, inflammation, gut health, and mental stress. The model integrates qualitative and semi-quantitative information and expert knowledge. The model was able to simulate the development of type-2 diabetes over a multi-month period following different food intake profiles, and the modulation of disease development induced by physical activity and mental stress-relieving lifestyles. We will further detail this model in a recently started EU project MISSION-T2D which will focus on the interplay of metabolism and low-grade chronic inflammation. In addition to the high-level model that typically will show trends over multiple months, a second aggregation level will be included that describes the integrated dynamics of all processes at the minute-day timescale. This model can be calibrated using data from nutritional challenge tests such as OGTT and OLTT. Since the models at the 2 aggregation levels will be interlinked, the overall model will allow to use model parameters derived from challenge test data, for simulation of long-term disease development expectancies. As a next step, genetic variance of key genes may be included e.g. as modulation of the mechanisms involved in the dynamic responses to challenge tests. Concluding, we have successfully made a first step towards a systems health model of metabolic syndrome and type-2 diabetes.

The impact of diet composition on health maintenance in insulin resistance sub-populations

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Insulin resistance (IR) is a risk factor for future type 2 diabetes and cardiovascular disease. Although in many individuals the insulin resistance develops simultaneously in multiple organs, the severity of insulin resistance may differ among the various tissues (e.g. pancreatic β-cells, liver, skeletal muscle and adipocytes). Consequently, various IR sub-populations exist. Importantly, the risk for and type of developing pathologies varies between these IR sub-populations. It is therefore important to identify new markers for high-risk IR sub-populations and initiate personalized programs. Also, the efficacy of interventions, most of which are organ specific (e.g. 8 weeks very low calorie diet (VLCD) for improving hepatic IR), may be improved by quantifying which organs to what extent are resistant to insulin and adjusting the intervention in accordance to the outcome. To initiate targeted and personalized lifestyle and nutritional interventions, knowledge on how these interventions impact on distinct insulin resistant tissues in IR sub-populations is required. Therefore, the following research question is addressed: what is the impact of diet composition on health maintenance in different IR sub-populations? By means of measurements of plasma glucose and insulin concentrations during an oral glucose tolerance test (OGTT) surrogate indexes that can selectively quantitate β-cells (Δinsulin (AUC 0-30) / Δglucose (AUC 0-30)), liver (Δinsulin (AUC 0-30) * Δglucose (AUC 0-30)) and skeletal muscle ((dG/dt) / mean plasma insulin concentration) insulin resistance are determined. The surrogate index for fat cell insulin resistance makes use of an additional parameter, fasting plasma NEFA (fasting plasma insulin * fasting plasma NEFA). This study is performed within the European Framework project called Diet, Obesity and Genes (DiOGenes). Healthy overweight volunteers with Body Mass Index (BMI)>28 kg/m^2 were subjected to an 8 weeks low calorie diet (800 Kcal/day) and those loosing >8% of their body weight were assigned to four groups with a high/normal protein, high/low glycemic index diet for a 6 months weight maintenance diet period. The research question will be addressed by using univariate and multivariate statistical methods. Clinical chemistry, lipidomics and low molecular weight profiles will be analyzed to examine new markers for IR sub-populations and examine the mechanisms underlying the potential distinct impact of diet compositions on health maintenance.

Intestinal DUOX2-mediated epithelial H_2O_2 production and its role in defence against bacteria

