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## PhytoHub version 1.0: a food metabolome database dedicated to dietary phytochemicals

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# **11th NuGOweek NUTRIGENOMICS OF FOODS**

8-11th September 2014,  
The Vesuvian Institute  
Castellammare di Stabia, Italy

## **Book of abstracts**



# Nutrigenomics of Foods

*– NuGOweek 2014 –*



# Nutrigenomics of Foods

## Book of abstracts

**NuGOweek 2014**

*Castellammare di Stabia, Napoli, Italy*

*8-11 September 2014*

*NuGO-week 2014 is organised in collaboration with CRA-NUT*

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# Welcome

Welcome to NuGOweek 2014 in Castellammare di Stabia!

The 11<sup>th</sup> conference in the successful series of annual meetings organized by the Nutrigenomics Organization will focus this year on *Nutrigenomics of Foods*. This main theme reflects the NuGO driven evolution of nutrigenomics science in the past 10 years, which started with the application of -omics technologies to nutrition research (transcriptomics, metabolomics, proteomics), proceeded to develop ways to deal with the resulting datasets (bioinformatics, data pipelining, pathway analysis), used them in mechanistic nutrition research (regulatory mechanisms, nuclear receptors, etc), eventually including extensive phenotyping to take into account individual genetic variation (personalized nutrition). This path has led to what we now call 'nutritional systems biology', integrating all relevant molecular processes leading to the whole body response to diet. Focusing on *Nutrigenomics of Foods* with nutritional systems biology approaches also allows to take into proper consideration the complexity of food matrices when analyzing the complexity of body responses to nutritional stimuli. The conference sessions summarize this path while tackling the role of food in nutrigenomic research: from old and new dietary profiles associated with disease prevention (MedDiet/Nordic Diet) to the molecular basis of the diet-health relationship (systems biology of foods), including the crucial roles of gut microbiota and genetic variation; from omics technologies to the growing need for datasharing through common research infrastructures, with a final look at the state of the art on personalization of diet and health.

NuGOweek 2014 is especially targeted to our younger students and post-docs, the next generation of nutrigenomics scientists, whom we have involved in organizing this conference from the very beginning. Some of them took the lead in designing and chairing a session with PhD students/post-docs as invited speakers, focusing on the applications of omics technologies to nutrigenomics. They have also put forward novel ideas for a fully digital poster session accessible from any mobile device and computer, which we are glad to experiment in parallel with the 'old fashioned' paper posters. This multimedia session is based on Pathvisio (<http://www.pathvisio.org>), an open source software for pathway visualization created by the BiGCat Bioinformatics group at Maastricht University, and allows poster visualization in pdf format with topics-based clustering. A link to the 'poster pathways' is highlighted in the NuGOweek 2014 webpage.

A few closing words on the conference site chosen for this NuGOweek in the Sorrento peninsula, one of the most beautiful and romantic spots of the Mediterranean region, overlooking the Vesuvius and the Gulf of Naples with its worldwide famous islands and ancient roman history. The Vesuvian Institute where we are holding our conference is an ideal site to explore the surrounding ruins of our ancient culture. Only a few train stops away from the archeological site of Pompei, it often hosts archeology students and professors from all over the world, who come here to experience the excitement of restoring ancient roman villas (*stabiae*). We hope that most of you will be able to stay an extra day or two to enjoy the magic history that permeates the air we breathe in Castellammare, and to appreciate the wide variety of foods which inspired the initial studies on the health promoting effects of the Mediterranean Diet which were conducted in this area by Ancel Keys in the '50s.

We hope you will enjoy Italy, Castellammare and the beauties of the Sorrento peninsula!

*Giuditta Perozzi*

on behalf of the Organizing and Scientific Committees

We are grateful for the financial support by our sponsors, and in particular to the Italian Ministry of Agriculture, Food and Forestry (MiPAAF) and the NUME project.

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**Latest evidences of the protective effects of Mediterranean diet on cardiovascular disease**

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The Mediterranean diet (MeDiet) is one food pattern reputed for its beneficial health. This diet is characterized by the abundant use of olive oil; high consumption of plant foods (fruits, vegetables, legumes, cereals, nuts and seeds); frequent but moderate intake of wine (especially red wine) with meals; moderate consumption of fish, seafood, fermented dairy products (yogurt and cheese), poultry and eggs; and low consumption of red and processed meat and sweets. Several epidemiological studies have pointed out that high adherence to MeDiet is associated with strong protection against cardiovascular disease (CVD). However, the highest level of scientific evidence only is obtained by the performance of randomized clinical trial that evaluate hard end-points as main outcome. The PREDIMED (PREvención con DIeta MEDiterránea) study assessed the long-term effects of the MeDiet on incident CVD in individuals at high risk. Participants were randomized into three diet groups: MeDiet supplemented with extra-virgin olive oil (EVOO), MeDiet supplemented with nuts, and control diet (advice on a low-fat diet). After 4.8 years, 288 major CVD events occurred in 7,447 participants. MeDiet+EVOO and MeDiet+nuts groups showed a 30% reduction in the incidence of CVD compared to the control group. Incident diabetes (273 cases) among 3,541 non-diabetic participants diminished by 40% in the MeDiet + EVOO compared to the control group. After 1 year follow-up, participants in the MeDiet+nuts group showed a significant 13.7% reduction in prevalence of metabolic syndrome, compared to reductions of 6.7% and 2.0% in the MeDiet+EVOO and control groups, respectively. Despite the total energy intake was higher in both MeDiet groups, compared to the low-fat diet group, body weight and waist perimeter decreased in the three groups, but especially in the MeDiet+EVOO group. Analyses of intermediate markers of cardiovascular risk demonstrated beneficial effects of the MeDiets on blood pressure, lipid profiles, lipoprotein particles, inflammation, oxidative stress, and carotid atherosclerosis. The PREDIMED results demonstrate that a high-unsaturated fat and antioxidant-rich dietary pattern such as the MeDiet is a useful tool in the prevention of cardiovascular disease. However, these protective effects of the traditional MeDiet may be even greater if we upgrade the health effects of this dietary pattern, changing the common olive oil used for extra-virgin olive oil, increasing the consumption of nuts, fatty fish and whole grain cereals, reducing sodium intake, and maintaining a moderate consumption of wine with meals.

**The 'modern' Mediterranean diet: a critical appraisal**

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The typical Mediterranean dietary pattern, described by the Seven Countries Study investigators in the sixties, was made in Southern Italy of a dish of beans with macaroni, much bread, lots of fresh vegetables, small portions of meat or fish twice a week, use of olive oil as almost unique condiment, daily use of red wine and fresh fruits for dessert. That model was associated with lower rates of heart attacks, paralleled by lower levels of serum cholesterol in Mediterranean countries. Later on it has become clear that, perhaps surprisingly, similar macro- and micro-nutrient distribution can be found in the typical diet of several counties in the Middle East Asia, Japan and North Africa. Indeed, an extensive downgrading of this model has taken place in the last few decades due to massive unfavourable changes progressively occurred in the dietary habits of most Mediterranean countries. Moreover, an in-depth evaluation of the original model has to recognize some inherent critical aspects and potential downsides, such as: the high grain intake from white rather than whole grain flour, the high caloric intake favouring overweight and obesity, the uncontrolled salt consumption, the often too high wine and total alcohol intake. The exposure to highly prevalent overweight and excess salt intake may be the reason for the high incidence of stroke relative to coronary heart disease in Italy compared to other Western countries. These considerations suggest the need for a re-modelling of this dietary pattern that accounts for both its inherent weaknesses and the unfavourable changes occurred with time, focusing in particular on more moderate use of fats (Including extra-virgin olive oil), preference to vegetable rather than animal protein, higher intake of omega-3 fatty acids through increased consumption of fish and nuts, reduced sodium (salt) and wine intake, higher intake of potassium, calcium and magnesium through increased consumption of fruits and vegetables.

**Effects of a healthy Nordic diet on mRNA gene expression in PBMCs after an OGTT (The SYSDIET study)**

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The metabolic syndrome (MetS) is a clustering of risk factors, increasing the risk of type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVDs). Obesity, insulin resistance (IR) and T2DM are associated with chronic inflammatory response and inflammation plays a pivotal role in atherosclerosis. Diet has a great impact on the risk of MetS, T2DM and CVD. Characteristics of a healthy Nordic diet are reduced SFA, enhanced PUFA and fibre intake as well as increased fruit, vegetable and berry consumption. Recently, a healthy Nordic diet has been shown to decrease ambulatory blood pressure, lipid profile and low-grade inflammation among subjects with MetS (the SYSDIET study). The aim of this sub-study of SYSDIET was to examine if genes involved in inflammation and lipid metabolism in peripheral blood mononuclear cells (PBMCs) were regulated by a 2hOGTT, and if a healthy Nordic diet could modify this response. A Nordic multi-centre randomized dietary study included subjects (n=213) with MetS, randomized to a healthy Nordic diet (HND) group or a control diet (CD) group applying an isocaloric study protocol. In this sub-study we included subjects (n=89) from three Nordic centres: Kuopio (n=26), Lund (n=30) and Oulu (n=33) with a maximum weight change of  $\pm 4$  kg, hs-CRP concentration  $\leq 10$  mg/l, and baseline BMI  $< 39$  kg/m<sup>2</sup>. PBMCs were isolated and the mRNA gene expression was performed using TaqMan Array Micro Fluidic Cards (Applied Biosystems) for RT-qPCR amplification. Therefore, 44 genes were analysed before and after a 2hOGTT at baseline and at the end of the intervention (18/24 weeks). Gene expression of inflammatory and lipid-metabolism related genes were regulated by 2hOGTT. Whereas fasting PBMC gene expression of TLR4, PDGFB, LDLR and PPAR $\delta$  was not different between the HND and CD group, TLR4, PDGFB, CD36, LDLR, and PPAR $\delta$  were significantly different expressed after the 2hOGTT when the HND and the CD were compared after the intervention, considering multiple comparisons using FDR ( $q \leq 0.02$ ). This study shows that PBMC gene expression of TLR4, PDGFB, LDLR and PPAR $\delta$  was significantly different after a 2hOGTT between the HND and CD group at the end of the intervention. Our results may suggest 2hOGTT response as a new sensitive tool to study effects of diet on gene expression in intervention studies.

**Genetic susceptibility to dyslipidemia and incidence of CVD by a diet quality index**

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Overall diet quality has previously been shown to associate with lower risk of cardiovascular disease (CVD) incidence and mortality in prospective studies, including the Malmö Diet and Cancer Study (MDCS). Here we examine the association between genetic susceptibility to dyslipidemia and incidence of CVD [including coronary (CHD) and ischemic stroke (IS) events] and if these associations differ depending on diet quality. We included 24,799 participants (62% females, age 44-74 years) from MDCS without history of CVD and diabetes at baseline. During a mean follow-up time of 15 years, 3,068, 1,732 and 1,336 incident CVD, CHD and IS cases were identified, respectively. Genetic risk scores (GRSs) were constructed by combining 80 validated genetic variants associated with higher triglycerides (TG) and LDL-C or lower HDL-C. Additionally the participants' dietary intake, assessed by a modified diet history method, was ranked according to a diet quality index that included six dietary components: saturated fatty acids, polyunsaturated fatty acids, fish and shellfish, dietary fibre, fruit and vegetables, and sucrose. Total index score was categorized as low (0-1, n=3,890), medium (2-4, n=17,724) or high (5-6, n=3,185). The risk of CVD, CHD and IS were assessed by Cox proportional hazard regression adjusting for age, sex and several lifestyle factors. The  $GRS_{LDL-C}$  (HR per SD=1.09, 95% CI:1.05-1.13,  $P=5 \times 10^{-6}$ ) and  $GRS_{HDL-C}$  (1.05 [1.01-1.08],  $P=0.02$ ) but not  $GRS_{TG}$  (1.03 [1.00-1.07],  $P=0.08$ ) were observed to associate with increased risk of CVD when adjusting for age and sex. No significant interaction between the GRSs and diet quality was observed on incidence of CVD ( $GRS_{LDL-C}$   $P=0.39$ ,  $GRS_{HDL-C}$   $P=0.85$  and  $GRS_{TG}$   $P=0.86$ ). When CHD and IS events were examined separately, we observed a significant interaction between  $GRS_{LDL-C}$  and diet quality index on IS incidence ( $P=0.01$ ). A high diet quality attenuated the association between  $GRS_{LDL-C}$  and risk of incident IS compared to a lower diet quality. We found some evidence of interaction between diet quality and  $GRS_{LDL-C}$  on IS but not for any of the GRSs on CVD or CHD risk. In addition to examine the impact of diet quality, further studies are needed to examine whether any of the individual diet quality factors, or other specific dietary factors may modify the associations between genetic susceptibility to dyslipidemia and incidence of CVD, CHD or IS.

**Mediterranean diet and breast cancer risk: the role of polymorphisms in the MnSOD and CAT genes**

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Oxidative stress could arise due to a cellular imbalance between the levels of both exogenous and endogenous oxidants and antioxidants, in combination with an altered activity of several enzymes involved in catalysing reactive oxygen species. Oxidative stress has an adverse effect on important cellular biomolecules and contributes to the development of several diseases including cancer. In contrast, the Mediterranean diet, rich in exogenous antioxidants and beneficial marine n-3 fatty acids, was found to lower the risk of breast cancer in Greek-Cypriot women. Single nucleotide polymorphism (SNPs) within genes encoding endogenous antioxidant enzymes, alter enzyme activity and might modify the association between dietary antioxidants and breast cancer risk. We aimed to investigate whether SNPs p.Val16Ala in manganese superoxide dismutase (MnSOD, rs4880) and c.-330C>T in catalase (CAT, rs1001179) genes, could modify the effect of a dietary pattern specific, to the Greek-Cypriot population, on breast cancer risk. This pattern was derived using Principal Component Analysis (PCA) and included high loadings of vegetables, fruits, legumes and fish, thus closely resembling the Mediterranean diet. The effect of intake levels of vegetables, fruits, legumes and fish as individual dietary variables was also examined. Genotyping was performed on subjects from the MASTOS study, a case-control study of breast cancer in Cyprus (1,109 cases and 1,177 controls), using Taqman assays. Women with a high adherence to the PCA-derived dietary pattern and the low-risk CAT CC (P-trend<0.0001) and CT (P-trend=0.012) variants had a significantly lower breast cancer risk, compared to women with a low adherence to the PCA-derived dietary pattern. Reduced breast cancer risk was also observed in the MnSOD Val/Val (P-trend=0.029) and Val/Ala (P-trend=0.004) carriers as well as in the CAT CC (P-trend=0.010) and CT (P-trend=0.005) carriers who had higher vegetables intake compared to the corresponding individuals with lower intake. Furthermore, the CAT CT genotype in combination with a high consumption of legumes was associated with a reduced disease risk (P-trend=0.021). The low-risk alleles for both CAT (CC, P=0.006) and MnSOD (Val/Val, P=0.029) when combined with high intake levels of fish were found to be protective against breast cancer risk. Finally, no statistically significant breast cancer-diet associations were observed for the carriers of high-risk alleles of both SNPs. Our study suggests that the antioxidative protecting effect of high adherence to a Mediterranean dietary pattern, on breast cancer status is enhanced by the effect of the MnSOD and CAT SNPs. These SNPs may act as effect modifiers on the association between Mediterranean diet and breast cancer risk.

**Healthy Nordic diet down-regulates the expression of inflammatory genes in adipose tissue**

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Healthy Nordic diet (ND) has a beneficial influence on the plasma lipid profile and systemic inflammation when compared with a Control diet (CD). Objective was to explore if ND has an impact on gene expression in abdominal subcutaneous adipose tissue (SAT). Obese adults underwent an 18-24 week randomized intervention study comparing ND with CD (the SYSDIET study). The subset of participants from Nordic SYSDIET study was included (n=56) with a maximum weight change of  $\pm 4$  kg, highly sensitive C-reactive protein concentration less than 10 mg/l at the beginning and the end of the intervention, and baseline BMI < 38 kg/m<sup>2</sup>. SAT biopsies were obtained before and after the intervention and subjected to global transcriptome analysis using Gene 1.1 ST Arrays (Affymetrix®). Altogether 128 genes were differentially expressed in SAT between ND and CD (nominal P < 0.01, false discovery rate 24%). The genes were over-represented in pathways related to immune response (adjusted P = 0.0076). Immune related pathways included leukocyte trafficking and macrophage recruitment (e.g. IRF1, CD97), adaptive immune response (IL32, IL6R), and reactive oxygen species (NCF1). Interestingly, the regulatory region of the 128 dietary associated genes was over-represented for binding sites for the transcription factor NF- $\kappa$ B. ND reduces inflammatory gene expression in SAT when compared with CD independent of body weight change. The change could contribute to beneficial influences of ND on systemic inflammation and consequently on the development of cardiovascular disease and type 2 diabetes.



**A healthy Nordic diet modulates gene expression related to T-cell immune function in PBMCs (SYSDIET)**

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Recent studies have demonstrated that a healthy dietary pattern is associated with a lower risk of metabolic syndrome attributed to beneficial influence on the plasma lipid profile and systemic inflammation. In order to increase the knowledge of the overall effect of a healthy diet on inflammation at a molecular level the transcriptome profile in peripheral blood mononuclear cells (PBMCs) of subjects with metabolic syndrome was analysed in a Nordic multi-centre randomized controlled dietary intervention study (SYSDIET) investigating the effect of a healthy Nordic diet (ND). Subjects (n=213) with metabolic syndrome were randomized to a ND group or a control diet (CD) group applying an isocaloric study protocol for 18/24 weeks. The main food items in the ND group were whole-grain products, berries, fruits and vegetables, rapeseed oil, three fish meals per week, low-fat dairy products, and avoidance of sugar-sweetened beverages. In this sub-study participants from three Nordic centers were included: Kuopio (n=20), Lund (n=18) and Oulu (n=18) with a maximum weight change of  $\pm 4$  kg, highly sensitive C-reactive protein concentration less than 10 mg/l at the beginning and the end of the intervention, and baseline BMI < 39 kg/m<sup>2</sup>. PBMCs were obtained before and after the intervention and total RNA were subjected to global transcriptome analysis using Gene 1.1 ST Arrays (Affymetrix®). Over 7,000 probe-sets were significantly down-regulated after intake of ND compared to CD. Functional annotation analyses showed that the most strongly dietary regulated gene transcripts (FDR q-value < 0.05, Fold change > 18%) were overrepresented in processes, pathways and networks related to immune response, and more specific to T cell-mediated immune function, such as T cell activation and T cell receptor signalling. A decreased mRNA level of TNF and IFNG in particular implies a reduced Th1 cell response. Furthermore genes with binding sites for the transcription factors TBX21 and NF- $\kappa$ B in their regulatory regions were down-regulated by ND. In conclusion, a ND significantly decreased the mRNA expression of a broad set of genes in subjects with metabolic syndrome, altogether indicating an anti-inflammatory response at molecular level linked to T-cell mediated immune function.

**Study on chronic intake of high and low-flavonoid F&Vs. LC-HR-MS/MS based metabolomics of urine**

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Nowadays, nutrition focuses on improving health of individuals through diet. Current nutritional research aims at health promotion, disease prevention, and performance improvement. Human intervention trials have provided evidence for protective effects of various (poly)phenol-rich foods against chronic disease, including cardiovascular disease, neurodegeneration, osteoporosis and cancer. However, overall impact of polyphenols on human metabolome is not fully known. It is clear, that dietary biomarkers require much further research in order to be better applied and interpreted. A single-blind, dose-dependent, parallel randomized controlled dietary intervention study was designed to measure the dose-response relation between high-flavonoid (HF), low-flavonoid (LF), and habitual F&V intakes and vascular health together with metabolomics profiling and cardiovascular disease (CVD) risk indicators. We aimed with a comprehensive metabolomic analysis of urine to examine thousands of compounds in search of nutritional biomarkers with their complete structural identification. Therefore we applied LC-HR-MS method using Orbitrap LTQ with its unique combination of linear ion trap and FT technologies, which enables rapid, sensitive and reliable detection of small molecules. Metabolomics data together with microvascular reactivity and arterial stiffness measurements support recommendations to increase F&V intake to >6 portions daily, with specific additional benefit from F&Vs that are rich in flavonoids.

**Addressing food security through the nutritional enhancement of foods**

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Addressing Food Security Through Nutritional Enhancement of Food Understanding the complex relationship between diet and health has become key to developing preventive strategies to reduce the rising incidence of chronic disease, globally. Understanding the relationship between diet and health requires multidisciplinary approaches that integrate the expertise of plant biotechnologists, geneticists, organic chemists and food technologists with researchers in the fields of experimental medicine and clinical epidemiology. Plants have long been viewed as potential green factories for nutritional biofortification of crops. I will describe some approaches to engineering metabolic pathways to enhance the production of phytonutrients in crops. Consumption of these new foods demonstrated that the biofortification achieved was sufficient to confer nutritional benefits against chronic diseases. Nutritionally-improved foods can be taken directly from preclinical trials with animal models, through human intervention studies to the market, to improve the well-being and quality of life in both developed and developing countries.

**Disintegration of food in the gastrointestinal tract: what do we know and where are the gaps?**

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Digestion provides nutrients and energy essential to the survival and growth of the organisms. It also leads to the release of components (bioactive peptides, fatty acids, minerals...) that can have a positive or negative effect on human health. So far, the digestive tract has very often considered as a black box with a poor understanding of the mechanisms of food breakdown and more research is needed on this topic if we want to better understand the effect of food on human health. One of the key step of the gastrointestinal digestion is the gastric phase. When the bolus enters the stomach, acid and digestive enzyme secretions will initiate hydrolysis of the macronutrients. Gastric emptying will be modulated by the caloric content of the meal and its osmolarity. Recent evidences have shown that the structure the food will adopt in gastric conditions is also an essential factor and more research is needed to determine how this structure is formed, how the digestive enzymes will diffuse, get access and hydrolyse their targets... We also need to get a better understanding on to what extent stomach contractions affect the breakdown of food matrices of different viscosities and structures. After gastric digestion, food constituents will be transferred into the small intestine where most of the dietary proteins and lipids will be digested thanks to highly efficient digestive enzymes (trypsin, chymotrypsin, pancreatic lipase) and bile salts and then further absorbed. More research is needed in order to determine the exact protective role played by the mucus layer, the structure needed for a nutrient to be absorbed, the interactions that occur between nutrients in the lumen, the half-life of food constituents when they will enter the bloodstream... Finally, undigested dietary components will reach the large intestine. We still don't know precisely the relative amount of food proteins and lipids that will reach the colon, how it will be handled and affect the intestinal microbiota and what will be the consequences for human health. Although the whole digestive process has been studied for years, gaps in knowledge on the precise mechanisms of food disintegration in the gastrointestinal tract are still numerous and deserve a better attention if we want to clarify the impact of food on human health.

**Foodomics: the link between food and nutrition**

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Nutrition science mostly relies on the mean values of macro and micro nutrients contained in the food products, as also reported on their relative labels. Nutritional tables are therefore a widely accepted tool for the assessment of food quality and its potential health pros and cons. Although tables are still an important vehicle of nutritional information, it has been demonstrated that the reported content of food compounds, especially for what minor components concerns, undergoes a great variance. Modern nutrition, aiming at establishing links between food molecules and their activity in the body, needs a complete molecular profile, as well as the magnitude of variance of the content of all relevant compounds, as the consequence of the effect of several factors affecting food quality, such as genotypes, ripening stage, kind of production system, storage time and conditions, etc. For this reason modern nutrition is being increasingly supported by omic sciences, comprising the high-throughput foodomics. Foodomics has been defined as a new comprehensive and holistic approach which applies the so-called omic techniques in food science in order to improve human nutrition. One of its branches, food metabolomics, includes all the approaches aiming to provide a quick and complete description of the entire set of metabolites constituting the food system. In effect, this methodology can allow, in a snapshot, the visualisation of the whole metabolic profile characterizing a determined food. This approach is particularly useful when food quality needs to be assessed without the biases introduced by the conventional targeted analysis. Being able to profile a food product, identifying its true complete content is, in effect, a key tool for modern nutrition, since it allows to account even for slight metabolites oscillations in the content of similar products. Results from several researches based on foodomics will be presented, describing the variance of the metabolic profiles of food products as depending on farming practices, like organic or biodynamic productions, different geographical origins and rearing conditions, processing technologies, and so on. The approaches so far considered could be usefully applied to catch even unexpected changes occurring when food technologists develop protocols to improve food quality or shelf-life. The assessment of these alterations in food composition is usually quite difficult to obtain with common analytical approaches, whilst more and more studies are nowadays employing metabolomics to describe, with a comprehensive picture, the overall changes occurring in the food, up to provide a sort of definition for the food matrix, as it evolves during food production and storage. This is particularly useful for the so-called novel or enriched foods, containing specific micronutrients with beneficial effects on human health: this enrichment needs to be proved not to affect the expected nutritional value of the hosting matrix. It is thus evident how this modern scientific approach can be useful to evaluate the food quality in a holistic manner, bringing to a complete and trustworthy nutritional information on food products and correctly defining the nutritional values of food.

**Dietary patterns: how to capture them and what do they mean?**

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In recent years the use of dietary patterns as a tool in nutritional epidemiology has emerged. This approach results in the identification of patterns of food intake and the resultant use of these dietary patterns to uncover diet-disease interactions has increased. In this regard dietary patterns have previously been used to uncover complex relationships that are more likely related to a combination of dietary factors rather than a single dietary factor. Classical approaches used to derive patterns can be based on a priori criteria or through a posteriori statistical method. The a priori approach uses for example dietary index scores defining diet quality based on previously dietary guidelines whereas the a posteriori is a quantitative approach where dietary patterns are derived through statistical analysis. An example of the statistical approach is cluster analysis and through this method subjects are characterised into dietary patterns. Other statistical approaches used to derive dietary patterns include Principal Component Analysis. More recently, the concept that nutrigenomics could be used to identify dietary patterns or classify subjects into dietary patterns has emerged. In this respect metabolomics analysis of biofluids to classify subjects into dietary patterns offers great potential. Our previous work has demonstrated that dietary patterns are reflected in urine and plasma metabolomics profiles. The challenge now is the use of such profiles to classify subjects into certain dietary patterns and to decipher if such an approach can be used to study diet-health interactions.

**Single nutrients/bioactives vs whole food: the epigenetics perspective**

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Within the past decade, epigenetic mechanisms and their modulation by food and dietary constituents have gained major interest in the nutrigenomics community. The term ‘epigenetics’ refers to modifications in gene expression caused by heritable, but potentially reversible, changes in DNA methylation and chromatin structure. Major epigenetic mechanisms include DNA hyper- and hypomethylation, histone acetylation and methylation, and non-coding (micro) RNAs. Data – mainly derived from *in vitro* analyses – have accumulated that single bioactive compounds or plant extracts affect epigenetic mechanisms. Examples include reversal of gene silencing through promoter methylation by the green tea polyphenol (-)-epigallocatechin gallate (EGCG), or inhibition of histone deacetylases (HDACs) by a metabolite of sulforaphane (SFN), a broccoli-derived isothiocyanate. Modulation of miRNA expression has been shown with curcumin, a constituent of curry. Stoner and colleagues investigated in colon cancer patients potential epigenetic effects of a dietary intervention with freeze-dried black raspberries rich in ellagic acid and anthocyanidins. They discovered reexpression of silenced genes of the Wnt signalling pathway through reduction of promoter methylation. These data were recapitulated in rodent models for ulcerative colitis. Comparative *in vitro* analyses revealed that anthocyanidins might be the active principle through downregulation of DNA methyltransferases (DNMTs). Extensive comparisons of single compounds vs whole food affecting the epigenome have not been performed yet. However, several recent reports indicate that combinations of bioactives targeting different epigenetic mechanisms might be more effective than each single compound alone, for example by combining green tea extracts with HDAC inhibitors. Implications for whole food consumption will be discussed.

**Identification of nutritional health biomarkers for metabolic flexibility**

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One recent focus of nutritional research is the identification of biomarkers reflecting the impact of food on human health. These biomarkers should be influenced by the diet already in healthy individuals and be nonetheless indicative, or even predictive, of the potential impact of specific foods on the development of metabolic diseases such as obesity. In order to identify such biomarkers, a crossover study was conducted including seven normal weight and seven obese subjects who consumed three caloric doses (500, 1000, 1,500 kcal) of a high-fat meal. The blood cell transcriptome was measured before as well as 2, 4 and 6 h after meal ingestion by using microarrays. Forty-five probe sets were differentially expressed between normal weight and obese subjects under fasting condition. Remarkably, the postprandial expression of this set of probes in the normal weight subjects having ingested the high-fat meal changed in the same direction (up- or down-regulated) as differentially measured under fasting conditions between the obese and normal weight subjects. Obese subjects, however, were no longer able to alter the expression of these probe sets postprandially. Furthermore, the obese subjects were significantly impaired in their ability to metabolically cope with increasing caloric doses of the high-fat meal. Our study design allowed the identification of potential nutritional health biomarkers which can be used to characterize the interaction between food and the human and, ultimately, to identify healthy diets for specific consumer groups.



**Dietary protein intake affects amino acid and acylcarnitine metabolism in infants aged 6 months**

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Early breastfeeding reduces the risk of later obesity compared to formula feeding. One possible cause is the lower protein content of human milk. We studied the metabolic response of a different formula protein supply in infancy. Serum samples were drawn from children of the Childhood Obesity Project (CHOP), a European multicenter study. Plasma amino acid and acylcarnitine concentrations were determined in 6-months old infants who were randomized to receive a higher (HP, n=286) or lower protein (LP, n=291) content formula. Breastfed infants (BF, n=187) were enrolled as a reference group. Twenty-nine metabolites differed significantly between the formula groups. Children with higher protein intakes showed increased plasma concentrations of branched-chain amino acids (BCAA) and their related oxidation products, short-chain acylcarnitines, compared to those with lower protein intakes and those who were breastfed (all adjusted  $P < 3.9 \times 10^{-4}$ ). This indicates alterations in the BCAA metabolism. Segmented regression revealed that with increasing BCAA, the ratio between acylcarnitines and BCAA decreased suggesting that excess BCAA escape their degradation. This alteration of the BCAA metabolism is reflected in a non-linear relation between blood urea nitrogen and BCAA. Elevation of BCAA in the HP group contributes to increased insulin levels shown previously. Furthermore, high BCAA levels seem to act as key mediators from higher protein intake to later obesity by modulating the  $\beta$ -oxidation. We report decreased long-chain acylcarnitines in HP fed infants (adjusted  $P < 0.014$ ), which indicates a lower  $\beta$ -oxidation. Aromatic amino acids, known to promote IGF-1 secretion, were also elevated in HP-fed infants (all adjusted  $P < 7.7 \times 10^{-4}$ ), probably by competitive mechanism with BCAA. In summary, higher protein intake strongly affects BCAA levels. This could contribute to an enhanced fat storage, increased secretion of growth factors and insulin signalling.

**Bioinformatics approach to prioritize food ingredients with the aim to design new functional foods**

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A specific nutritional intervention, possibly in combination with specific supplements, can be very effective in individuals who are at risk of developing a chronic disease. These so called functional foods can be helpful in reducing health risk factors and may thereby contribute to prevention of disease. However, it is not always clear how to combine specific food ingredients to optimally target specific disease processes. We developed an integrative bioinformatics approach using multiple databases with the aim to identify complementary dietary components with potential health-beneficial effects. We focused on cerebrovascular accident (CVA) specifically that is known to develop chronically and is multifactorial in origin. First, we collected all documented risk factors and biomarkers related to the most important CVA-associated processes, such as hypertension, lipid metabolism, and obesity. From there, we identified SNPs and associated genes that are most relevant in these processes. Next, we generated a bioinformatics workflow, that includes information from an extensive food database to extensively map and mechanistically explain a Disease-SNP-Food interaction network. Prioritization of the components in this network together with their pathway-related components provides a top-list of food ingredients which potentially target specific disease processes and work complementary in the prevention of CVA. In the next phase the efficacy of ingredients from the prioritized list will be evaluated in a specific mouse models.

**Assessment of dietary patterns with metabolomics**

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Accurate assessment of dietary intake is necessary for understanding the relationship between nutrition and disease. Traditional methods of dietary assessment are prone to errors such as under-reporting. In recent years dietary biomarkers have been proposed as a means for unbiased and objective measures of dietary intake and metabolomics offers a means to identify novel biomarkers. Classification of subjects into dietary patterns has emerged as a useful tool in nutrition epidemiology. These patterns have been used to uncover complex disease processes that are more likely related to a combination of dietary factors rather than a single dietary factor. The objective of the research was to investigate the link between dietary intakes patterns and metabolic profiles. Urine samples and dietary intake data from the National Adult Nutrition survey (NANS) were used in the analysis (n=600). Dietary data was collected using 4-day weighed food diaries. Samples were analysed using <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy. A total of 567 subjects were included in the study, 287 males and 280 females. The mean age of the total group was 47±16 (years). The mean BMI was 30±14 (kg/m<sup>2</sup>). The subjects were clustered into 2 metabolic profile groups using k-means cluster analysis. Cluster 1 had a significantly higher mean BMI and age than cluster 2 (P=0.047 and P=2.01×10<sup>-22</sup> respectively). There was a significant relationship between gender and cluster group with females more likely to be represented in cluster 1 (P=0.005). Cluster 1 had a significantly lower mean (±SD) daily energy intake (1,963.47±607.68 kcal) compared to cluster 2 (2,110.79±624.28 kcal). Examination of the food group intake across the 2 clusters revealed that cluster 1 was characterised by significantly higher intakes of wholemeal, brown breads, breakfast cereals and porridge, low fats and skimmed milks, yoghurts, potatoes, fruit, fruit juices and smoothies, fish, fish products and fish dishes and cluster 2 had significantly higher intakes of savouries, cheese, chips and processed potatoes, savoury snacks, meat products, alcoholic beverages, confectionary and high energy beverages. Heatmap analysis was used to link metabolites and dietary intake data. Correlations showed that metabolites found at higher concentrations in cluster 1 are positively correlated with the food groups consumed at higher percentages in cluster 1 while being negatively correlated to the food groups associated with cluster 2. For example betaine, which showed positive correlations to fruit and potatoes and negative correlations to confectionary, chips, processed potatoes and high energy beverages. Metabolites found at higher concentrations in cluster 2 are also shown to be positively correlated to the food groups associated with cluster 2 and negatively correlated to the food groups associated with cluster 1. The use of metabolomics results in the identification of food groups that can be linked to metabolic profiles. The use and reliability of metabolic profiles in dietary pattern intake assessment will further be explored and is set to have a large impact in nutrition research.

**Endoplasmic reticulum stress contributes to tocotrienol induced apoptosis in HeLa cells**

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The term vitamin E is usually utilized by nutritionists to describe eight molecules, subdivided into two groups, tocopherols (TOCOs) and tocotrienols (T3s) further subdivided into four forms characterized by a different number of methyl groups in the chromanol ring and indicated as  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ - forms. A number of reports have shown that specific T3s are able to affect the growth of several lines of tumour cells by a mechanisms that is not shared by TOCOs. However, the molecular mechanism(s) involved in T3s activity is still unclear. In the recent past we proposed a novel mechanism for T3s activity that involves, at least in part, estrogen receptor beta (ER $\beta$ ) signalling. In fact, according to a set of studies *in silico*, followed by *in vitro* binding experiments coupled with cell culture we demonstrated that in breast cancer cell (MCF-7 and MDA-MB-231), T3s treatment increases ER $\beta$  translocation into the nucleus and significantly inhibits ER $\alpha$  expression and signalling. The transcriptomic data-set obtained within these studies, interrogated by bio-informatic tools, 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. This study conducted in HeLa cells, void of any canonical form of ERs, indicates that T3s activity is mainly ascribable to the induction of a cellular stress at the level of the endoplasmic reticulum (EndoR). Our study, starting from the interrogation of transcriptomic platforms, demonstrates that treatment with  $\gamma$ - and  $\delta$ -T3 is associated to specific Ca-dependent signals, to the expression and activation of IRE1- $\alpha$  and other molecules involved in the Unfolded Protein Response (UPR), the core pathway to cope with EndoR stress in eukaryotic cells, finally leading to apoptosis.