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Dual oxidases (DUOX) belong to the NADPH-oxidases and are responsible for regulated hydrogen peroxide (H_2O_2) release of epithelial cells. Expression of DUOX along the mucosal surfaces of the gastrointestinal tract or major airways proposes a role in host-defence by creating a pro-oxidative environment adjacent to the membrane. In intestinal epithelial cells, the uptake of bacteria-derived peptides is mediated by the peptide transporter PEPT1. From a RNA interference (RNAi) screen in the model organism *Caenorhabditis elegans* we obtained evidence that PEPT1 and the sodium-proton exchanger NHE3/NHX-2 possess some co-regulation in the intestine in the context of ROS-production. We now hypothesize that this interplay includes DUOX2 as part of a distinct microenvironment on the gut surface that affects the microbiota-surface interaction. We are characterising the role of DUOX in the intestine in view of bacterial growth and adherence and assess whether and how enzyme function depends on NHE3 and PEPT1 by using the model organism *C. elegans*, the human colon carcinoma cell line Caco-2 and murine tissue samples. Cell culture experiments with the human colon carcinoma cell line Caco-2 were performed to study the interplay of the homologous mammalian proteins by analysis of H_2O_2 production. Therefore, the H_2O_2 production of differentiated Caco-2 cells was utilized after treatment with various PEPT1 substrates or NHE3 inhibitors. The DUOX activity was significantly reduced in presence of the specific NHE3 inhibitor S1611, indicating a functional coupling of these two membrane proteins. Immunohistochemistry in addition to H_2O_2 measurements were performed to analyse whether DUOX expression and function in small intestine and colon from wild-type and Pept-1$^{-/-}$ mice are altered. Initial immunohistochemical stainings indicate modifications in the expression pattern of DUOX at the apical membrane in the distal colon of Pept-1$^{-/-}$ when compared to wild-type mice. RNAi feeding experiments using the *C. elegans* strain MZE91R expressing PEPT-1::dsRed were performed to examine changes in PEPT-1 protein expression in the absence or the presence of NHX-2 or Ce-DUOX/BLI-3. Gene silencing of nhx-2 induced a significant reduction in PEPT-1 expression at the apical membrane, while Ce-DUOX/bli-3 RNAi silencing had no impact on PEPT-1 protein expression. Infection of MZE91R with the pathogenic *Enterococcus faecalis* strain OG1RF showed a 25% increase in PEPT-1::dsRed protein abundance compared to heat inactivated *E. faecalis* or non-pathogenic *Escherichia coli* OP50 after 24 hours. The findings provide first evidence for the functional interplay of the three brush border membrane proteins DUOX2, NHE3 and PEPT1 in host defense to prevent adhesion/invasion of pathogenic bacteria as for example of *E. faecalis*.

Effects of short-term standardized diet on the urinary metabolomic profiles of exercising humans

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Concerning the interpretation of nutritional metabolomics data, an understanding of short-term lifestyle effects is very important. The extent to which the metabolome can be normalized by standardized protocols is not yet fully described. We examine: (1) the intra-and interindividual variation in normal human metabolic profiles and focus on time of sample collection as a possible mean of reducing daily physiological variation; (2) and investigate the effect of standardizing diet and exercise on altering variation. Design Urine was collected from 24 (5 men, 19 women) healthy volunteers in phase I: for 7 days in a real life situation and daily documentation of lifestyle factors; phase II: 3 days in a standardized diet and exercise protocol. Data on dietary intake have been analysed by a nationspecific software and nutrition database. Exercise has been monitored and standardized by published protocols and pedometers. Samples have been analyzed by using ^{1}H nuclear magnetic resonance spectroscopy followed by multivariate data analysis. Considering all samples collected over the entire period of 10 days we obtain a good discrimination (accuracy 98.9%). The last three days are clearly separated from the first seven days. Clear changes in the level of metabolites are detectable when comparing the first 7 days with the following ones. Creatinine ($P<0.005$), citrate ($P<0.020$), hippuric acid ($P<0.005$), m-HPPA ($P<0.008$), trigonelline ($P<0.005$), TMAO ($P<0.003$) and acetone ($P<0.048$) resulted to be significantly different in phase II of the study. For all metabolites level changes no defined trend during the first 7 days can be observed. Unification of the changes is visible starting from the ninth day (second day of diet). This confirms earlier observations that the metabolome standardization occurs within first day after the introduction of the control diet. Even a highly standardized diet requires collection and analysis of several independent samples in order to reveal the stable individual metabolomics status. In general individual recognition is possible with a very high overall accuracy and a natural gender separation occurs. Discrimination between subjects remains equally good even on a standardized diet. The effect of exercise is modest. Some significant metabolites are highlighted. After standardization the spreading tends to diminish, it seems that controlled diet levels off the homeostasis for these metabolites for all individuals. In summary, the degree of discrimination is: individuals > standard diet > exercise. Urine as well known representative of a sensitive metabolic profile will be added by information on salivary and fecal metabolic profiles in open and standardized protocols. This study will help to consider further investigations on changeable lifestyle factors to develop more focused strategies for prevention of chronic diseases.

Iron deficiency reduced muscle tRNA thiomodification in a rat model

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In eukaryotes, the wobble base of the tRNAs for Glu, Gln and Lys are universally modified to 5-methyl-2-thiouridine derivatives (xm^5s^2U), such as 5-methoxycarbonylmethyl-2-thiouridine (mcm^5s^2U) in cytoplasmic tRNAs. The 2-thio modification xm^5s^2U plays a critical role in protein synthesis. It is known that the 2-thio group of mnm^5s^2U is required for efficient codon recognition on the ribosome. In addition, such modification in tRNAGlu acts as the identifying element for specific recognition by glutaminyl-tRNA synthetase. In yeast and humans, the mitochondrial cysteine desulfurase NFS1 is essential for the post-translational modification of thiouridine in both mitochondrial and cytosolic tRNAs. There is also a link between FeS cluster biogenesis and s^2U. In a rat model, NFS1/IscS is reduced in iron deficiency anemic animals. Therefore, iron deficiency may affect the thio-modification of tRNAs. In this study, we investigated the effect of iron deficiency anemia on the expression and thio-modification of tRNA in both liver and skeletal muscle. Male weanling Wistar rats were rendered to different degree of iron deficiency anemia in 5 wks using a dietary iron depletion-and-repletion method. At the end of study, rats were divided into 5 groups by their final hemoglobin levels: <6 g/dl, 6-8 g/dl, 8-10 g/dl, 10-12 g/dl, and >12 g/dl. Small molecule RNAs were extracted from liver and gastrocnemius muscles. Total tRNAs for Glu, Lys, Arg and Ser were measured with DIG-labeled probe and Northern blotting. Thio-modified tRNAs for these amino acids were measured similarly but with APM (N-acryloylamino) phenylmercuric chloride)-Northern blotting. Furthermore, nucleosides prepared from total tRNAs were analyzed by HPLC, and mcm^5s^2U were quantified using 8BrG as an internal standard. The results showed that in the cytosol of the muscle, the expression of total tRNAs for Lys, Glu and Lys were related to hemoglobin levels in a dose-dependent fashion, and they were significantly reduced by 50% in the anemic rats (tested by one-way ANOVA and Duncan's multiple range test at $P<0.05$). The level of thio-modified tRNA for Lys were reduced by 20% in rats of Hb<6 g/dl, while that for Arg and Glu were reduced by 20% in rats of Hb<10 g/dl. The level of mcm^5s^2U also decreases with decreasing hemoglobin level in a dose-response relationship. Neither the expression of total tRNA for Ser, nor the thio-modified tRNAs in the liver were affected by iron deficiency. Our results confirms that iron deficiency anemia has a tissue-specific effect on thio-modified tRNA, that total and thio-modification of tRNA for Lys, Arg and Glu are significantly reduced in the muscle but not in the liver. This is probably the first study to link iron status to tRNA modifications.

The future of diet: health research in Europe – JPI: 'healthy diet for a healthy life'

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Except for the EU Framework programs, Europe's research funding landscape is still deeply compartmentalized with overlap in research efforts in the different countries as well as lack of coordination to strengthen scientific excellence. Joint Programming should be the answer to tackle this problem by which Member States engage in defining, developing and implementing a common strategic research agenda and funding based on a common vision in close collaboration with the DG Research in Brussels drafting the Horizon 2020 from 2014 onwards. One of the areas a JPI has identified and substantiated is 'A healthy diet for a healthy life' where 22 member states and associated countries are working together and coordinated by the Netherlands. This JPI should be implemented, using a methodology similar to that used in the European Technology Platforms, which have been successfully developing a common research strategy 'Food for Life' for the main industrial sectors, going through three stages. (1) Developing a common vision for the Joint Programming Initiative; (2) Defining a Strategic Research Agenda (SRA), specific objectives and related deadlines; (3) Implementing the SRA and monitoring results so as to ensure maximum impact. The JPI is centred on three main areas; Diet and Physical Activity, Diet and Food and Diet and Chronic diseases. The vision of the JPI on 'A healthy diet for a healthy life' is that in 2030 all Europeans will have the motivation, ability and opportunity to consume a healthy diet from a variety of foods, have healthy levels of physical activity and the incidence of diet-related diseases will have decreased significantly. The first three Joint Actions are now being implemented. Determinants of Diet and Physical Activity (DEDIPAC) was launched on March 7 in Berlin. It's objective is to set up a knowledge hub for European researchers to understand the most effective ways of improving public health through interventions targeting diet and physical activity. Two other initiatives: one on Biomarkers and one on a phenotypical nutritional database to combine nutritional research are currently under development and will be launched later in 2013. In June further topics will be selected for Joints Actions later in 2014-2015. These Joint Actions are driven by a group of volunteer countries based on variable geometry. This gives participating member states not only the freedom to choose which research topics fit best their national agenda but also allows countries to participate to the extent of their own capacities. Joint programming will contribute significantly to the construction of a fully operational European Research Area on the prevention of diet-related chronic diseases and strengthen leadership and competitiveness of the food industry by effectively integrating research in the food-, nutritional-, social- and health sciences to increase knowledge and deliver innovative, novel and improved concepts. The National, Joint and European research efforts in this field will be harmonised through a continuous dialogue between the Joint Programming Initiative and the European Commission.