**Resting metabolic rate, substrate oxidation and biochemical indicators in obesity women**

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Obesity is a disease characterized by excessive accumulation of body fat, with a multifactorial origin, such as behavioural, genetic and environmental. Studies show that hundreds of genes having capacity to influence on body weight regulation and energy metabolism, which may depend about 40% on the genetic background of the individual. Thus, they become strong evidence of association between obesity and gene expression related to energy and lipid metabolism. This study aimed to compare resting energy expenditure, carbohydrate oxidation and lipid and biochemical indicators among women with grade III obesity and normal weight. This is a cross-sectional study with a sample of women that were seen in Hospital – FMRP / USP, classified into two groups, according to the Body Mass Index (BMI): G1=obese (BMI $\geq$ 30 kg/m<sup>2</sup>) and G2=normal weight (BMI between 18.5 kg/m<sup>2</sup> and 24.9 kg/m<sup>2</sup>). Anthropometric data (weight and height), resting metabolic rate (RMR) and biochemical parameters (glucose and lipid) were collected. To review the RMR, we used indirect calorimetry for 30 minutes by phone QUARK RMR – COSMED, Rome, Italy and the Weir equation:  $(XVO_2 \cdot 3.941) + (1.106 \times VCO_2) \times 1440$ . To calculate substrate oxidation were used this formula: glucose oxidation (g/min) =  $4.55 VCO_2 - 3.21 VO_2 - 2.6 N_2$  and lipid oxidation (g/min) =  $1.67 VO_2 - 1.67 VCO_2 - 1.92 N_2$ . T test for independent samples was used to comparisons between groups (P<0.05). Were evaluated 28 women, 10 normal weight and 18 obese. Individuals with obesity had higher BMI (49.3 $\pm$ 11.6 vs 21.8 $\pm$ 1.7 kg/m<sup>2</sup>, P<0.001), resting metabolic rate (2,217.6 $\pm$ 371.9 vs 1,333.5 kcal $\pm$ 157.6 kcal, P<0.001), lipid oxidation (232.1 $\pm$ 74.3 vs 94.7 $\pm$ 40.3 g/ml, P<0.001), although lower carbohydrate oxidation (-35.2 $\pm$ 94.3 vs 111 $\pm$ 77.7 g/ml, P<0.001) when compared with normal weight. Biochemical indicators, individuals with obesity had higher triglyceride concentrations (115 $\pm$ 42.2 vs 49 $\pm$ 1.4 mg/dl, P<0.001) and lower HDL-cholesterol (35.9 $\pm$ 5.7 vs 51.5 $\pm$ 3.5 mg/dl, P<0.002) than those with normal weight. No differences between concentrations of glucose, total cholesterol and LDL-cholesterol were evidenced (P>0.05). Our results show that individuals with obesity have a greater resting metabolic rate, lipid oxidation and triglyceride concentrations. The next step we will evaluate expression of genes associated with obesity and adipogenesis in individuals with obesity and correlate to different expression patterns of resting metabolic rate and biochemical profile.

**Effects of *Anethum graveolens* and *Brassica oleracea* on cholesterol homeostasis in hepatic cells**

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Dill (*Anethum graveolens* L.) and kale (*Brassica oleracea* L., ssp. *acephala* Dc) are commonly consumed vegetables considered anti-hypercholesterolemic in folk medicine. This study was designed to elucidate the mechanisms underlying the cholesterol-lowering effect of selected vegetable extracts at the molecular level, using cultured human hepatocytes HepG2 as a model system. The vegetable extracts were supplemented to culture media at concentrations of 100 µg/ml medium for 24 h. Gene expression analysis of sterol regulatory element binding protein (SREBP)-1 and -2, 3-hydroxy-3-methyl coenzyme A reductase (HMG-CoAR), and LDL receptor (LDLR) was performed by quantitative real-time PCR. The expression of corresponding proteins was carried out by western blot analysis. Dill and kale extract supplementation caused significant increases in SREBP-1, but not SREBP-2, mRNA levels. Notwithstanding the increased SREBP-1 transcription, no modification in the level of the encoded protein (both uncleaved and cleaved form) was observed. HMG-CoAR and LDLR gene expression are upregulated when the nuclear, cleaved form of SREBP protein is expressed at superphysiologic levels; in our experiments no modification of nuclear SREBP levels were observed upon supplementation, and accordingly HMG-CoAR and LDLR gene transcription was similar to controls in all supplemented cells. To our surprise, HMG-CoAR protein levels were increased by dill supplementation. This effect could appear in contrast to its reported cholesterol-lowering effect, but it must be considered that cholesterol levels can be regulated by different mechanisms in an *in vitro* system. The observed increased protein expression of LDLR in kale supplemented cells appeared related not to the synthesis but to the proteolytic end of the receptor modulation. We acknowledge that results in whole organisms may diverge from those in the cultured cells in part because of liver or other organ metabolism of ingested phytochemicals mixtures and agents, and therefore results in the cultured cells could be misleading if taken in isolation. Nonetheless, this study represents a first contribution for the evaluation of kale and dill extract mechanism of action, and could have potentially important implications for screening or evaluating cholesterol-lowering nutritional agents. This study was funded by FP7 EU Project BASEFOOD 'Sustainable exploitation of bioactive components from the Black Sea Area traditional foods' (grant agreement no. 227118). The authors thank Nadiya Boyko (Uzhhorod National University, Ukraine), and Bike Kocaoglu and Osman Hayran (Yeditepe University, Turkey) for providing kale and dill.

**Polyphenols from wild blueberry reduce lipid accumulation in THP-1 derived macrophages**

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Polyphenols, such as phenolic acids and anthocyanins (ACNs), are widely studied for their protective effects against oxidative stress and inflammation. Recently, *in vitro* and *in vivo* studies suggest that polyphenol compounds may modulate lipid metabolism by preventing atherogenic and atherosclerotic processes. The molecular pathways involved in the prevention of lipid accumulation are not well known; however, polyphenols seem to be able to modulate the transcriptional activities of different nuclear receptors that control lipid metabolism, including peroxisome proliferator-activated receptors, sterol regulatory element-binding proteins, liver X receptors. In the present study, we examined the effect of an ACN-rich fraction, obtained from a wild blueberry (WB) powder, on the capacity to counteract lipid accumulation in macrophages derived from monocytic THP-1 cells. The cells display characteristics similar to those in foam cells of atherosclerotic plaques. In addition, it was tested the capacity to affect lipid accumulation of malvidin-3-glucoside (Mv-3glc; the most representative compound of the ACN-rich fraction) and syringic acid (SyAc; metabolic product of Mv-3glc). Cells were incubated with a solution of free fatty acids (500  $\mu$ M oleic/palmitic acid) and different concentrations (from 0.05 to 10  $\mu$ g/ml) of ACN-rich fraction, Mv-3glc and SyAc. Lipid accumulation was measured in a fluorescence spectrophotometer by using the fluorescent dye Nile red and the number of fold increase respect to the control (without fatty acids, FA) was calculated. Results were analysed by ANOVA. Post-hoc analysis of differences between treatments was assessed by the Least Significant Difference (LSD) test with  $P \leq 0.05$  as level of statistical significance. Lipid accumulation was reduced at all concentrations of ACN-rich fraction tested with a maximum reduction at 10  $\mu$ g/ml (-27.4%;  $P < 0.0001$ ), while the maximum effect with the single compounds was observed at the concentration of 0.05  $\mu$ g/ml (-63% and -65%,  $P < 0.0001$ , respectively for Mv-3glc and SyAc). These preliminary results demonstrated a potential role of polyphenol compounds in the regulation of lipid accumulation as evidenced in THP-1 macrophages. Moreover, the effects were observed also at the low concentrations, supporting a possible contribution at doses comparable with those achievable *in vivo*. The molecular mechanisms involved in such modulation are going to be investigated.

**Vascular and nutrigenomic effects of grapefruit naringenin consumption in post-menopausal women**

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Recent epidemiological studies have reported that a high flavanone intake, provided by the consumption of citrus fruits and derived products, is associated to a reduced risk of coronary heart disease and stroke. In addition, a large number of animal studies support potential cardiovascular protective properties of flavanones. However clinical evidence accounting for a benefit in consuming dietary flavanones for cardiovascular health is less consistent due to the low number of intervention studies in humans. The present clinical study aimed at (1) characterizing the specific role of naringenin in the effect of a long-term consumption of grapefruit juice on vascular function in humans and (2) analysing changes in PBMCs transcriptome profile induced by grapefruit naringenin. 52 healthy post-menopausal women were enrolled in randomized, controlled, cross-over trial. For two periods of 6 months, subjects consumed 340 ml/d of grapefruit juice (212 mg naringenin-glycosides) or an iso-energetic control beverage mimicking the composition of the juice but without naringenin. The effects on endothelium-dependent vasoreactivity (assessed by FMD), arterial stiffness (assessed by PWV) and blood pressure as well as on conventional systemic biomarkers of CV risk factors were evaluated. Furthermore, a global gene expression profiles in peripheral blood mononuclear cells was performed using microarrays. The obtained results showed that grapefruit juice consumption lowered arterial stiffness in conduit arteries compared to the control drink; this effect was specifically related to the presence of naringenin in the juice. Thereby, modulation of arterial stiffness could be an interesting target by which grapefruit juice may exert beneficial effect on vascular health. The PBMCs nutrigenomic analyses showed that the intake of naringenin through grapefruit consumption regulated the expression of genes involved in inflammatory processes as well as in interaction between vascular endothelium and circulating immune cells. These molecular mechanisms may be potentially involved in the lowering effect of grapefruit naringenin on arterial stiffness.



**The glycemic effect of milk-derived bioactives on pancreatic  $\beta$ -cells**

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Recent literature has demonstrated an association between milk intake and protection against type 2 diabetes. Therefore, milk may be a rich source of food bioactives with potential benefits in the context of type 2 diabetes. The objective of the present study was to investigate the effect of milk-derived bioactives on insulin secretion from pancreatic  $\beta$ -cells and the potential mechanism driving the insulin secretion. In this study, BRIN-BD11 pancreatic  $\beta$ -cells and primary islets isolated from C57BL6 standard mice were treated with a bioactive milk sample at a concentration 1 mg/ml. The milk bioactives were derived from bovine colostrum whey fractions. Insulin secretion was carried out with milk-derived bioactives in an acute manner. Following exposure for 24 h, metabolic profiling and functional assays including intracellular calcium and plasma membrane potential were performed in BRIN-BD11 cells. Metabolomics analysis was performed using GC-MS. Statistical analysis was carried out using IBM SPSS Statistics 20 and GraphPad Prism 5. Acute insulin secretion from both BRIN-BD11 cells and primary islets were significantly increased in the presence of 1 mg/ml milk-derived bioactives by 3.01 fold and 8.61 fold, respectively, compared to positive controls. A positive dose response was also observed in both BRIN-BD11 cells and primary islets. No detrimental effect was observed in both cell types following 24 h treatment with the milk-derived bioactives. Intracellular calcium and plasma membrane potential of BRIN-BD11 cells treated with the milk-derived bioactives were significantly increased ( $P=0.002$  and  $0.049$ , respectively), compared to control conditions. The area under the curve (AUC) of intracellular calcium increased significantly from x to y following exposure to the milk-derived bioactives ( $P=0.023$ ). Metabolomics analysis revealed no significant change in amino acids, TCA cycle intermediates and fatty acids. The results illustrated that the milk-derived bioactives has no detrimental effect on pancreatic  $\beta$ -cells and a positive effect on acute insulin secretion from pancreatic  $\beta$ -cells. The milk-derived bioactives under study here promoted insulin secretion through enhanced plasma membrane potential and intracellular calcium levels. Future experiments will determine their effects *in vivo*. This study was funded by CSC-UCD Scholarship and Food for Health Ireland.

**Changes in anthocyanins profile of anthocyanin enriched bakeries**

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PATHWAY-27 will evaluate the effectiveness of docosahexaenoic acid (DHA), anthocyanins (AC) and beta-glucan (BG) alone or in combination with two other bioactives, chosen for known/claimed effectiveness in reducing some risk factors of Metabolic Syndrome (MS). Determination of changes in AC profile of AC enriched bakeries is one of the outputs of this project. Buns and biscuits were enriched with three different concentrations of AC. Extraction of AC from food matrix was optimised using four different solvent systems. The extraction efficiency was determined by measuring total anthocyanin content using pH-differential method. Sepabeads SP850 ion exchange resin was used for column chromatography. Separation of AC was performed by HPLC. The color of AC enriched buns was completely purple and some slightly green and brown spots were also detected in the crust. Anthocyanin enriched biscuits had an excellent taste and flavour and the texture was not too compact. Nevertheless, biscuits had brown colour in the outer layer and a purple and green spots inside as in the buns. Among the extraction solvents methanol containing 0.1% formic acid was the most efficient solvent. Amount of AC extracted with methanol containing 0.1% formic acid was 3.71, 1.62 and 1.45 times greater than those of extracted with water containing 0.1% formic acid, methanol containing 1% formic acid and ethanol containing 1% formic acid respectively. The recovery of AC from buns enriched with three different concentrations of AC was changed in between 4.95 to 27.71%. This value for biscuits enriched with three different concentrations of AC was in between 36.80 to 54.66%. When compared the HPLC chromatogram of AC source and buns, the main findings were the disappearance of the first peak of AC source in the chromatogram of buns and new peaks, possibly originated from wheat and other ingredients or occurred during fermentation, in buns. However, determination of the same anthocyanin profiles for biscuits and AC source indicated that food-processing conditions during biscuit production had no effect on the anthocyanin profile, but other peaks were also identified. The authors participate in the FP7 EU Project PATHWAY-27 'Pivotal Assessment of the Effects of Bioactives on the Health and Wellbeing, from Human Genome to Food Industry' (grant agreement no. 311876).

**Metabolomics for identifying novel markers of n-3PUFA intake in mice and humans**

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Omega-3 polyunsaturated fatty acids (n-3PUFA) are considered to be anti-inflammatory, hypolipemic and anti-diabetogenic via the production of series 3 eicosanoids derived from the COX and LOX pathways and the modulation of fatty acid oxidation. We used a comprehensive metabolite profiling in a mouse feeding study and in a human supplementation trial to find novel marker metabolites reflecting an increased dietary n-3PUFA intake. Six-week-old male C57BL/6J mice were fed a control diet for 2 weeks and at the age of 8 weeks were randomly assigned to 3 groups fed either the control diet with 13 kJ% fat, a high-fat diet with 48 kJ% fat or a 48 kJ% high-fat diet enriched with n-3PUFA (EPAX 1050-TG, Goerlich Pharma International, Germany). At the end of the experimental period, organs and plasma were analysed for changes in around 200 metabolites represented by lysophosphatidylcholines (LPC), diacyl phosphatidylcholines (PCaa), alkyl:acyl phosphatidylcholines (PCae), sphingomyelins (SM) via LC-MS/MS. The same metabolites were quantified in dry blood spots collected before, during and after a supplementation of daily 360 mg of EPA and 240 mg of DHA/day for 24 days in 15 human volunteers. Participants collected samples 3 days before, during the supplementation and for 18 days after the supplementation every second day. Mouse data revealed a distinct signature of metabolites in each organ (liver, 2 adipose tissue depots) and in plasma associated with the increased n-3 intake. However, some metabolites coherently changed in all compartments irrespective from the 'organ-specific signature'. In liver for example approximately 30% of analysed PC's changed significantly with several EPA or DHA-containing PC-species showing markedly increased levels. From the 38 PCae derivatives measured, more than 50% had increased levels in the n3-PUFA group leading to an increased total hepatic PCae content. Several of these PCae species increased similarly also in plasma. The total hepatic SM also changed with SM24:1 for example displaying a 3-fold increase. In human dry blood spots the composition of PCaa, PCae and SM species responded with similar changes as found in mouse plasma. Particularly, the concentrations of PCaa36:5, LPC20:5 and SM22:3 rapidly increased after start of supplementation, reached a plateau after 10 days with 2- to 3-fold higher levels and rapidly declined over the wash-out period. In summary, we identified in mice and humans a distinct and almost identical novel pattern of lipids that changed upon an increased intake of n3-PUFA. Why those metabolites – with PCaa36:5 in prominent position – are so responsive is currently not known.

**Assessment of the effects of bioactives on health and wellbeing: clinical protocol design**

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PATHWAY-27 will evaluate the effectiveness of docosahexaenoic acid (DHA) alone or in combination with two other bioactives, beta-glucan (BG) and anthocyanins (AC) in reducing some risk factors of Metabolic Syndrome MS. These compounds will be used as ingredients of bioactive-enriched foods (BEF), enriching 3 different widely-consumed food matrices (dairy-, bakery-, egg products) and not as pure compounds. This will allow a better understanding of possible synergisms and bioactive-matrix interactions. BEFs to be tested in clinical studies have been designed, selected and produced by different Pathway' partners. The aim of this multi-centre, randomized, double-blind, parallel pilot study is to identify the BEF achieving the greatest effect on lipid parameters (reduction in serum triglycerides or increase in HDL-C). The selected BEF will then be tested in a subsequent, larger interventional study. Three different matrices containing DHA, BG and AC given alone or of DHA associated with BG or AC will be tested. 300 men and women at risk for MS will be investigated (either one or two of the following criteria should be met: elevated waist circumference, elevated fasting triglycerides, reduced fasting HDL-C, elevated blood pressure or hypotensive treatment or elevated fasting glucose). MRI, ULE, CRNH will investigate BEFs based on either bakery, dairy or egg products, representing a different food matrix. Each pilot study will be conducted on 100 volunteers for a period of 4 weeks. Participants will be divided in 5 groups receiving BEF enriched with DHA, BG, or AC alone or DHA+BG, or DHA+AC. At baseline and after 4 weeks of intervention, fasting blood samples will be collected for further analysis. Additionally, blood pressure and anthropometric data will be determined. The 3 most effective BEF (one for each matrix) having the most significant impact on end-points selected for this study will be used in a larger randomized, double-blind, placebo-controlled study. The aim will be to understand the mechanisms underlying the effects observed on primary and secondary endpoints related to the consumption of BEF. Omics approaches will be used to examine metabolic changes and potentially identify new markers of effects.

**Effect of isocaloric high-protein diet in subjects with type 2 diabetes: animal versus plant protein**

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Previous studies observe favourable as well as adverse effects of high-protein diet in subjects with type 2 diabetes. However, there is not enough data about the origin of the protein and its influence on metabolism. In our study, we evaluated in details the effect of a high-protein diet in type 2 diabetic subjects and also compared proteins of animal and plant origin. Individuals with type 2 diabetes (HbA<sub>1c</sub> 6-11%) were randomised to either an isocaloric high-animal protein or an isocaloric high-plant protein diet (25-30% protein, 40% carbohydrates, 30-35% fat) for 6 weeks. The high-animal protein diet was rich in meat and dairy foods, while the high-plant protein group received products with high amount of legumes and pea protein. Laboratory analysis of routine markers and metabolic hormones were performed before and after the intervention. Further, an *ex vivo* stimulation of whole blood samples with 100 ng/ml Lipopolysaccharide (LPS) for 20 h was performed to investigate the effect of the diet on the cytokine secretion. The study was already completed by ten subjects (age 65±6 years, BMI 31.0±4.2 kg/m<sup>2</sup>, HbA<sub>1c</sub> 7.3±0.5%). Cholesterol as well as LDL and HDL levels were lower after the high-protein diet. Triglycerides were not different from baseline in either group. The HbA<sub>1c</sub> decreased after the intervention, however significant changes were found only in the plant protein group. Furthermore, a decrease of blood glucose and insulin level during meal tolerance test was observed again only in the plant protein group. On the other hand, NEFA in blood were diminished in the animal but not in the plant group. Notably, the *ex vivo* experiments showed an increase in the LPS-induced TNF $\alpha$  secretion in all participants. Moreover, the IFN $\gamma$  secretion was higher, but only in the plant protein group. Our preliminary data suggest that a 6-week high-protein diet leads to a decrease of blood cholesterol, LDL and HDL levels in subjects with type 2 diabetes. Furthermore, a diet with high-protein amount of plant origin has a beneficial effect on glucose metabolism. However, a slight pro-inflammatory effect of the high-protein diet is observed.