Horizon 2020 – the new EU Framework Programme for Research and Innovation

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Horizon 2020 is the financial instrument implementing the Innovation Union, a Europe 2020 flagship initiative aimed at securing Europe's global competitiveness. Running from 2014 to 2020, the EU's new programme for research and innovation is part of the drive to create new growth and jobs in Europe. The proposed support for research and innovation under Horizon 2020 will strengthen the EU's position in science, strengthen industrial leadership in innovation, and help address major concerns shared by all Europeans such as climate change, developing sustainable transport and mobility, making renewable energy more affordable, ensuring food safety and security, or coping with the challenge of an ageing population. Horizon 2020 will tackle societal challenges by helping to bridge the gap between research and the market. This market-driven approach will include creating partnerships with the private sector and Member States to bring together the resources needed. International cooperation will be an important cross-cutting priority of Horizon 2020. In addition to Horizon 2020 being fully open to international participation, targeted actions with key partner countries and regions will focus on the EU's strategic priorities. Horizon 2020 will be complemented by further measures to complete and further develop the European Research Area by 2014. These measures will aim at breaking down barriers to create a genuine single market for knowledge, research and innovation.

NRC – the NuGO revolution in cohort research

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The Nutrition Researcher Cohort (NRC, www.nugo.org/nrc) is a research initiative that develops a new concept of nutrition and health research, based on sharing of standardized self-quantification data. A new generation of devices and measurements developed primarily for consumer use, unlocks new research and healthcare possibilities. These measurements vary from tracking personal activity, weight and food intake to blood measurements and direct to consumer genetics. NuGO is creating a cohort, NRC, that will collect such information varying from genomic, phenotypic, observational, diet, health-related and other forms of information from volunteer individuals. The primary, but not exclusive focus of the research will be to explore the connection between diet, health, cognition, lifestyle, genotype and phenotype. Importantly, all personal health information that is donated to the NRC will be fed back to the participant via a personal health portal. The NRC will lead to a new relationship between research and healthcare; study subjects are not just passive data-and-blood-donating humans but they receive direct benefit from participating in research by personalized health information (and possible advice) based on their personal data. Ideally, the whole world would become a self-quantifying cohort concerning personal healthcare. In practice, all participants in this cohort self-report on a series of measurements (DIY or performed in a lab) at the frequency and intensity that he or she chooses, respecting a minimal set of parameters, and by communicating on this, the NRC comes to a series of SOPs on what and how to measure variables. For the first 'crowd science study' we will ask researchers only to join. In this stage researchers can help improve the concept in a straightforward way. In a later stage the NRC will be opened up for a much broader audience. Researcher participants that are interested can join at http://ci.dbnp.org.

Protein content in the maternal diet during gestation may affect liver S-adenosylhomocysteine levels

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The flow of the one-carbon cycle is regulated by the intake of several nutrients involved in methionine metabolism. We hypothesize that the flow of this cycle in the rat liver may also be influenced by the protein and/or folic acid content of the maternal diet during gestation or by the fat content of the diet after weaning. To verify the hypothesis, pregnant rats were fed a diet with normal protein and normal folic acid levels (a modified casein-based AIN-93G diet), a protein-restricted and normal folic acid diet, a protein-restricted and folic acid-supplemented diet, or a normal protein and folic acid-supplemented diet. The progeny were fed either the modified AIN-93G diet or a high-fat lard-based diet. Liver S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) concentrations in the progeny were measured using high-performance liquid chromatography (HPLC). In the 10-week-old progeny SAM concentrations were only affected by sex ($P<0.001$), with higher levels in the males. Prenatal protein restriction resulted in a 20% decrease in SAH concentrations ($P<0.001$). Higher fat content in the postnatal diet was associated with lower SAH concentration ($P<0.001$). Interaction between protein content in the maternal diet and sex ($P<0.05$) influenced the SAM-to-SAH ratio, the highest values of which were observed in the male progeny of dams fed the low protein diet. Our study demonstrates that liver SAH concentrations and SAM-to-SAH ratio may depend on sex and the protein content of the maternal diet during gestation.