**Application of food metabolomics for the development of standardized food matrices**

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Epidemiological evidence indicates that there is an inverse association between consumption of cruciferous vegetables (such as broccoli, Brussels sprout and cauliflower) and risk of cancer and cardiovascular disease. These vegetables contain numerous bioactive compounds (vitamins, minerals, glucosinolates and phenolic compounds) that have been considered responsible for their health-promoting properties. However, the interactions and the possible synergies between these molecules and the complexity of the food matrix make difficult to understand the real biological role of the single bioactive compound. Broccoli sprouts have a particularly high content of bioactive molecules, whose concentration in plants responds to changes of environmental growth conditions. Then, acting on growth condition, it is possible to affect the overall bioactive molecules network. In this work, we searched for the best growth conditions and inducers able to increase the content of different bioactive molecules (glucosinolates, phenolic compounds, flavonoids, anthocyanins, vitamins and  $\beta$ -carotene) in broccoli sprouts. Targeted food-metabolomic and multivariate analysis allowed us to identify 'light plus sucrose' as the best inducer of the bioactive network. Then, broccoli sprouts grown in the dark or in the 'light plus sucrose' were used to produce a juice. The targeted and untargeted metabolomics analysis revealed large, but reproducible, differences between the two juices. This standardized compositional diversity can be used as a tool to investigate the biological role of different bioactive molecules embedded in their food matrix, both in *in vitro* (cellular model) and in *in vivo* models (animal model and humans).

**Physiological levels of caffeic acid counteracts endothelial cell dysfunction caused by high glucose**

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Food bioactive molecules are signals that are detected by cellular sensor systems and play important role in the control of gene and protein expression. Bioavailability is one of the most important points to consider when assessing the biological role of nutritional molecules as their circulating concentration and molecular structure determines the specificity and the entity of the cell signalling pathways activated. A number of epidemiological studies suggest that a moderate and regular coffee consumption is associated with a substantial decrease of the risk of type 2 diabetes. Coffee represents a rich source of phenolic compounds, in particular chlorogenic acids that are derivatives of ferulic, p-coumaric and caffeic acids. Despite the high concentration of chlorogenic acids in coffee, the amount of free plasmatic caffeic acid does not exceed nmolar levels, even just after coffee consumption. In order to study the molecular effects of caffeic acid on vascular dysfunction induced by hyperglycemia, we set up an *in vitro* model using the endothelial cells Ea.hy926, maintained in 25 mM glucose mimicking hyperglycemic condition, in the presence of physiological concentration of caffeic acid (10 nM). Since one of the main functions of the endothelium is to act as selective barrier between plasma and the interstitial space, one of the most evident consequences of high glucose induced dysfunction is the loss of the selective permeability and the uncontrolled flow of molecules through the interstitial space is associated to a pro-inflammatory response and to cell death. Our observations indicate that physiological concentrations of caffeic acid counteracts the reduction of the trans-endothelial electrical resistance and the increase of FITC-dextran permeability associated with high glucose. Moreover, caffeic acid protects cells from high glucose-dependent apoptosis. The activation of the transcription factor NFκB has been shown to be associated with the activation of a proapoptotic pathway in endothelial cells incubated with high glucose; In order to gain a specific insight on this process we assessed the expression of about 90 genes specifically involved in NFκB activation, among which MAPK signalling, PKC-dependent complex and the apoptotic pathway. Our data reveal that caffeic acid significantly prevents most of the gene expression changes induced by high glucose. These results are in agreement with the reduced nuclear translocation and activation of NFκB. Furthermore, the antiapoptotic effects have been confirmed by the decrease of the activation of the caspase 3, 7, 8 and 9 and by the increase of Bcl2 activation. Noteworthy, in spite of the very low concentration considered in our experimental design, closely replicating normally achievable physiological levels, our data show that caffeic acid counteracts endothelial cell dysfunction associated to high glucose and indicate the reduction of NFκB activation as a key step in the mechanisms underlying this activity.



**Unexpected metabolic effects of agrimony tea**

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*Agrimonia eupatoria* L. (agrimony) is an herb widely used by the traditional medicine for various treatments – liver and gall bladder diseases, mild diarrhoea, pulmonary and gastrointestinal inflammatory diseases, and even in diabetes or obesity. Traditional medicine reports that the herb possesses anti-inflammatory properties. The mechanism of these effects is unclear and often attributed to the antioxidant properties of the contained polyphenols and flavonoids. This pilot study aims to assess the metabolic effects of *A. eupatoria* tea (AET) consumption on lipid and glucose metabolism and explore possible relation to its anti-inflammatory potential in a case-control study involving normal (BMI<25) and overweight subjects (BMI≥25). The intervention included 40 healthy volunteers, aged between 20 and 60 years. 23 of them were normal weight (NW) and 17 – overweight subjects (OW). They consumed AET (2.5 g dried plant material in 200 ml boiling water) once a day for a period of 25 days. Blood samples were collected before (day 0) and at the end (day 25) of the intervention. Fasting glucose, total cholesterol, HDL-Cholesterol (HDL-C), LDL-Cholesterol (LDL-C) and triacylglycerol (TAG) levels were measured using a biochemical analyser and commercial kits. The levels of adipokines leptin and adiponectin and inflammatory markers, such as C-reactive protein (CRP) and interleukin-6 (IL-6) were determined with ELISA kits. Tea consumption had a positive impact on carbohydrate metabolism measured by a significant decrease in fasting blood glucose levels with 4.13% in NW group and 18.16% in OW group ( $P<0.001$ ). Interestingly, the intervention seemed to affect lipid metabolism in an unexpected manner. Increased triglyceride (TAG) levels by 12.25% ( $P<0.05$ ) in NW and by 5.3% in the OW group were established as a result of AET consumption. LDL-C decreased in both groups, but the changes were not statistically significant. Total cholesterol levels and HDL/LDL ratio remained unchanged. Furthermore HDL-C levels decreased by 4.7% ( $P<0.01$ ) for NW and 2.35% ( $P<0.05$ ) for OW. Surprisingly, as a result of the intervention adiponectin levels were found to decrease by 17% in NW ( $P<0.001$ ) and by 20% in OW group ( $P=0.058$ ), while leptin levels increased by 25% in NW ( $P<0.05$ ) and 17% in OW group. The changes regarding pro-inflammatory markers were statistically non-significant – IL-6 decreased, while CRP increased in both NW and OW groups. In support to results of previous studies based on rat models, we established that Agrimony has a (strong) hypoglycemic activity and therefore has possible applications in preventive medicine. Elevated TAG could be due to the insulin-like effect on blood glucose levels in both normal and overweight individuals. The non-consistent and reverse changes in pro-inflammatory cytokine IL-6 and in CRP may be a result of high rate lipid metabolism. This is a first report about seemingly unfavourable changes in the lipid profile after consumption of very high polyphenols and flavonoids containing Agrimony tea.



**Heart rate variability in women with grade III obesity**

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Reduced heart rate variability (HRV) is present in obesity indicating predisposition to chronic degenerative diseases such as cardiovascular involvement. There have been few studies evaluating the role of genetic and biochemistry associated with HRV in individuals with grade III obesity. In this study, we evaluated the linear and nonlinear indices of the HRV in women with grade III obesity. We studied 55 individuals, which were divided into two groups: 23 women with grade III obesity and 32 eutrophics. For analysis of HRV indices, HRV was recorded beat by beat with all individuals in supine position for 30 minutes. We analysed following linear indices: SDNN (Standard deviation of all normal RR intervals recorded in a time interval, expressed in ms); pNN50 (Represents the percentage of adjacent RR intervals with a difference of duration greater than 50 ms.), RMSSD (is the root-mean square of differences between adjacent normal RR intervals in a time interval, expressed in ms); LFms2 (Low Frequency component, ranging between 0.04 and 0.15 Hz, which is due to the joint action of the vagal and sympathetic components on the heart, with a predominance of the sympathetic ones), and HFms2 (High Frequency component, ranging from 0.15 to 0.4 Hz, which corresponds to the respiratory modulation and is an indicator of the performance of the vagus nerve on the heart), as well as the ratio between LF and HF components (LF/HF). In relation to nonlinear indices we evaluated SD1, SD2, SD1/SD2, approximate entropy (-ApEn),  $\alpha_1$  and  $\alpha_2$ . The statistical analysis was obtained by Kolmogorov-Smirnov and independent t test (SPSS 17.0). Our study shows obese patients with reduced values of SDNN ( $43.5 \pm 18.2$  vs  $58.7 \pm 19.2$  ms;  $P=0.005$ ), RMSSD ( $37 \pm 22.7$  vs  $50.4 \pm 22$  ms;  $P=0.031$ ), HFms2 ( $586 \pm 768.6$  vs  $1049.1 \pm 774.7$  ms<sup>2</sup>;  $P=0.033$ ), LFms2 ( $448 \pm 382.3$  vs  $1,053.6 \pm 855.3$  ms<sup>2</sup>;  $P=0.001$ ) SD1 ( $26.1 \pm 16$  vs  $36.5 \pm 16.8$  ms;  $P=0.026$ ) and SD2 ( $55.3 \pm 22.3$  vs  $74.4 \pm 24$  ms;  $P=0.003$ ) compared with eutrophics. Our results shows changes in linear and nonlinear domains of HRV in obese women, indicating increased risk of cardiovascular complications. The next step we will associate these results with whole transcriptome to identify molecular pathways involved in chronic degenerative diseases.

**Regulation of eNOS and endothelin-1 by grape seed polyphenols: role of sirtuins**

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Antihypertensive effects of grape seed polyphenols have been reported. This beneficial effect has been attributed to an enhancement of the availability of vasodilatory factor nitric oxide (NO). In the present study, mRNA levels of different genes implicated in endothelial dysfunction (ED), characteristic of many pathologies such as hypertension (HPT), have been studied in hypertensive cafeteria (CAF) fed rats. Twenty-four rats fed CAF diet for 18 weeks were acutely intragastrically administered water or 375 mg/kg of a grape seed extract rich in low molecular weight polyphenols (LM-GSPE) and sacrificed 6 hours post-administration. Blood pressure was recorded before administration and just before sacrifice. Aorta gene expression was performed for endothelial NO synthase (eNOS), arginase 1, Krüppel-like factor 2 (KLF2), sirtuin 1 (Sirt1), ET-1, angiotensin II receptor type 1 a and b (ART1a, ART1b) and NADPH oxygenase 4 (NOX-4). Antihypertensive effect was verified for CAF fed rats treated with LM-GSPE. Gene eNOS and Sirt-1 mRNA expression were found upregulated in LM-GSPE-administered rats, indicating that grape seed polyphenols increase NO availability via eNOS mRNA expression which could take place through Sirt-1 induction. In addition, expression of vasoconstrictor ET-1 was downregulated in these animals. No effects were found in arginase 1, KLF-2, NOX-4 or angiotensin II receptors expression. The molecular mechanism implicated in the antihypertensive effect of grape seed polyphenols would be mediated by an increment of eNOS and a decrease in ET-1 expression. The cardioprotective effect on eNOS and ET-1 expression suggests that grape seed polyphenols are excellent functional endothelial regulators, which could be useful as nutritional supplements or in pharmaceutical formulations.

**Are green tea extracts enough to change gene expression related to resting energy expenditure?**

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Anti-obesogenic effects of green tea (*Camellia sinensis*) polyphenols can be explained by impact of the increase in thermogenesis, decrease fat absorption and change in appetite. It known that green tea supplementation increased 4% of expenditure energy in 24 hour, these results confirms the effect as thermogenic changing the resting energy expenditure. Green tea is characterized chemically by presence of numerous polyphenolic compounds known as catechins. Recently, it has demonstrated that epigallocatechin-3-gallate (EGCG) is the most abundant catechin and is considered the most bioactive component, representing 50-80% of catechins. Other catechins found are: epicatechin-3-gallate (ECG); epigallocatechin (EGC); and epicatechin (EC). *In vivo* studies demonstrated the effect of these catechins in gene expression related to lipid metabolism and glucose production enzymes. Thus, it becomes stronger evidence that green tea is beneficial for the prevention of obesity and hypercholesterolemia by inhibiting the hepatic expression of lipogenic enzymes in association with decrease gene expression the transcription factor playing a central role in the gene expression of lipogenic enzymes. The objective of the present study was to determine amount of catechins present in available commercial products and their possible uses in clinical practice. Three commercial green tea extracts were analysed, and for each product were evaluated 10 capsules. The following chemical markers ECGC, CA (caffeine) and ECG were quantified by liquid chromatography high efficiency using the Shimadzu Prominence chromatograph, UV detection Photodiode Array mod. SPD-M10A, reverse phase column Shimadzu C-18 (250×4.6 mm ID, 5 mM). For product 1, the average content in each capsule was EGCG=10.14±0.14 mg; CA=4.27±0.06 mg and ECG=2.81±0.04 mg. The product 2 analysis showed EGCG 2=38.35±2.0 mg; CA=24.06±1.27 mg; ECG=11.16±0.59 mg and product 3 showed EGCG=31.81±1.22 mg, CA=15.15±0.58 mg, ECG=8.25±0.31 mg. The product 2 showed the highest content of more bioactive component (EGCG) so it was chosen for our study. The next step we will supplement obese women for 8 weeks to investigated gene expression of genes related to energy metabolism and obesity and correlate with resting energy expenditure.

**Zn depleted intestinal cells susceptible to inflammatory challenges: protection by food bioactives**

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Differentiated human intestinal Caco-2 cells respond to an inflammatory stimulus induced by TNF $\alpha$  by activating a zinc-dependent survival pathway involving modulation of transcription and translation of Inhibitor of Apoptosis Proteins (IAPs), and NF $\kappa$ B activation. Thus, Caco-2 cells subjected to mild zinc depletion followed by TNF $\alpha$  treatment represent a good model of intestinal inflammation that can be utilized to investigate the molecular mechanisms underlying protective effects of different food components. Epidemiological studies indicate a positive correlation between high fruit and vegetable consumption and reduced risk of several chronic degenerative diseases, and different classes of bioactives are considered responsible for these outcomes. In the present work we have studied the effects of *Brassica oleracea* var. *botrytis* sprouts juices in the intestinal inflammatory model, since *Brassica* vegetables are rich in secondary bioactive metabolites with anti-inflammatory properties. To this aim, Caco-2 cells were pre-incubated with broccoli sprout juices and then challenged with the inflammatory stimulus under conditions of zinc depletion. Juices obtained from sprouts grown under two different controlled environmental conditions were compared, namely L-juices, derived from growth of the sprouts in light + sucrose, and D-juices, grown in the dark. Results indicated that both juices protected zinc deprived intestinal cells from inflammatory injury, as shown by reduced damage to epithelial monolayer integrity. The L-juice, however, exerted a significantly higher protection than the D-juice and allowed complete recovery from the inflammatory insult that was normally irreversible. We could exclude that such effect might be due to the presence of free zinc in the juice. Metabolomic analysis showed that L-juices contained higher levels of bioactive metabolites, in particular anthocyanins and sulforaphane, which is the prevalent isothiocyanate, as compared to D-juices. Morphological analysis and caspase-3 activity assays demonstrated that protection by L-juices was mediated by reduced apoptosis and increased expression of cIAP2 at the protein level. Further studies will address the mechanisms of action underlying the protective effects of broccoli sprouts on inflammatory stress, using transcriptomic approaches to identify the signalling pathways and the molecular targets involved. Moreover, comparison of cell transcriptomic and juice metabolomic data will allow to identify the specific classes of bioactives responsible for the observed effects. This work was supported by Italian Ministry of Agriculture, Food & Forestry (MiPAAF) grant 'NUTRIGEA' (DM 30281 23/12/2009).

**Glucagon-IGF-1 bioactivity interaction: a link between high protein diet and cancer development**

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Accumulating evidence suggests the important role of high protein diet, characterized by increased glucagon secretion, in cancer development. Otherwise, IGF-1 plays a critical role through PI3K/Akt signalling as an apoptosis inhibitor and proliferation stimulator, two physiological processes implicated in tumour growth. Our goal was to identify the interaction between glucagon and IGF-1 bioactivity taking in consideration changes in insulin as an important confounder. In our study, we investigated GH-IGF-1 system responses to intramuscular glucagon administration in 13 patients with T1DM (6 males, 7 females; body mass index [BMI]  $24.8 \pm 0.95 \text{ kg/m}^2$ ), 11 obese participants (OP; 5 males, 6 females; BMI  $34.4 \pm 1.7 \text{ kg/m}^2$ ), and 13 healthy lean participants (LP; 6 males, 7 females; BMI  $21.7 \pm 0.6 \text{ kg/m}^2$ ). The interaction between glucagon and GH-IGF-1 system on the transcriptional level was studied using mouse primary hepatocytes stimulated with glucagon with or without growth hormone. In comparison with baseline levels, glucagon administration decreased significantly IGF-1 bioactivity in all tested groups ( $P < 0.01$ ). Despite serum total IGF-1 and IGFBP-3 concentrations remained unchanged during glucagon injection, both of IGFBP-1 and IGFBP-2 were up-regulated ( $P < 0.01$ ) in all study groups. *In vitro*, surprisingly, glucagon increases IGF-1 and decreases IGFBP-3 expression significantly but did not affect the transcription of both IGFBP-1 and IGFBP-2. These results indicate that glucagon decreases IGF-1 bioactivity in humans independently of endogenous insulin levels. This glucagon-mediated reduction in bioactive IGF-1 may be related to higher levels of IGFBP-1 and IGFBP-2 but not to changes in total IGF-1 or IGFBP-3. Furthermore, we speculate that glucagon-mediated reduced bioactive IGF1 may be responsible for the positive effect of high protein diet, characterized by increased blood glucagon levels, on tumorigenesis.

**Acute ingestion of Indian cress increases PYY secretion and affects cytokine production**

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Glucotropaeolin (benzyl glucosinolate) is the major glucosinolate in Indian cress (*nasturtium*; *Tropaeolum majus* L.). Its highly reactive breakdown product, benzyl isothiocyanate (BITC), demonstrated antibacterial, antitumorigenic, and anti-inflammatory properties *in vitro* and *in rodent* studies. However, there is little information on the distribution, metabolism and bioavailability in human individuals. The aim of this study was to investigate acute effects of benzyl glucosinolate on the hormone and cytokine secretion in humans. 15 healthy volunteers were randomly recruited for the study. All participants avoided foods containing glucosinolates for the entire experimental period. After overnight fasting, participants consumed 10 g freeze-dried Indian cress dissolved in water (intervention day) or only water (control day). Blood and urine samples were taken every hour, and several BITC metabolites such as BITC-gluthatione (BITC-GSH), BITC-cysteinylglycine (BITC-CysGly), BITC-cysteine (BITC-Cys), BITC-N-acetyl-L-Cysteine (BITC-NAC) and BITC-lysine (BITC-Lys) were analyzed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Laboratory analysis of routine markers and metabolic hormones were performed before and after the intervention. For the *ex vivo* experiments, whole blood samples taken 4 h after the consumption of Indian cress were stimulated with 100 ng/ml Lipopolysaccharide (LPS) for 24 h, and cytokine secretion was measured using Luminex technology. Results: Blood levels of BITC metabolites (BITC-GSH, BITC-CysGly, BITC-Cys, BITC-NAC, BITC-Lys) in plasma and urine peaked 3-5 h after Indian cress consumption. Acute ingestion of Indian cress had no effects on biochemical parameters including cholesterol, triglyceroles, liver enzymes, C-reactive protein, as well as on plasma insulin and GIP levels. However, an anorectic hormone PYY produced mainly by enteroendocrine l-cells in the distal gastrointestinal tract was increased 2-5 h after the consumption of Indian cress. Interestingly, an incretin hormone GLP-1 synthesized by the same cells was also increased 1 h after consumption. The LPS-induced production of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) were significantly reduced 24 h after intervention day compared to control whereas the anti-inflammatory cytokine interleukin-10 (IL-10) was not affected. The present data demonstrated that acute ingestion of Indian cress leads to large increase of BITC metabolites, reduction of pro-inflammatory cytokine production and increases plasma PYY secretion in humans. This supports the anti-inflammatory properties observed in *in vitro* experiments and might explain the glucosinolate-induced reduction of food intake found in some animal studies. Further studies are needed to determine mechanisms of benzyl glucosinolate effects in humans.

**Dwarf elder fruit infusion suppresses LPS induced MCP-1 and ICAM-1 gene expression**

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Scientific researches focused on herbal medicine as a possible way to treat inflammation related disorders become more popular recent years. Dwarf elder (*Sambucus ebulus* L., SE) is an anti-inflammatory herb used in ethno medicine of many European and Middle Asian countries for treatment of gastrointestinal bacterial infections, wound healing and immunostimulation. Fruits are rich in polyphenols including anthocyanins with strong antioxidant properties which may contribute to their anti-inflammatory potential. Therefore with the aim to explore the anti-inflammatory properties of the SE fruits the effect of SE fruit aqueous infusion (FAI) on lipopolysaccharides (LPS) induced, monocyte chemotactic protein 1 (MCP-1) and intercellular adhesion molecule 1 (ICAM-1) gene expression was studied in J774A.1 macrophages. Macrophages were treated with increasing concentrations of SE FAI or salicylic acid (SA) (positive control) +/- LPS (*Escherichia coli*, 026:B6). SE FAI used for treatment was prepared from ripe fruits. To evaluate the effect of SE FAI and SA on cell viability 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid (MTT) reduction assay was performed. Real time quantitative polymerase chain reaction (RT-qPCR) was used to measure MCP-1 and ICAM-1 gene transcription levels. Relative gene transcription levels were calculated using  $2^{-\Delta\Delta Ct}$  method. SE FAI and SA in applied concentrations were not toxic for cell viability. Low concentrations of SE FAI in cells non-treated with LPS induced slightly the gene expression of MCP-1 ( $P < 0.01$ ). Pretreatment with increasing concentrations of SE FAI reduced significantly LPS induced transcription levels of MCP-1 approximately by 63.69% ( $P < 0.01$ ), and ICAM-1 by 92.9% ( $P < 0.05$ ) similarly as SA (96%,  $P < 0.05$ ), compared with LPS treated control group. Expression of both genes was suppressed in a dose dependent manner. This study proves the traditional use of SE fruit as a remedy in cases of inflammation-related disorders. The suppression of MCP-1 and ICAM-1 gene expression may be pointed as a part of anti-inflammatory mechanism of action for SE FAI. Recent study added new data about a natural product with a potential in treatment of inflammation related disorders such as various infections, wounds, atherogenesis and other cardiovascular diseases.



**HepG2 cells as a model to study how bioactive compounds modulate pathologies of metabolic syndrome**

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Metabolic Syndrome (MS) refers to the clustering of several risk factors (cardiovascular and metabolic) including dyslipidaemia, hyperglycaemia and increased blood pressure, where abdominal obesity and insulin resistance represent core parameters. There is a growing interest in the design of natural strategies to prevent these pathologies based on the use of dietary bioactive compounds with beneficial activities. This emphasises the necessity to know the mechanisms of action of the molecules to have useful cellular models for their evaluation. The aim of this study is to develop a set of assays on HepG2 cells to determine the effect of bioactives on lipid metabolism and insulin resistance on hepatocytes. Three model bioactive compounds with healthy benefits were selected: docosahexaenoic acid (DHA), beta-glucan (BG) and anthocyanins (AC). To consider the bioavailability and the cellular metabolism of the three bioactives, we supplemented to HepG2 cells their metabolites and therefore DHA, propionate (PRO) from BG colonic fermentation and protocatechuic acid (PA) from the metabolisms of AC. Cells were supplemented with DHA alone or in combination with PRO or PA to elucidate their function and their interaction. A screening of the physiological non-cytotoxic concentration of bioactives, alone or in combination, was done to set up conditions. To evaluate the effect of supplementations on lipid metabolism, the lipid accumulation was measured. All supplementations caused a modification in the fatty acid profile, and a significant decrease in the fatty acid content was observed in cells supplemented with PRO and PA compared to control cells. The positive effect of PRO and PA was clearly evidenced also in the decrease of lipid accumulation. The potential modulation of insulin resistance was assessed by the evaluation of glycogen storage. The preliminary results have shown a tendency of DHA to restore the levels of glycogen, although further analysis should be done. Results herein reported show how bioactives are able to modulate lipid accumulation and glycogen storage in HepG2, two key end-points in pathologies of MS. Deeper studies will be developed to determine the molecular mechanism of these effects. This research demonstrates HepG2 cells as a good model for the evaluation of bioactives with preventing effect on MS. Acknowledgements. The authors participate in the FP7 EU Project PATHWAY-27 'Pivotal Assessment of the Effects of Bioactives on the Health and Wellbeing, from Human Genome to Food Industry' (grant agreement no. 311876).



**Bakery matrix effect in the DHA bioaccessibility**

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Knowledge of the bioavailability of the compounds is a very important factor in evaluating the nutritional of an enriched matrix with these compounds. Additionally to determine the total content of them in the food, it is also necessary to identify the fraction available and/or absorbed and utilized by the body. The bioactivity depends on the digestion processes, absorption of nutrients and their availability for the metabolic functions. For this reason, for a better characterisation of the bioactive compounds it is recommendable to evaluate their bioavailability and their absorption. The bioavailability is the result of three steps: (1) digestibility and solubility of bioactive compound in the gastrointestinal tract (bioaccessibility); (2) hydrolysis of nutrients to obtain low m.w. molecules; (3) the absorption and transport to the circulation, and d) the incorporation to their functional target. *In vitro* screening methods have been developed for the determination of nutrient bioaccessibility and bioavailability from foods, providing useful information. Bioavailability, depends on digestion, nutrient release from the food matrix, absorption by intestinal cells, and transport to body cells. On the other hand, Bioaccessibility is dependent on release from the food matrix, and hydrolysis of high m.w. molecules through the digestion process, while bioavailability is the amount of bioaccessible nutrients that are absorbed by intestinal cells, and transported to body cells. Docosahexaenoic acid (DHA) is considered beneficial for human health. The present study focuses on DHA bioaccessibility, and aims to evidence the impact of o the bakery matrix (buns and biscuits) on it. For this purpose, the bioaccessibility of DHA during gastrointestinal passage was carried out by following guidelines developed by COST Action FA 1005. A standardised static *in vitro* gastro-intestinal digestion has been performed, which has included an enzyme treatment in three stages: a first stage with amylase, a second stage with pepsine at pH2 (gastric digestion) and a third stage with pancreatic and intestinal enzymes at neutral pH (intestinal digestion). After digestion, DHA concentration was evaluated in the whole soluble fraction of the digestate, and in the <3 Kda fraction, containing low m.w. molecules supposed to be available for absorption. Additionally the stability of the DHA incorporated in bakery products has been evaluated, by determining the DHA concentration in three different points along the shelf life of both products. Our results evidence that DHA is stable during shelf-life of buns and biscuits, and although the different bakery matrices don't affect the DHA bioaccessibility, the concentration of the bioactive molecule in the digested samples is much lower than in the not digested food. This confirms the need of the use of the *in vitro* system gastrointestinal digestion as a rapid and cost-effective model, in order to evaluate the effective role of foods as carrier of bioactive compounds.

**Needs and difficulties of the food industry/SMEs in establishing and submitting health claims**

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The authors participate in the FP7 EU Project PATHWAY-27 'Pivotal Assessment of the Effects of Bioactives on the Health and Wellbeing, from Human Genome to Food Industry' (grant agreement no. 311876) The process of 'creating' a health claim show there are many strict requirements and rules, and that companies in the food industry must follow a complicated and complex procedure to meet the requirements established by the National and EU authorities (i.e. EFSA) for health claim substantiation. Based on the PATHWAY-27 project general strategy, publication and implementation of guidance documents will be prepared that will inform and assist the food industry sector, especially SMEs, to produce bioactive-enriched foods with supportive health claims according to the EU legislation. For the development of new guidance documents, first of all the expectations, requirements and needs of the stakeholders need to be analysed and evaluated. A questionnaire was developed within the scope of the project. The general aim of the questionnaire survey was to collect and identify the needs and difficulties of the industry/SMEs in establishing and submitting health and nutrition claims for food products enriched with health-promoting bioactives. It also elicited feedback from SMEs regarding clear indicators for measuring innovation and competitiveness. 125 valid questionnaires were collected from 17 countries. As a conclusion of the survey, the use of health and nutrition claims has strong contribution to the success of the companies, but product development with health and nutrition claims is very difficult task for them. It was found out that the main difficulties are not the extraction, intake and stabilization of the active substance, but the following issues: Scientific barriers: Lack of markers, to be used, accepted by EFSA; difficulties in establishing the relationship between the food/bioactive substance and the claimed effect; difficulties in setting up the experimental design and carrying out human intervention studies; lack of existing human intervention studies set-up related to the presence of bioactive substance. Technical/technological barriers: Lack of guidelines/supporting documents; lack of specialized human resources; difficulties in knowledge on conducting randomized controlled trials and statistics; lack of communication with the national and/or EU authorities; lack of specific technology for carrying out the necessary tests; difficulties in food characterization; lack of tools; lack of applicable technology for carrying out the necessary tests; difficulties in communication within the company. Economic barriers: Cost of conducting human intervention studies if no relevant data are available; return of investment not guaranteed, length of the process of authorization, costs of preparing the health claim dossier; rising input costs; lack of internal resources. Therefore, the conclusions of the survey gave countenance to the fact that new guidelines and best practice on preparation of scientifically correct standard dossiers are necessary to obtain a positive evaluation by EFSA.

**Intestinal microbiota in inflammatory disorders: the immune-gut axis**

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The increasing incidence of chronic disorders is considered to be the consequence of environmental and individual risk factors. Inflammatory processes are key mechanisms in the etiopathology of immune-mediated pathologies including multiple sclerosis, Type 1 diabetes, allergies, colon cancer and inflammatory bowel diseases (IBD). A major focus of research into disease mechanisms underlying chronic inflammation is the gut microbial ecosystem and its reciprocal interaction with the intestinal immune system. High-throughput sequence analysis identified changes in community structure and function of the intestinal microbiota associated with various pathologies supporting the idea that microbe-host interactions are key in disease initiation and/or progression. The 'Old Friends' hypothesis provides a further challenging concept for the explanation of increases in chronic inflammatory diseases suggesting the lack of adequate microbial exposure and subsequent defects in immune Regulation in modern industrialized societies. Genome-wide association studies identified 163 susceptibility loci in IBD with substantial overlap between other immune disorders or infections providing clear evidence for a central role of intestinal bacteria in the pathogenesis of chronic inflammatory disorders. Despite the fact that a variety of susceptibility genes suggest a role for microbial triggers in the pathogenesis of Crohn's disease as one of the two major IBD phenotypes mostly affecting the small intestine, diet emerged as an important disease modifier in relevant models. In this context, composition and activity of the intestinal microbiota is largely affected by diet and, both intermediates might play an interrelated role in orchestrating disease risk and activity. Functional evidence for the specific interplay of disease-relevant bacteria (so called 'pathobionts') with dietary factors substantiates the hypothesis that the tripartite interaction of diet, intestinal microbiota and host susceptibility shape chronic inflammatory pathologies in the gut and peripheral organs.

**The role of diet in the immuno-metabolic plasticity of the gut: a systems nutrition approach**

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The intestine is the major organ for the uptake of nutrients and other food components. The absorption of nutrients from the lumen is generally highly efficient. However due to more recent dramatic changes in our lifestyles and food intake behaviour and the related health problems we became increasingly interested in the role of the intestine as an essential gatekeeper between the host, foods and the microbiota. We have in particular been focussing on the regulation of intestinal genes and functional properties upon adaptive responses to nutritional challenges in order to better understand the mechanisms how the intestine adapts its capacity to resorb nutrients from foods. One of the most important challenges that has received significant attention is dietary fat and in particular in its recently recognized interaction with the gut microbiota. We performed several comprehensive whole genome transcriptome analysis studies to characterize the differential regulation of intestinal gene regulation at different locations of the small intestine. Briefly we found that saturated fat (but not unsaturated fat), stimulating obesity and fatty liver disease, affects gut microbiota composition (reduced microbial diversity and increased *Firmicutes*-to-*Bacteroidetes* ratio) by an enhanced overflow of dietary fat to the distal intestine. Unsaturated fats are more effectively taken up by the proximal and middle part of the small intestine, likely by more efficiently activating nutrient sensing transcription factor systems and their target genes. This will contribute to the prevention the development of early pathologies (e.g. NASH). I will furthermore summarize in my talk the recent developments about how different dietary components modulate gene transcription in the intestine. These insights into diet-induced gene regulation and changes in chromatin activity allow us a better understanding of how bioactive components are involved in the regulation of the metabolic plasticity and resilience capacity of our organs, a role vital for our health.

**NutriGenoMilk: from bacterial genomics to human metabolism**

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The first interaction of the offspring with bacteria takes place during birth as it interacts with the mother's vaginal microflora. Then, the diet plays a paramount role in establishing and maintaining the gut microbiota, which modulates metabolic and immune processes in humans. Fermented products compose about one third of the foods ingested by humans and, as such, are likely to significantly influence health by acting on the composition and activity of the gut microbiota. The processing of food along the chain 'fermentation by technological microorganisms' → 'digestion by the GIT and the gut microbiota' → 'intestinal transport and metabolic activation by enterocytes and metabolic organs' offers a complex sequence of biochemical modifications of the food matrix, which are mediated by both prokaryotic and eukaryotic cells. These modifications can be investigated with an analytical strategy that combines foodomics, bacterial genomics and nutrigenomics. This strategy promises to accelerate the translation of molecular knowledge available on fermented foods into biological information that is relevant to human health. Among the fermentable foods, milk is a natural and technologically relevant vector that delivers bacteria, as well as products of fermentation, to the human organism. Milk also shares a significant fraction of its metabolome with other biofluids, in particular blood serum, what makes it a matrix of choice to identify biomarkers of food. Finally, milk possesses immunomodulatory properties, in addition to nutritional properties, which may be at play while identifying biomarkers of efficacy in intervention trials. We have, therefore, undertaken a research program aimed at linking the genome of selected strains of lactic acid bacteria to the metabolome of the corresponding fermented dairy products and, ultimately, to the phenotype (blood cell transcriptome, serum metabolome, faecal microbiome) of subjects having ingested these products. The current status of this research program is presented and illustrated with *in vivo* and *in vitro* data spanning the food processing chain described above.

**Epigenetic regulation of inflammatory molecules mediated by changes in SCFA producing GI microbiota**

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Genetic and lifestyle factors as well as diet contribute to metabolic syndrome. Studies in both, mice and humans have identified alterations in the composition of the intestinal microbiota in response to high fat diet. Changes in the composition of gut microbiota are believed to contribute to an increased permeability of the intestinal wall and low grade inflammation. Changes in gut microbiota and diet are also known to induce alterations in the synthesis of short chain fatty acids such as butyrate by various bacterial groups. Butyrate synthesis was recently shown to affect inflammation regulated by Treg cells in the gut involving epigenetic mechanism. We therefore, compared changes in gut microbiota with epigenetic regulation of molecules involved in inflammatory responses of metabolic syndrome, especially free fatty acid receptor FFA3, Toll like receptors (TLRs), TNFA and IL-6. We studied obese and type 2 diabetes patients in a four month intervention period in comparison to a lean control group. Intervention involved Victoza for type 2 diabetics and nutritional counselling for both intervention groups. Diets were assessed by FFQ. Microbiota were analysed for abundance and diversity by PCR-DGGE, qPCR and 454- pyrosequencing. Epigenetic methylation of 3-7 CpG sites in promoter regions of TLR2, TLR4, FFAR3, TNF- $\alpha$ ; and Line1, as a marker for global methylation, were analysed using bisulfite conversion and pyrosequencing. In type 2 diabetes and obesity the diversity of band patterns was decreased compared to healthy controls. *Firmicutes/Bacteroidetes* ratio, abundance of lactic acid subgroups and Enterobacteria increased during the intervention period in type 2 diabetes. In contrast, the ratio of *Firmicutes/Bacteroidetes* was decreasing in obese patients with weight loss. Decreased bacterial diversity and abundance also correlated with abdominal discomfort in patients claiming food intolerances. We identified a significant decreased methylation in CpG promoter regions of TLR2, TLR4, FFAR3 and TNF $\alpha$  and IL-6 in obesity but also decreased CpG methylation in diabetics, (TLR2: obese: 2.96%, TLR4: obese: 4.30%, FFAR3: diabetes: 31.75%; obese: 32.51%). CpG methylation of Line 1, reflecting genome wide methylation, did not show significant changes between the groups. We also we found a significant correlation between an increased BMI/WHR and decreased methylation of TLR2, TLR4, FFAR3 and IL-6. Our results suggest that a different composition in microbiota in obesity and type 2 diabetes modulate the epigenetic regulation of inflammatory molecules, including CpG methylation in parallel to histone modification. Interactions between microbiota and epigenetic regulation may involve NF-kB signalling from TLRs as well as SCFA binding to SCFA-receptors. Modulation of SCFA synthesis by diets may be therefore a way to interfere with the epigenetic regulation in inflammatory responses. The significant correlations of anthropometric measurements with TLR2, TLR4, FFAR3 or could develop into useful markers.