Time-resolved metabolomics of berry meals show different excretion kinetics for their markers

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While the prevalence of cardiovascular disease has been decreasing in the last decade, the incidence of type 2 diabetes is still rising at a global level. Berry consumption has been shown to improve CVD surrogate markers, the cluster of which partly defines the pre-diabetic state. In order to correctly assess the association between foods and their biological effect, reliable dietary assessment methods are required. In the present study, an untargeted metabolomics approach has been applied with the aim of identifying exposure and possibly effect markers in urine after consumption of sugar-sweetened strawberry and sea buckthorn meals. A randomized controlled single-blinded cross over study has been conducted in 16 overweight men. Each subject had a randomized sequence of the three intervention meals: strawberry, sea buckthorn and control. Urine samples were collected on each test day, at different time points (baseline, 0-1 h, 1-2 h, 2-24 h) and analyzed by UPLC-qTOF-MS. Principal component analysis has been used to explore the dietary exposures. Thereafter, partial least squares discriminant analysis (PLS-DA) has been applied to discriminate the most important features, for the different test meals, at each time point. Strawberry and sea buckthorn berry gave rise to 11 and 15 potential exposure markers, respectively. Different urinary postprandial patterns have been observed after intake of the two berry meals. Strawberry exposure gave rise to early markers, with a peak in excretion at 2 h. In contrast, sea buckthorn intake mainly gave discriminant features in the 2-21 h and pooled 24 h samples, suggesting longer-term markers. However, a few potential exposure markers for sea buckthorn were also found at 1-2 h. Potential characteristic exposure markers have been distinguished in human urine after consumption of sea buckthorn and strawberries. The markers have clearly distinct time-sequences of excretion. We speculate that markers observed only later than 2 h might originate from either lipid-derived components in sea buckthorn or specific microbial metabolites. Identified markers will be presented.

miR-107: a metabolically relevant microRNA regulated by lipids, atorvastatin and fenofibrate

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miRs have emerged as new key players in energetic metabolism. Moreover, they have been shown to be potential targets for the treatment of metabolic disorders. Recently, some reports suggested that some bioactive compounds of the diet, such as n-3 polyunsaturated fatty acids (n-3 PUFAs) or some polyphenols are able to modify the miR expression profile in different tissues. In this work, we performed a screening of miRs modified by cholesterol and n-3 PUFAs in an *in vitro* model of enterocytes. We selected miR-107 to further analyze its response to diet and lipid-lowering drugs such as Atorvastatin (Ator) and Fenofibrate (Fen). Differentiated Caco-2 cells were treated with micelles containing cholesterol (200 μM) docosahexanoic acid (DHA) or conjugated linoleic acid (CLA) (250 μM) for 24 and 48 hours. miR expression was analyzed by microarray and significantly expressed miRs were selected by Significant Analysis for Microarray (SAM) and t-Student test. Results were confirmed by RT-qPCR. C57BL6/J wild-type (WT) mice (6-weeks old) were fed a chow or a High Fat Diet with a 2.5% cholesterol (HFD-chol) for 16 weeks. The expression of miR-107 was analyzed in different tissues. The promoter region of miR-107 was analyzed for the detection of putative transcription factor (TF) binding sites using TESS y TFSearch. Differentiated Caco-2 cells were treated with Ator or Fen using different dosages for 24 hours and the expression of miR-107 and PANK1 was measured by RT-qPCR. 27 miRs were significantly modified by DHA, 11 by CLA and 2 by cholesterol. After functional analyses of their targets, we observed that most represented pathways were related with cardiomyopathies and neuronal function. miR-107 was up-regulated with DHA and the RT-qPCR analyses showed that it was also upregulated by CLA and cholesterol. miR-107 was preferentially expressed in the brain and the kidney of WT mice, and was upregulated in the white adipose tissue (WAT) and the gut of mice fed a HFD-Chol. miR-107 is located into the intron 5 of PANK1, although the expression of both was discordant. miR-107 expression was modified by Ator and Fen, as well as PANK1 expression, although with different dosages and exposure times. These results suggest that miR-107 is regulated by its own promoter. The *in silico* analysis of the putative miR-107 promoter region showed the presence of putative binding sites for metabolically relevant TFs such as PPARA, C/EBPα, SREBP, and CREBP. In conclusion, dietary lipids modify the expression of microRNAs in an *in vitro* model of enterocytes, being DHA the most effective one. Diet-modified miRs were predicted to be involved in cardiomyopathies and neuronal functions. miR-107 was upregulated by dietary lipids and a HFD. miR-107 expression is also modified by Ator and Fen and may be regulated by its own promoter that has potential binding sites for metabolically relevant transcription factors.