**A regulatory role for probiotic yoghurt on metabolic health in healthy men: a pilot study**

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With a third of the human diet made up of fermented foods, health-promoting properties of fermented dairy products represent a major area of research with potential implications for public health policy and food industry development. Despite evidence from numerous studies that describe metabolic and immune-modulating properties of fermented dairy products, there remains controversy concerning the validity of these proposed properties, with the mechanisms underpinning their effects not yet well-established. The specific physiological effects of fermented dairy products directly depends on lactic acid bacteria activity; either through bioactive metabolites released during milk fermentation or later on through their activity within the gut and, in both cases, by directly influencing the hosts metabolic profile and by changing intestinal microflora balance. Our pilot study aimed to assess the effect of probiotic yogurt consumption on parameters of metabolic health. The dynamic response to the acute and chronic ingestion of a liquid yoghurt enriched with the widely used probiotic *Lactobacillus rhamnosus* GG (LGG) was evaluated using clinical indicators of metabolic health in parallel with different '-omics' approaches. In this double blinded cross-over study, fourteen healthy male volunteers were recruited and randomised to test a yoghurt containing the LGG and the control, an acidified non-fermented milk. Both products were tested after a single dose (800 ml) and after two weeks of daily consumption (400 ml/day). In the first single dose test, the post-prandial response to the product was evaluated over six hours. In the second test, the effect of daily consumption of each product on fasting analyses was evaluated as well as the postprandial response to a high fat meal known to induce a transient inflammatory response. A two week run-in phase preceded the beginning of the study with three week wash-out phases between testing each product. Dietary restrictions were in place throughout the study with three days controlled diet prior to every test day. Milk was provided during the run-in and wash-out phases (400 ml/day). To establish the mechanisms by which a probiotic yoghurt may induce physiological changes following ingestion, an extensive approach of evaluation has been chosen. Nutritional assessments in the postprandial phase of ingestion will primarily address the immediate impact of the food metabolome on the serum metabolome. Conversely, nutrodynamic assessments will consider the chronic effect of the product on parameters of metabolic health, intestinal microflora changes, response to inflammatory stress and fasting serum metabolome. Furthermore, the identification of specific biomarkers and metabolic pathways that are regulated by probiotic yoghurt intake may enable a more targeted selection of probiotic strains to increase the efficacy of probiotic yoghurt in improving the metabolic health.

**Fermented food microbiota: a metagenomic analysis to search bacterial genes related to host health**

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Lactic Acid Bacteria (LAB) represent the predominant microflora in fermented foods. Foodborne LAB have received increasing attention for their natural probiotic properties, but also as potential reservoir of antibiotic resistance (AR) determinants, which may be horizontally transferred to opportunistic pathogens within complex microbial communities such as the gut microflora. We have previously reported isolation of AR LAB from an Italian fermented cheese using a culture-dependent microbiological approach. Although all AR isolates were detected only in raw ingredients, a parallel, culture-independent, PCR approach on total DNA extracted from cheese revealed the presence of AR genes also in the final product. We therefore turned to a metagenomic approach, based on the construction of a fosmid library containing microbial genomic DNA extracted from the food matrix. To maximize yield and purity, and to ensure that genomic complexity of the library is representative of the total original bacterial population, we have defined a suitable protocol for total DNA extraction from cheese which can also be applied to other lipid-rich foods. Key steps were optimized to obtain high quality DNA, which was also proven to be exclusively of microbial origin. Functional screening of the metagenomic library on different antibiotics allowed recovery of ampicillin and kanamycin resistant clones with low frequency, suggesting that metagenomics represents a sensitive approach, enabling to identify rare species carrying the gene of interest within complex microbial communities. This work can therefore be considered a pioneering example of metagenomic applications to food microbiota and allows to extend the methodology to other traditional fermented products, greatly contributing to a deeper understanding of metabolic functions encoded by foodborne bacteria that can be beneficial to human health.



**The effect of the colonic metabolites, p-cresol on *in vitro* models of colorectal carcinogenesis**

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Colorectal cancer (CRC) is a major cause of morbidity and mortality worldwide each year. Proteolytic bacteria in the colon produce many genotoxic, mutagenic and carcinogenic substances resulting in colon dysfunction. Tyrosine is fermented to produce p-cresol (PC) which may be absorbed and sulphated in the liver being recovered in urine where it is considered a biomarker of protein intake. Curiously our group recently observed increased concentrations of para cresol in the urine of the aged relative to the young. This may reflect a widely reported shift in microbiota composition towards proteolytic strains in the bowel, but its functional effects on host health are unclear. Investigation the effect of PC on *in vitro* models of colorectal carcinogenesis with a view to evaluating the potential effect of this metabolite on CRC risk. DNA damage in Caco-2 cells was assessed via the FPG and EndoIII modified comet assay. Cell cycle progression was determined with PI staining and flow cytometry. Cell differentiation was assessed by change in transepithelial electrical resistance across very post confluent (17 day) Caco-2 monolayers. PC induced DNA damage at concentrations typical of those expected in the gut (1.5 and 3 mM). Cell proliferation was increased after 48 h treatment with PC at higher concentrations coupled to an increasing abundance of cells S and G2/M phase. PC also significantly decreased TER across Caco-2 cells treated 3.0 mM after 6 h indicative of a loss of tight junction integrity and a less differentiated phenotype. These data suggest that the PC might act as both a tumour promoter and a mutagen within the colonic environment, further studies are needed to confirm the potential contribution of this protein metabolite to carcinogenesis.

**Gut microbiota among adults and the impact of intervention with probiotic sobya on ten biomarkers**

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The role of probiotic food products has gained attention from consumers as novel adjuvants to chemical drugs to combat gastro intestinal disorders and therapeutic use towards cholesterol-lowering activities and cardiovascular diseases. The study aims to characterize and identify gut microbiota in the faeces of Egyptian adults. The impact of intervention with fermented sobya or with flavonoid rich fruits on the balance of human resident microbiota and on other blood biomarkers -associated health parameters had been studied. Design of the study: The subjects consisted of adults of both sexes (24.4 years). Fresh stool samples were collected and frozen immediately at -70 °C. Supplements: flavonoid rich fruits: apricots; pomegranate and sobya a probiotic fermented food product rich in bioactive peptides. The subjects were divided into five groups, each f group consumed one of the supplements daily for three weeks. (1) 200 gram apricots; (2) pomegranate juice (250 g); (3) traditional fermented sobya (180 grams) providing diverse Lactobacilli ( $5.7 \times 10^9$  cfu) and yeasts ( $1.82 \times 10^8$  cfu); (4) Pomme granate (75 g) and sobya (100 g), (5) control group. Laboratory investigations Collection of all biological samples including stool and blood at base line and after 21 days. The genomic DNA was extracted from all stool samples using commercial kit. Bacteria Specific primers were used for the identification of 17 faecal bacteria genus/ strain. Standard protocol was used to complete quantitative real time polymerase chain reaction (Q-RT PCR). Faecal short chain fatty acid concentrations were determined by Gas chromatographic technique. The lipid profile, nitrate/ nitrite were assayed in the serum samples using commercial kits. Serum homocysteine was determined by tandem spectrometry. Results: At baseline, (17) bacterial genus / strains were identified in the faeces of the subjects. faecal butyrate averaged 12.1  $\mu$ moles/g fresh faeces, making up one fifth of the total faecal short chain fatty acids. Consumption of sobya for three weeks effectively increased the counts of Lactobacilli and *rhamnosus rhamnosus*, total bifidobacteria, *Bacteroidates*, *Faecalibacterium prausnitzii*; with concomitant decrease in the count of *Enterobacteriaceae* and *Escherichia coli*. Significant association was found between the modulation in gut microbiota following the consumption of fermented sobya and increases in faecal short chain fatty acids concentrations and in lowering of pH values. Following the intervention with sobya, the serum analysis showed also improvement in the lipid profile expressed by a shift towards a higher ratio of high density cholesterol / low density cholesterol compared to the respective base line figures. The intervention with pomegranate juice has positive effectiveness to that of sobya. The combination of sobya and pomegranate juice didn't have a synergistic effect. Based on the data obtained for serum nitrate / nitrite and serum homocysteine levels the mechanism of action had been interpreted and will be discussed.

**Rat microbial catabolic pathway for grape seed flavonoids**

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Flavan-3-ols and their oligomeric forms proanthocyanidins (PAs) are the most abundant polyphenols in the human diet and, as with other polyphenols, they exert several beneficial health effects *in vivo*. Dietary PAs are poorly absorbed in the small intestine and reach the colon where gut bacteria enzymes can hydrolyze them and break down the polyphenolic skeleton to get small molecular metabolites which can reach systemic circulation. However, the PAs microbial metabolism has been poorly described in *in vivo* studies. The aim of this study was to determine the colonic biotransformation pathway of grape seed PAs in rat plasma after an acute administration of a grape seed PA extract (GSPE). For this, rat plasma flavonoids and their colonic metabolites were analysed by HPLC-MS/MS at 2, 4, 7, 24 and 48 h after the ingestion of GSPE (1000 mg/kg). Results showed that non-metabolised flavanols peak plasma concentrations at 2 h after GSPE administration, whereas the colonic metabolites appeared in plasma later in the time indicating their gradual colonic biotransformation as: valerolactone<phenylpropionic acids≈phenylacetic acids<benzoic acids. This study proposes an *in vivo* metabolic pathway of rat grape seed PA microbial metabolites.

**Impact of supplementation with a food-derived microbiota on obesity-associated inflammation**

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Obesity and its linked disorders have become a serious public health problem worldwide. Obesity represents a complex pathology associated with several metabolic alterations, characterised by low grade chronic inflammation promoted by immune cells, such as lymphocytes and macrophages, infiltrating and populating adipose tissue. Gut microbiota has recently attracted much attention as a crucial factor involved in obesity development, since alterations of intestinal microbial composition involving bacterial phyla and classes associated with improved energy extraction from the diet, were identified in obese human subjects as well as in animal obesity models and shown to affect host metabolism and energy storage. Probiotics are live microorganisms that, when administered in adequate amount, confer health benefits to the host, prevalently acting on immunomodulation. Based on these considerations, probiotic supplementation has been suggested to be able to counteract obesity-associated immune alterations. The interplay between food and gut microbiota has also been suggested to act in this direction. Traditional fermented products represent a natural source of live bacteria, including strains with probiotic features capable of transiently colonising the animal and human gut. The aim of the present work was to evaluate whether supplementation with a complex foodborne bacterial community can counteract obesity associated inflammation in C57BL/6J mice fed a 45% high fat diet for 90 days and supplemented with a mixture of natural lactic acid bacteria (LAB) strains derived from a traditional fermented dairy product. As controls we used either the characterised probiotic strain *Lactobacillus rhamnosus* GG (LGG) or PBS. Flow cytometry analysis of leucocyte subpopulations in epididimal white adipose tissue revealed that bacterial supplementation induced an increase in T-regulatory cells, as well as a decreased macrophage number, suggesting anti-inflammatory effects. These results are associated with decreased levels of pro-inflammatory cytokines in cultured adipocytes, such as Tumor Necrosis Factor (TNF)- $\alpha$ , Regulated on Activation-Normal T cell Expressed and Secreted (RANTES), and Granulocyte Macrophage – Colony Stimulating Factor (GM-CSF). Transcriptomic analysis and bacterial species profiling in faecal microbiota are in progress to further investigate inflammatory status in the intestine, stress response pathways in the liver as well as the contribution of gut microbiota.

**Distal gut microbiota structure and function differs between healthy adolescents from Egypt and USA**

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Cultural traditions, diet, and lifestyles of ethnic group living in different geographic locations can serve as a source of variability in human gut microbiota. To examine the differences in gut microbiome of geographically distinct human populations, we have carried out phylogenetic and functional gene analysis of the distal gut microbiota of healthy adolescents from United States and Egypt using next generation high-throughput sequencing. Based on amplicon sequencing of small ribosomal subunit gene, non-supervised dimensionality reducing ordination techniques such as principal components analysis and phylogenetic principal coordinates analysis generated a statistically significant separation of samples from the US and Egyptian groups ( $P < 0.001$ ). While both microbiomes were dominated by the phyla *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*, distinct differences were observed at the genus level. Gut microbiome from the Egyptian group was enriched in members of genera *Prevotella* and *Succinivibrio* compared to the US group. In contrast, significantly higher levels of *Bacteroides* and *Akkermansia* were observed in the US samples. Functional gene analysis of the gut microbiomes revealed increased abundance of fructose transport and amino acid metabolism in US kids and higher poly- and oligosaccharide metabolism in Egyptian children, likely an effect of differences in consumed diets. The distal gut microbiomes of US and Egyptian adolescents can be distinguished based on key differences in their functional and phylogenetic profiles.

**Analysis of large biomedical datasets: diseases, medical records, and genetics**

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Whereas countless highly penetrant variants have been associated with Mendelian disorders, the genetic etiologies underlying complex human diseases remain largely unresolved. Here, we examine the extent to which Mendelian variation contributes to complex disease risk by mining the medical records of over 110 million unique patients. We detect thousands of Mendelian-Mendelian and complex-Mendelian comorbidity associations, revealing a ‘Mendelian code’ that maps each complex phenotype to a unique collection of loci. Using genome-wide association results, we demonstrate that common variants associated with complex diseases are enriched in the loci indicated by this code. Finally, we infer genetic models from the clinical datasets and show that Mendelian variants contribute non-additively to complex disease risk. Overall, our findings suggest a vast network of putative genetic interactions among Mendelian loci, provide a complementary approach for the mapping of complex disease loci, and generate specific predictions for the etiologies of these diseases.

**The end of single SNP studies?**

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The focus on individual single nucleotide polymorphisms (SNPs), copy number variations (CNVs), or other single genomic structural variations (e.g. insertion/deletions or INDELS) is based implicitly on the one gene – one enzyme hypothesis of Beadle and Tatum. They demonstrated that a mutation in a single gene could eliminate enzyme activity and produce a change in phenotype. More sophisticated, yet conceptually similar approaches, were successfully applied to the study of monogenic diseases. Single gene- and genome wide (GWAS) association- studies have identified over 13,000 potential candidate SNPs meeting a predefined P-value threshold ( $5 \times 10^{-8}$ ) which are associated with a large number of different phenotypes. Individual variations or collections of statistically significant structural variations explain only a small fraction of the phenotype, which may be explained in part because of environmental variables were not included or measured in the analysis. Excluding external factors that influence internal biological processes generates an incomplete system at best, likely an inaccurate understanding of the interactions between environment and genetic makeup, and from a practical standpoint, misses an opportunity to identify modifiable factors that influence health. However, gene-environment association studies also identify variants with small effect size, suggesting that most, if not all phenotypes are the result of the contributions of many genes interacting with many environmental facts. Analysing complex phenotypes or response to nutrients in foods requires systems thinking, strategies, and methodologies. The conceptual basis of systems nutrition approaches is illustrated with examples from past and new reports of gene-environment interactions.

**Identification of cofactor-requiring enzymes with high genetic differentiation between 1000 Genomes**

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The aim of this research project is to identify genetic polymorphisms (SNP) within genes coding for cofactor-requiring enzymes that are highly differentiated between various human populations. Since most cofactors are derived from (micro)nutrients and cofactor-requiring enzymes are involved in multiple processes, inter-population differences in dietary habits and in metabolism could thus have a broad biological impact. Multiple databases (i.e. EBI CoFactor database, Uniprot, ExPASy database and MaCie) were mined with the aim of identifying human genes coding for cofactor-requiring enzymes. A list of SNPs found in the coding regions of those genes was constructed. The allele frequencies in unrelated individuals that participated to the 1000 Genomes project (n=1,092) was obtained from public databases. Indexes of genetic differentiation (Wright's inbreeding coefficient,  $F_{st}$ ) were then calculated within the entire cohort and for pairwise comparisons between populations from the African, American, East Asian and European continents. 1,698 cofactor-requiring enzymes were found and included in this analysis. We observed that most SNPs found in the coding region of cofactor-requiring enzymes have relatively low genetic differentiation (avg.  $F_{st}$ =0.064), which falls below the average whole-genome  $F_{st}$  of 0.13. However,  $F_{st}$  indexes had a wide distribution that ranges from 0 to 0.80 with the highest differentiated SNPs found in pairwise comparisons between African and East-Asian populations. Biological function and pathway enrichment analyses with genes / SNPs falling within the 99<sup>th</sup> percentile for each pairwise comparison revealed significant enrichment for different neurological and metabolic processes. Functional analyses of those SNPs are ongoing. The results of this study will enable the understanding and prediction of the outcome of nutrient deficiencies and interventions aimed at improving nutrient adequacy and may help guide population-specific nutritional recommendations and interventions.



**Effects of FADS2 genotype on fatty acid status & response to whole diet intervention in older adults**

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The objective of this research is to establish the impact of fatty acid desaturase-2 (FADS2) genotype on fatty acid status and response to intervention in older adults (the Nu-Age study). Nu-Age (EU FP7) is a five centre trial involving 1,250 older adults (aged 65-79 years) with the aim of examining the impact of a yearlong whole diet intervention (including advice on intakes of n-3 fatty acids Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)) on chronic low grade inflammation and cardiovascular health. A total of n=140 participants are included in the current analysis. The FADS2 enzyme is responsible for the desaturation of fatty acids in the bioconversion of essential fatty acids (EFAs) to long chain-polyunsaturated fatty acids (LC-PUFAs), including EPA and DHA. 10 tagging SNPs were selected using HapMap, including: rs1535, rs174589, rs2524299, rs174605, rs174616, rs174570, rs526126, rs968567, rs174602 and rs498793. Allele frequencies were calculated using the Hardy-Weinberg principle and were shown to be similar to the European allele frequency for all SNPs analysed. Results to date show the impact of several of these FADS2 SNPs on the plasma concentrations of several fatty acids, with the rs1535 intron SNP emerging as being particularly important. Participants with the homozygous minor genotype for rs1535 had significantly ( $P<0.05$ ) higher linoleic acid (LA) plasma levels and significantly ( $P<0.05$ ) lower arachidonic acid (AA), EPA and total n-3 status, as well as significantly lower desaturase activity (measured by a product-to-precursor ratio of AA/LA) compared to participants with either the homozygous major genotype or the heterozygous genotype. This suggests that the rs1535 allele is associated with lower FADS2 expression and/or activity. As the rs1535 SNP tags for 17 different FADS2 SNPs, it is possible that the rs1535 itself is not the functional SNP but may simply be serving as a marker for an associated genotype. The impact of the other FADS2 SNPs on fatty acid status will be further discussed as well as results on the impact of the FADS2 genotype on response to the Nu-Age yearlong whole diet intervention. (Nu-Age stands for 'New dietary strategies addressing the specific needs of elderly population for a healthy ageing in Europe' and is supported by the European Commission under the Food, Agriculture and Fisheries, and Biotechnology Theme of the 7<sup>th</sup> Framework Programme for Research and Technological Development.).

**FTO genotype influences insulin resistance in the Amerindian but not Caucasian population in Chile**

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Obesity is one of the major determinants of type 2 diabetes (T2D), presumably through its effect on insulin resistance. Previous studies have reported that variants in the FTO gene increase obesity risk, but their impact on risk of T2D-related traits differ by ethnicity. Therefore we aimed to analyse the effect of the FTO genotype on obesity-related and metabolic markers in Amerindian and Caucasian populations from Chile. We investigated associations between the FTO SNPs, rs8050136 (A/C) and rs17817449 (G/T) and quantitative trait measures in a cross-sectional population sample from Chile (n=409, 56% females) including adults of both native Amerindian (Mapuches) and Caucasian ethnicity. These quantitative traits included anthropometric (BMI, waist circumference (WC), body fat), metabolic (glucose, Insulin, HOMA<sub>IR</sub>, lipids profile and liver function markers), social and lifestyle variables, including dietary intake and physical activity. Both FTO SNPs were in perfect linkage disequilibrium, therefore we report the results for the SNP rs8050136 (A/C) only. FTO genotype was significantly associated with body weight ( $\beta$ : 3.1 kg SE: 0.8 per copy of the risk allele,  $P=0.0002$ ), BMI ( $\beta$ : 0.95 kg/m<sup>2</sup> SE: 0.2,  $P=0.0009$ ), WC ( $\beta$ : 2.4 cm SE: 0.9,  $P=0.008$ ), body fat ( $\beta$ : 1.85 kg SE: 0.4,  $P<0.0001$ ), insulin ( $\beta$ : 3.46 pmol/l SE: 0.5,  $P<0.0001$ ) and HOMA<sub>IR</sub> ( $\beta$ : 0.72 SE: 0.28,  $P=0.0001$ ). We found an 'ethnicity  $\times$  FTO' interaction effect on HOMA<sub>IR</sub> ( $P<0.0001$ ), such that the effect of FTO genotype on HOMA<sub>IR</sub> was significant for Mapuches ( $\beta$ : 1.25 SE: 0.2,  $P<0.0001$ ) but not for Caucasians ( $\beta$ : 0.15 SE: 0.1,  $P=0.162$ ). All results were adjusted for age, sex, environment, smoking status, socio-economic status and physical activity. In addition, BMI was included as a covariate when metabolic markers were the outcome. Our results reveal that FTO genotype has a large effect on insulin resistance in Mapuche Amerindians, independent of adiposity/BMI. If generalizable, this observation may suggest the existence of modifier genes that reduce the influence of FTO genotype on insulin resistance in Europeans. Such genetic differences may contribute to the larger increase in diabetes risk following urbanisation observed in non-White ethnic populations.

**A worldwide, trans-ethnic analysis of FTO gene and risk of type 2 diabetes: a meta-analysis**

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FTO gene variants have been associated with risk of type 2 diabetes (T2D) in several ethnic groups but there is no systematic assessment of the evidence. Therefore, the objectives of this study were to undertake a systematical review the effect of FTO genotype on T2D risk and to explore how BMI might modulate this association in trans-ethnic populations worldwide. Four databases (MEDLINE, Embase, Scopus, and Cochrane) were searched using the following criteria: (1) 'FTO' gene or 'fat mass and obesity associated gene'; and (2) T2D and metabolic-related terms (diabetes, glucose insulin, HbA1c, lipids profile, etc.). Data were pooled as Odd Ratios (OR) with their corresponding 95% confidence intervals and analysed using a random effects model. The study protocol has been registered in PROSPERO (CRD42014009467). Data were pooled from a total of 507,570 individuals (143,224 T2D cases and 364,351 normoglycaemic controls) from 74 case-control studies conducted in 19 countries worldwide (mean age 54.3±6.7; mean BMI 26.2±2.5). The unadjusted pooled analysis for all populations showed that FTO genotype are associated with increased T2D risk (OR: 1.14 [95%CI: 1.12 to 1.16], P<0.0001). This association remained significant after adjusting for age, sex and BMI (OR<sub>adj</sub>: 1.08 [1.06 to 1.11], P<0.0001). When the adjusted analysis was stratified by ethnicity, the associations were significant for South Asians (38,084 cases, 52,183 controls; OR<sub>Adj</sub>: 1.11 [1.07 to 1.16], P<0.0001), East Asians (51,439 cases, 60,884 controls; OR<sub>Adj</sub>: 1.10 [1.06 to 1.14], P<0.0001), and White-European populations (43,929 cases, 239,700 controls; OR<sub>Adj</sub>: 1.07 [1.04 to 1.10], P<0.0001), but not for the Latino population (2,588 cases, 2,930 controls; OR<sub>Adj</sub>: 1.10 [0.97 to 1.26], P=0.138). FTO genotype appeared to be protective against T2D among Africans and Afro-Americans (2,680 cases, 11,120 controls; OR<sub>Adj</sub>: 0.89 [0.84 to 0.96], P=0.002). This meta-analysis indicates that FTO variants are associated with significantly increased T2D risk in South Asians, East Asians and White-Europeans, but further work needs to be undertaken for Latino and African populations.

**Impact of PCFT and MTHFR genes on lipid and homocysteine concentrations in elderly Polish women**

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The relationship between homocysteine and lipid metabolism has been recently underlined, though its mechanisms are not yet thoroughly known. Proton-coupled folate transporter (PCFT, gene symbol SLC46A1) mediates intestinal folate absorption and folate transport across the choroid plexus. A recent genome-wide association study identified two single nucleotide polymorphisms (SNP) in PCFT gene, in the form of G to A substitutions (rs37514694 and rs739439), that were significant predictors of plasma HDL cholesterol in a Caucasian population. The methylenetetrahydrofolate reductase (MTHFR) gene codes for the key enzyme of folate metabolism, and C677T polymorphism leads to a reduction in the enzyme activity and increase of plasma total homocysteine (hcy) concentration. The aim of the present study is to evaluate the impact of these three SNPs on plasma lipid profile, folate and hcy concentration in elderly Polish women. 122 women over 60 years of age were recruited from the University of the Third Age and the Social Welfare Home in Poznań. Folate intake was assessed using a food frequency questionnaire. The serum folic acid level was determined immunoenzymatically, and plasma hcy was measured using the HPLC method. Blood biomarkers were analysed using a Vitalab Flexor biochemical analyzer. Genotyping was performed using the PCR-RFLP method. The minor allele frequencies were 0.17, 0.20, and 0.36 for rs37514694, rs739439, and C677T polymorphisms, respectively. One-way ANOVA showed no association between the analysed PCFT polymorphisms and the levels of biomarkers. Similarly, there were no interaction effects between the gene polymorphisms and folate intake. The C677T polymorphism was associated with HDL and triacylglycerol (TAG) concentrations ( $P < 0.05$ ), T-allele carriers had lower HDL and higher TAG levels than did CC homozygotes. Polymorphism of PCFT does not contribute to homocysteine or lipid metabolism, but MTHFR polymorphism may affect HDL and TAG concentrations in elderly Polish women.

**Polymorphism in perilipin gene and food intake in obese patients underwent bariatric surgery**

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The current epidemic of obesity resulted in increased number of surgical procedures and variations of responses of individuals after surgery can be determined by many factors including feeding behaviour, defined by the intake of energy, carbohydrates, proteins and lipids. Located in adipocytes, the perilipins (PLIN) are proteins that regulate lipid metabolism through modulation of lipolysis in adipose tissues. Associations between polymorphisms (SNPs) of PLIN gene and increased risk for obesity have already been described, however, their interactions with food intake require greater attention. This study evaluated the influence of polymorphism 11482G/A in the perilipin gene on food intake in obese patients after bariatric surgery and whether this interaction is associated with weight loss in the postoperative period of 36 months. Anthropometric (weight and body mass index; BMI) and food consumption (energy and macronutrients) data of 150 individuals who underwent the surgical technique of gastric bypass Roux-Y for more than 36 months were studied. Genotypic analysis was performed by allelic discrimination method on PCR equipment (Polymerase Chain Reaction) in real time, and the SNP 11482G>A (rs894160) in PLIN gene was genotyped using TaqMan Pre-Designed SNP Genotyping Assays (Applied Biosystems kit, Foster City, CA). T test for independent samples was used for comparison between genotypes, considering  $P < 0.005$ . It was observed that 33.3% of patients had genotype GG, 60% GA and 6.7% AA. In the preoperative period, we did not found differences in weight ( $135.7 \pm 21.8$  vs  $138.6 \pm 24.1$  kg,  $P = 0.463$ ), BMI ( $51 \pm 5.6$  vs  $51.5 \pm 8$  kg/m<sup>2</sup>,  $P = 0.710$ ), carbohydrate intake ( $89 \pm 216.3$  vs  $200.2 \pm 98.6$  g,  $P = 0.318$ ) and protein intake ( $92.1 \pm 44.7$  vs  $91.1 \pm 55.8$ ,  $P = 0.907$ ) among patients without (GG) and with (GA + AA) the mutated allele. However, it was observed that in patients with homozygous mutated allele (AA), there was a lower energy ( $1,316.4 \pm 267.4$  vs  $1,620.4 \pm 752.7$  kcal,  $P = 0.009$ ) and lipid ( $34 \pm 7.7$  vs  $47.5 \pm 29$  g,  $P < 0.001$ ) consumption than in patients GG + GA. After 36 months of surgery, no differences in weight ( $85.9 \pm 18.7$  vs  $86.7 \pm 18.7$  kg,  $P = 0.803$ ), BMI ( $32.3 \pm 6.5$  vs  $32.3 \pm 6.3$  kg/m<sup>2</sup>,  $P = 0.984$ ), energy intake ( $1,238.1 \pm 437.2$  vs  $1,189.6 \pm 473.9$  kcal,  $P = 0.565$ ), carbohydrate intake ( $165.9 \pm 62.1$  vs  $156 \pm 67.7$  g,  $P = 0.410$ ) protein intake ( $56.8 \pm 20.9$  vs  $58.6 \pm 29.3$  g,  $P = 0.725$ ) and lipid intake ( $41.5 \pm 24.4$  vs  $38.1 \pm 2$  g,  $P = 0.430$ ) among patients without (GG) and with (GA + AA) the mutated allele were found. The comparison between patients with mutated allele in homozygous (AA) and those with genotype GG + GA also showed no difference in food consumption and anthropometric data. The data showed the influence of the mutated allele in homozygosis in lower energy and lipids consumption in grade III obese patients before bariatric surgery. Financial support: FAPESP and CNPq.

**Interaction between the Apo B ins/del SNP and dietary intakes on serum ghrelin in diabetic patients**

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A great deal of evidence suggests that ghrelin is involved in the development of metabolic syndrome and type 2 diabetes (T2DM). The ghrelin concentration influenced by genetic variation and environmental factors. Apo B insertion/deletion polymorphism is one of probable genetic factors. Apo B is a protein that has a central role in lipid metabolism. Diet is a main environmental factor interacting with this gene to modulate the serum ghrelin concentration, consequently, type 2 diabetes risk. The aim of this study was to determine whether this polymorphism apo B insertion/deletion modifies the association between dietary intake and serum ghrelin level in diabetic patients. We have examined the interaction between the apo B ins/del polymorphism and the macronutrient intake (total fat, specific fatty acids, cholesterol, carbohydrate and protein) in their relation to the ghrelin concentration in 276 men and 423 women with type 2 diabetes in a cross-sectional study in Tehran, Iran. Fasting ghrelin concentration was measured. Dietary intake was assessed using a semi-quantitative food frequency questionnaire. The polymorphism was determined by electrophoresis on polyacrylamide gel after PCR amplification. Data was analysed by SPSS version 18. The allele frequency of this del and ins were reported 0.18 and 0.82, respectively. We confirmed a dominant effect of the apo B ins/del polymorphism (ins/ins vs ins/del+del/del). Results showed that serum ghrelin concentration was not significantly difference in the model dominant ( $P=0.91$ ). We found a highly significant interaction between apo B ins/del polymorphism and carbohydrate intake in determining ghrelin concentration in crud model ( $P=0.041$ ) that remained statistically significant after adjustment for covariates in multivariate regression model, including physical activity, antilipid medication, total energy, BMI and dietary fibre ( $P=0.023$ ). Thus, the del allele carriers showed lower ghrelin concentration only when carbohydrate  $\geq 54\%$  ( $P=0.07$ ), whereas no difference was observed in subjects with low carbohydrate ( $<54\%$ ) intake ( $P=0.26$ ). For the first time to our knowledge, there is a gene-diet strong interaction between apo B ins/del polymorphism and dietary carbohydrate on serum ghrelin levels in patient with type 2 diabetes.

**ApoE genotype affects PBMC gene expression profiles at baseline and in response to fish-oil**

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Apolipoprotein E (ApoE) is part of several types of lipoproteins. People who carry the ApoE4 SNP have an increased risk of cardiovascular disease. Fish-oil supplementation containing large amounts of n-3 PUFAs may help in the prevention of cardiovascular disease, though this finding is not reported consistently. Interindividual differences in the response to n-3 PUFAs may be explained by genetic variation. In this respect, the ApoE gene is a likely candidate. In this study, we aimed to increase our understanding of the pathways affected by the ApoE4 allele that could potentially be responsible for the increased risk of cardiovascular disease. In addition, we aimed to gain further insight in the role of ApoE4 allele in the response to fish-oil supplementation. 23 Subjects received 6 months of fish-oil supplementation containing 1,800 mg of EPA and DHA per day. ApoE genotype and whole genome PBMC gene expression at baseline and after supplementation was measured. Firstly, the effect of the ApoE4 SNP on whole genome PBMC gene expression was studied and, secondly, the differences in the PBMC gene expression response to 6 months of fish-oil supplementation between carriers and non-carriers of ApoE4 was examined. ApoE4 allele carriers had a higher expression of cholesterol biosynthesis genes and interferon target genes. Furthermore, fish oil supplementation was able to reduce the increased expression of interferon target genes in ApoE4 carriers. As a result, after the intervention, interferon-regulated genes were no longer differentially expressed between carriers and non-carriers of ApoE4. Based on the interferon-related gene expression data, we hypothesize that fish-oil supplementation may particularly benefit ApoE4 carriers.

**Haplotype in UCP2 gene is associated with percentage of excess weight loss after bariatric surgery**

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Studies have associated polymorphisms (SNP) in UCPs genes with obesity and body weight changes. This study aimed to investigate whether the haplotype 866G/A (rs659366) and Ala55Val (rs660339) in UCP2 gene is associated with as variations in weight loss after the Roux in Y gastric bypass (RYGB). 150 patients with grade III obesity undergoing RYGB were evaluated in preoperative, 1, 2 and 3 years after surgery. Genotyping was performed by the allelic discrimination in real-time PCR (TaqMan SNP Genotyping). For statistical analysis we used Mann Wittney test and logistic regression. We observed that 60.7% (n=91) of individuals showed the dominant model of haplotype (at least one mutated allele in both polymorphisms) and 18.7% (n=28) the recessive model (mutated allele in homozygous in both polymorphisms). We observed that homozygote recessive individuals (TT/AA) showed less weight than the individuals with only one mutated allele for each polymorphism (\_T/\_A) at the one year ( $88.8 \pm 18.6$  vs  $93.2 \pm 21.2$  kg;  $P=0.027$ ), two years ( $83.5 \pm 20.4$  vs  $86.5 \pm 18.1$  kg;  $P=0.022$ ) and three years postoperative ( $83 \pm 19.8$  vs  $87.2 \pm 18.4$  kg;  $P=0.028$ ); and greater percentage of excess weight loss (%EWL) during the first year postoperative ( $61.2 \pm 12.6$  vs  $58.3 \pm 17.8$  kg;  $P=0.037$ ). Thus, individuals with \_T/\_A genotypes showed greater %EWL than individual without mutated allele (CC/GG) ( $60.6 \pm 15.6$  vs  $56.2 \pm 18.8$  kg;  $P=0.011$ ) at the first year after RYGB. The presence of mutated alleles T and A were associated with the greater percentage of excess weight loss one year after RYGP. Financial support: FAPESP and CNPq.



**Polymorphism in the GNAS1 gene is associated with greater triglycerides in obese individuals**

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The specific role of 393T/C polymorphism in the GNAS1 subunit of protein G in the obesity is not fully elucidated, and the increased activity of subunit alfa 2 implies to the protein a possible regulatory role in adipogenesis. This study aimed to investigate whether the 393T/C polymorphism in the GNAS1 subunit of protein G (rs7121) are associated with the anthropometric and biochemical profile in grade III obesity patients. This is a transversal study that 150 patients with grade III obesity were undergoing anthropometric (weight and body mass index – BMI) and biochemical (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) evaluation. Genotyping was performed by the allelic discrimination in qPCR (TaqMan SNP Genotyping). For statistical analysis we used Mann Whitney test and logistic regression. We observed that 38% of the patients have the genotype TT, 48% TC and 14% CC. We did not found difference between the weight (138.3±23.1 vs 136.8±26.9 kg, P=0.726), BMI (51.5±7 vs 51.1±7.8 kg/m<sup>2</sup>, P=0.709), total cholesterol (183±34.7 vs 183.7±37.6 mg/dl, P=1.000), LDL cholesterol (114.2±31.2 vs 114.7±31.3 mg/dl, P=0.954) and HDL cholesterol (40.8±10.3 vs 39.7±8.1 mg/dl, P=0.590) of patients with (TC+CC) and without (TT) the mutated allele. However, we observed that patients with the mutated allele in homozygous (CC) showed greater triglycerides concentrations than TT+TC patients (169±75.2 vs 144.1±87.1 mg/dl, P=0.045). The presence of mutated allele C in homozygous results in greater triglycerides concentration in patients with obesity grade III, suggesting the role of protein G in adipogenesis. Financial support: FAPESP and CNPq.

**Pro12Ala polymorphism and its relation with glycemia and lipidic profile after bariatric surgery**

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The PPAR $\gamma$ 2 gene, expressed in adipose tissue, participates in the regulation of adipogenesis, fat acids transport, glucose and lipids metabolism. This study aimed to investigate the association between Pro12Ala (C>G) polymorphism in PPAR $\gamma$ 2 gene and variation in glycemia and lipidic profile after Roux en Y gastric bypass (RYGB). Glycemia, LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c), total cholesterol (TC) and triglicerydes (TG) were evaluated preoperatively and 12 months after surgery. Genotyping was performed by allelic discrimination in real-time Polymerase Chain Reaction using pre-designed TaqMan SNP Genotyping Assays kits. 83 subjects (78.3% women, aged 41.4 $\pm$ 10.5 years) were evaluated. Genotyping showed 78.3% homozygous for the C allele and 21.7% heterozygous. Individuals CC and CG have similar glycemia, LDL-c, HDL-c, TC and TG, preoperatively. Individuals CC showed decreasing of 23.3 $\pm$ 32.4 mg/dl of glycemia, 26.3 $\pm$ 2.4 mg/dl of TC, 19.9 $\pm$ 5.1 mg/dl of LDL-c, 64.8 $\pm$ 53.1 mg/dl of TG, and increasing of 6.9 $\pm$ 0.1 mg/dl of HDL-c while individuals CG showed decreasing of 13.5 $\pm$ 13.6 mg/dl of glycemia, 23 $\pm$ 16.3 mg/dl of TC, 18.3 $\pm$ 11.8 mg/dl of LDL-c, 64.4 $\pm$ 52.3 mg/dl of TG, and increasing of 7.5 $\pm$ 0.3 mg/dl of HDL-c. However, there was no difference in changes in glycemia and lipidic profile between the different genotypes. The Pro12Ala polymorphism was not associated with improvement in glycemia and lipidic profile after 12 months of RYGB. Financial support: FAPESP and CNPq.