Discovery of novel nutrient benefits for increasing health and resilience

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In 1948, the WHO defined health as 'a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity'. More than half a century later, we continue to assess the health benefits of a diet by measuring risk markers of disease. In our laboratory we developed an innovative proteomics and pathway analysis approach to better assess the ability of diet to prevent, rather than reduce, occurrence of disease. Our approach is based on the concept of resilience: our ability to mount a protective response that restores an (adapted) equilibrium upon stress. In a series of studies we convincingly showed that dietary bioactives boost the production of resilience products. In relevant animal models we found, for example, that extra virgin olive oil significantly up-regulated a range of protective hepatic antioxidant enzymes, including thioredoxin reductase, thioredoxin peroxidase 2, peroxiredoxin 3, superoxide dismutase and glutathione-S-transferase, in Apoe$^{-/-}$ mice. This response would offer endogenous protection against subsequent chemical and/or oxidative challenges. We also discovered that olive phenolics up-regulate protein and activity levels of hepatic aldehyde dehydrogenase 2, a key enzyme of cardioprotection, in a vitamin E-deficient rat model. Furthermore, we showed that the dietary fatty acid cis9, trans11-conjugated linoleic acid (CLA) significantly increased levels of five different post-translationally modified forms of hepatic HSP70 in Apoe$^{-/-}$ mice. Exposure to mildly stressful stimuli can induce HSP70 expression, protecting cells against exposure to more severe stress, and high levels of human HSP70 have been associated with a low cardiovascular disease risk. In addition, long chain n-3 polyunsaturated fatty acids significantly increased antioxidant enzyme expression in the hearts of a rat model. As polyunsaturated fatty acids are prone to enzymatic and non-enzymatic lipid peroxidation, they are believed to increase ROS concentrations just enough to activate transcription leading to increased production of antioxidant enzymes and heat shock proteins, but not enough to cause cell death. In humans, blood cell proteomics proved extremely valuable when elucidating the cellular oxidative stress pathways that are affected by dietary bioactives. We found, for example, that consumption of cis9,trans11 CLA caused significant changes in the human platelet proteome. The focal adhesion pathway appeared an important resilience mechanism which may contribute to the anti-atherogenic effects of dietary cis9,trans11 CLA in animal models. Our unique approach to assess the role of dietary pro-oxidants (rather than anti-oxidants) in the maintenance of health (rather than reduction of disease) may significantly improve our understanding of the protective mechanisms that underlie the beneficial effects of specific food bioactives. Indeed, the use of disease risk markers may not be relevant or indeed difficult to modify by lifestyle interventions in a relatively healthy population. Ultimately, the use of novel resilience markers and signatures could significantly improve our ability to prove the efficacy by which pro-oxidant dietary bioactives improve lifelong health.

Hypoglycemic activity of Agrimony tea in normal and overweight subjects

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Recent studies suggest that *Agrimonia eupatoria* L. (Agrimony) is perspective therapeutic for social significant diseases such as type 2 diabetes and obesity. Also there are evidences about its capacity to prevent mass gain and fat accumulation in rat models. We aimed to evaluate the effects from Agrimony tea consumption on Body mass index (BMI), fasting glucose and lipid profile parameters in human subjects with BMI≤25 (normal weight, NW) and with BMI>25 (overweight, OW). Study included n=21 NW and n=16 OW volunteers, aged between 20-60 years. Subjects consumed 250 ml/day Agrimony tea for period of 25 days. BMI and waist/hip ratio (WHR) were calculated and fasting blood samples were collected at the beginning and at the end of the period. Standardized biochemical methods were used to measure the blood glucose and lipid profile parameters. We observed a significant decrease in fasting blood glucose levels with 4.9% in NW group ($P<0.05$) and 18% in OW group ($P<0.001$), along with increased triglyceride (TG) levels by 10.7% ($P<0.05$) in NW and by 6.45% in OW group, while total cholesterol and HDL/LDL ratio remained unchanged. In support of studies, involving experimental animals these results confirm the hypoglycemic activity of Agrimony and its possible application in preventive medicine. Established also by other studies higher TG levels still need further analyses and explanation.

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