**The role of PPAR $\alpha$  intron polymorphism in power performance**

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To date several genes have been associated with a strength/power performance e.g. ACTN3, CNTF, VDR, ACE or PPARA, underlining the importance of genetic component on strength/power-related phenotype. Peroxisome proliferator-activated receptor alpha coded by PPARA gene is highly expressed in the heart, liver, kidney, and skeletal muscles, activating genes involved in fatty acid metabolism. PPARA control expression of several genes which are involved in control of enzymes of fatty acid metabolism. We investigated the effect of PPARA intron 7 G/C polymorphism (rs4253778) on relative peak power per body weight (Pmax/kg) in a group of 81 elite Czech ice hockey players (18-36 y) during the 30-second Wingate Test (WT30) on bicycle ergometer. We found statistically significant differences in Pmax/kg values in WT30 between carriers and non-carriers for C allele ( $14.6 \pm 0.2$  vs  $13.9 \pm 0.2$  W/kg;  $P=0.029$ ). Previously, there was found an association with the reduced PPARA expression with regard to 7 G/C polymorphism. We hypothesize that resulting physiological changes in C allele carriers may represent possible metabolic advantage towards anaerobic metabolism in trained individuals. Our results indicate that PPARA 7C carriers exhibited higher speed-strength measures in WT30.

**C-reactive protein genetic polymorphism, polyunsaturated fatty acid intake and inflammatory pattern**

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To investigate the association between C-Reactive Protein (CRP) genetic polymorphism, polyunsaturated fatty acid (PUFA) intake and inflammatory pattern. Data were obtained from a population-based cross-sectional study involving a random sample of residents from São Paulo city, Brazil, aged between 20 and 59 years of both sexes (n=262). Dietary intake was estimated by two 24-hour dietary recalls. Information about life style was obtained using a questionnaire. Anthropometric measures were collected and blood samples drawn after overnight fasting. From blood samples, eleven plasma inflammatory biomarkers were determined by multiplex immunoassay and the genomic DNA was extracted for genotyping by the TaqMan® Open Array® System for the CRP (rs1417938) polymorphism. The chi-square test was used to determine whether genotype distribution followed the Hardy-Weinberg equilibrium. Multivariate Cluster Analysis (K-means) was performed to group the individuals according to eleven inflammatory biomarkers and generate inflammatory profiles. Subjects were divided into two clusters, representing Low (LI; n=169) and High (HI; n=93) level of inflammation. To determine the prevalence ratio (PR) between the single nucleotide polymorphism (SNP) and cluster groups, a general linear model using Poisson distribution and robust variance was applied, adjusted for confounders factors including age, body mass index, smoking status, alcohol consumption, moderate physical activity and skin color. The interaction between SNP and saturated fatty acids intake was tested with PUFA as the dichotomous variable (using median intake levels as cut-offs). A 2-tailed  $P < 0.05$  was considered significant. Genotype distributions did not deviate from the Hardy-Weinberg equilibrium ( $P > 0.05$ ). The HI cluster differed significantly in age, waist circumference, blood pressure, inflammatory biomarkers and smoking status compared to the LI cluster. No difference in PUFA intake was observed between cluster groups or in SNP genotypes on crude analysis. In the adjusted model, when stratified by total PUFA and omega-6, the SNP did not have a significant PR. However, among subjects with higher omega-3 intake, the allelic variant had a lower prevalence in the HI cluster (PR=0.63; 95%CI=0.40-0.99;  $P=0.047$ ) compared with the dominant genotype, but no significant genotype prevalence was evident among subjects with lower omega-3 intake. No interaction was observed between SNP and total PUFA, omega-6 and omega-3 intake. These results suggest that CRP (rs1417938) gene polymorphism with higher omega-3 fatty acid intake is associated with a lower prevalence of the inflammatory pattern. Financial support: FAPESP (grants: 2012/20401-7 and 2013/01741-4).

**Polyunsaturated n-3 fatty acids intake, ADIPOQ genetic variants and systemic inflammatory pattern**

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The objective was to investigate the association of five genetic variants of the adiponectin gene (ADIPOQ), polyunsaturated fatty acids (PUFA) intake and systemic inflammatory pattern. Data were obtained from a population-based cross-sectional study involving a random sample of residents from São Paulo city, Brazil, aged between 20 and 59 years (n=262). Dietary intake was estimated by two 24-hour dietary recalls. Single nucleotide polymorphisms (SNP) in the ADIPOQ gene (rs17300539, rs16861209, rs2241766, rs266729 and rs1501299) were genotyped by the Taqman® Open Array® system. Linkage Disequilibrium between SNP was calculated using Haploview software. Cluster analysis was used to group individuals according to similarities based upon eleven plasma inflammatory biomarkers. The relationship between SNP and clusters (called Inflammatory and Non-inflammatory), and likewise between PUFA intake and clusters and gene-PUFA interaction effects, were derived from a generalized linear regression with Poisson distribution and robust variance, adjusted for age, body mass index, gender, smoking status, alcohol consumption, physical activity and skin color, and expressed as Prevalence Ratio (PR) and 95% CI. To investigate gene-diet interaction, the PR between clusters and genotypes was stratified by percentiles of PUFA intake. Statistical significance was set at  $P < 0.05$  for all statistical tests. All SNP were in Hardy-Weinberg equilibrium. The Inflammatory cluster had higher age, body mass index and prevalence of smoking habits in comparison with the Non-inflammatory cluster. Among subjects with low n-3 PUFA intake (below the median), the rs2241766 G allele, rs16861209 A allele and rs17300539 A allele were associated with increased prevalence of the Inflammatory cluster (PR 95%CI): 1.79 (1.05-3.05); 1.78 (1.07-2.96); 1.79 (1.05-3.05), respectively), but there were no genotype associations among subjects with high n-3 PUFA intake (above the median). In agreement, n-3 PUFA-gene interaction was statistically significant for SNP rs2241766 and rs17300539 after adjustment ( $P=0.030$  and  $P=0.032$ , respectively). Moreover, an increased prevalence of the Inflammatory cluster was found among rs16861209 A allele carriers, only when n-6/n-3 ratio was below the median and after adjustment ( $P=0.049$ ). The same association between the rs16861209 A allele and Inflammatory cluster was observed when n-6 PUFA intake was below the dietary reference intake (PR (95%CI): 1.70 (1.10-2.63);  $P=0.024$ ), and remained significant after adjustment. Interaction between n-6 PUFA intake adequacy and SNP rs16861209 was significant after adjustment ( $P=0.015$ ). These results suggest that dietary PUFA intake may modulate the association between ADIPOQ genetic variants and systemic inflammatory pattern. Financial support: FAPESP (Grants: 2012/20401-7 and 2013/01740-8).

**ApoE variants and obesity-related traits in Mexican school children**

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Apolipoprotein E (ApoE) has a key role in lipid metabolism. Variation in this gene have been related with the risk for cardiovascular disease (CVD) and these effects may differ by sex. ApoE is expressed in adipose tissue and some investigations suggest that variation in this gene may have a role in obesity-related traits. Physical activity is a factor that contributes to energy balance and body fat deposition. The aim of this study was to analyse the association between the genetic variation in ApoE and obesity-related traits in Mexican school children, and to investigate the possible interaction between ApoE variants, physical activity and sex in this age group. Methods: The study was conducted in children between 8-10 y of age, with informed consent of their parents. A sample of 603 children who fulfilled the inclusion criteria was included in the study. Nutritional assessment of children was conducted using dietary, clinical and body composition methods. Measures of waist circumference (WC), height, weight and skinfolds were performed according to standard methods. A questionnaire to determine the pubertal score of children was applied. Body composition was measured by bioelectric impedance using a RJL portable equipment. DNA was isolated from saliva and ApoE genotypes rs429358 and rs7412 were analysed by allelic discrimination. Physical activity was assessed by accelerometry using a 7 d protocol in a subsample of 354 children. Analysis: main effects of variables influencing BMI, WC and percentage of body fat (%BF) were analysed by GLM using genotypes and sex as fix factors, and age, physical activity and anthropometric measures as covariates, according to the model. Interactions between ApoE genotype, physical activity and sex were explored. The mean  $\pm$  SD values for age, BMI and WC were  $9.06 \pm 0.8$  y,  $19.3 \pm 4.0$  kg/m<sup>2</sup> and  $68.6 \pm 11.9$  cm, respectively. Genotypes were in HW equilibrium. According to BMI for age and sex, approximately 48% of the participants were overweight or obese, above the national rate. Significant main effects were found for ApoE isoforms and total minutes of physical activity ( $P=0.027$ , partial  $\eta^2=0.029$  and  $P=0.036$ , partial  $\eta^2=0.017$ , respectively) influencing waist circumference (WC) and height/waist ratio. Carriers of the ApoE4 isoform had lower WC values as compared with other isoforms. Animal studies have suggested that ApoE4 is related to lower risk for obesity, as compared with ApoE3. No significant interactions between ApoE genotype and sex, or quantity of physical activity were found in the present study. These findings reflect the high prevalence of overweight in Mexican children. Variation in ApoE was associated with WC and showed a small effect size. ApoE4 is considered of higher risk for CVD however, in this study, carriers of this genotype had smaller WC. Further studies are required to investigate the role of variation of ApoE in human obesity.

**JPI a Healthy Diet for a Healthy Life: its role in co-ordinating research in the area food & health**

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The increased incidence of diet and lifestyle-related diseases is a major concern across Europe, leading to an intense research focus on the complex relationship between diet, exercise and health. The Joint Programming Initiative (JPI) a Healthy Diet for a Healthy Life (HDHL), offers the ideal framework to pursue this type of research, which requires large population studies and controlled trials, as well as a long-term follow-up strategy. Joint Programming entails a voluntary partnership between Member States (and Associated Countries) of the European Union and beyond and aims to tackle major societal challenges by combining and coordinating national research programmes and, thereby, making better use of Europe's public R&D resources. The JPI HDHL is working across 23 Member States and associated countries as well as Canada and New Zealand to improve our understanding of how individual, social and environmental determinants influence food and physical activity choices. The JPI HDHL published its Strategic Research Agenda in 2012 and its first Implementation Plan in 2014. The first pillar of our strategic research agenda is about trying to understand the most effective ways of improving public health through interventions targeting diet and physical activity. Research will include studies which aim to improve our understanding of the different biological, psychological and socio-cultural factors that impact on health, and how they interact. The second key pillar of the SRA is on diet and food production, with the goal of developing healthy, high quality, safe and sustainable foods, building on analysis of their effect on health. The final pillar of the SRA is focussed on understanding diet related diseases – preventing diet-related diseases and increasing the quality of life and delivering a healthier diet. Infrastructure is critical to delivering on the objectives set out in the SRA of the JPI HDHL. But now is the time for action and since 2012 the JPI has launched 3 joint actions – one of which is a nutritional phenotype data-sharing initiative entitled ENPADASI. The plan for the initiation of a further 5 joint actions under the JPI HDHL will be discussed during the presentation.

**Do we need a nutritional bioinformatics infrastructure?**

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Nutrition and health research is complex, as it needs to deal with the multiple subtle effects of an ever changing variety of dietary compounds against the 'background' of large inter-individual variation due genetic variation and lifelong exposure. Thus, nutrition science may be the prime example where genome, phenome and exposome meet, and as such nutrition and health research is real systems biology, with nutrigenomics an attempt to incorporate the adequate technologies and concepts. Many examples of complexity have been presented in nutrition research, and they all show the need for harmonization in many levels, open access to data and results, and the need for a shared 'toolbox'. Some of these requirements are being tackled by infrastructural efforts in basic biological sciences (Elixir, BBMRI, etc). Some are nutrition specific and still need improvement (food composition and intake quantification, the focus on health vs disease, etc.). A number of (European) initiatives are shaping the future towards a Nutritional Bioinformatics Infrastructure. NuGO has initiated standardized data sharing. The JPI HDHL is funding a showcase in this area. The FP7 project EURO-DISH is preparing a roadmap. These initiatives now converge and may lead to the creation of a bright future for nutrition and health research. In the meantime, innovative research shows both the beauty and the struggles in this area. I will demonstrate both the infrastructural efforts and the research examples and initiatives.



**PhytoHub version 1.0: a food metabolome database dedicated to dietary phytochemicals**

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The 1<sup>st</sup> international workshop on the ‘Food metabolome and biomarkers for dietary exposure’, organized in Glasgow last year, identified as a priority the development of databases and libraries of spectra for the food metabolome. The food metabolome comprises all metabolites present in human biofluids and tissues that directly derive from the digestion and metabolism of food chemicals. Exploration of the food metabolome through mass spectrometry untargeted profiling has opened new avenues for the discovery of intake biomarkers, also a key priority in nutrition research as described in the JPI HDHL strategic agenda and recent BioNH call. A large proportion of the food metabolome consists of metabolites of phytochemicals such as polyphenols and terpenes. Identification of these metabolites in metabolomic profiles is often a bottleneck in biomarker discovery, as their standards are not commercially available and their integration in online databases and libraries of spectra is still very limited. As part of the ANR PhenoMeNep project, we have designed PhytoHub ([www.phytohub.eu](http://www.phytohub.eu)), dedicated to all phytochemicals commonly ingested with the diet. Chemical data include name, chemical structure and identifiers, synonyms, physico-chemical properties. Data also include the taxonomy, foods of origin (linked to FooDB), compound of origin (if a metabolite), biofluid location and literature references. Known metabolites are manually extracted from the literature and references are attached. Since the metabolism of many phytochemicals has not been studied yet, *in silico* prediction of metabolism will be used. We are developing an in-house tool from (1) a compilation of all biotransformations (and combinations) occurring in humans (including gut microbiota biotransformations) for each chemical class and (2) analysis of functional groups on precursor phytochemicals. A list of predicted metabolites will be generated for each phytochemical. Mass spectral data come from literature, other databases on phytochemicals and experimental data from our collaborative platforms. Hopefully *in silico* prediction of mass fragmentation will be added in the future. An efficient relational design supports a powerful and intuitive web interface. For a queried monoisotopic mass or molecular formula, the database will return a list of metabolites or phytochemical precursors, along with their spectral data and possible dietary and metabolic origins. For a queried food, it will return a list of metabolites likely to be present in biofluids after consumption. MS-MS and custom advanced searches are also possible. PhytoHub is the first database to collate information on phytochemical metabolites from a metabolomics standpoint, and should facilitate identification of unknowns in non-targeted profiling. The version 1.0 of PhytoHub is online, with a first dataset on the 240 most consumed terpenes and their known metabolites. It will be updated monthly. Any willingness to contribute to this freely accessible resource is welcome so that the database can be as complete, accurate and useful as possible.

**Role of CPS1 and urea cycle in weight maintenance: results from the Diogenes project**

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The mechanisms involved in weight maintenance are diverse and not yet completely elucidated. Diogenes is a Pan-European randomized controlled dietary intervention study, in which 891 families with at least one overweight/obese parent were included. Eligible adults (CID 1) underwent an 8-week weight-loss phase following a low-energy diet. Those who achieved at least 8% weight loss (CID 2) were assigned to a 6 or 12 months weight maintenance diet (CID 3). Genome wide association studies showed a SNP trait association for Glycine levels within the CPS1 gene, which is the rate-limiting enzyme for the Urea cycle. Other studies on the Diogenes project have shown decreased Urea levels in subjects with successful weight loss maintenance. The aim of the present study was to investigate the role of Glycine and CPS1 related pathways in weight maintenance. Gene-metabolite interaction networks were built from String and DrugBank databases. Network analysis, trans- and cis-eQTL, and regression models, were applied to investigate the role of Glycine connected pathways in weight maintainers and weight maintenance resisters. Network visualization and gene ontology functional annotation were performed with Cytoscape and clueGO plug-in, respectively. Regression analysis on RNAseq transcriptomic data, from adipose tissue, was performed in the R software using the stats package. eQTL analysis on plasma genomic data was performed in the R software with the package MatrixEQTL. Gene-metabolite interaction networks of Glycine and CPS1 (608 nodes, 4635 edges) showed functional enrichment in lipid metabolism biological processes. Cis-eQTL analysis showed 18 SNPs associated with CPS1 gene expression. Linear regression predicting BMI fold change in response to gene expression fold change, within the genes in the CPS1-Glycine network, showed 42 genes significantly, or marginally significantly (adjusted P-value<0.1), correlated with BMI for CID 3 vs CID 1, and 12 genes for CID 3 vs CID 2. Weight maintainers showed a lower CPS1 expression at CID 1 (t-test P-value=0.006), and a lower CPS1 expression fold change between CID 2 and 1 (t-test P-value=0.028) compared to weight maintenance resisters. An overlap between genes (fold change CID 3 vs 2) correlating with change in BMI, and cis-eQTL, was found for 6 genes, 2 of which are part of the Urea cycle. The present study highlights a possible role of the CPS1 gene, and related pathways, in weight maintenance.

**The zinc-proteome interaction network as a model to identify nutrient-affected pathways**

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Zinc is an essential micronutrient that plays fundamental roles in cellular metabolism. It acts mostly through binding a wide range of proteins, affecting a wide spectrum of biological processes including cell division, growth and differentiation. Complete annotation of Zn-containing proteins showed that zinc-binding proteins represent about 10% of the human proteome, with over 300 enzymes containing Zn ions within their catalytic domains. Also hundreds of key regulatory proteins, including transcription factors require Zn for their activity. In this study the whole set of Zinc Binding Proteins (ZBNP) together with their direct interactors were listed and defined as the Zn Proteome (ZNP). We interrogated pathway analysis tools to identify the cellular processes that are predicted to be affected by zinc availability. Network and functional enrichment analyses highlighted biological processes potentially affected by deregulated zinc homeostasis. This computational approach was also tested on a real case study: the possible involvement of ZNP network proteins in Crohn's Disease pathogenesis was assessed in a previously published dataset including genes transcriptionally regulated in the intestinal mucosa of patients affected by this condition. This analysis produced a network of pathways likely to be influenced by zinc and associated with Crohn's disease. These results highlighted a central role for zinc in tissue remodelling that occurs upon gut inflammation, pointing at novel pathways that could be worsened by Zn dyshomeostasis and by impaired Zn fluxes in specific districts. Overall, our computational approach and its case study application emerge as a valid tool to provide novel insights into pathological conditions as well as to drive new mechanistic research in under-investigated areas by providing inputs for new study designs.

**Modulation of adipocytes differentiation and proadipogenic genes expression by different bioactives**

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Obesity, considered as one of the most easy to recognize but most difficult to treat medical conditions, is the main dysfunction of adipose tissue. It is characterized by excess body fat accumulation due to an increase in size and number of differentiated mature adipocytes. De novo generation of fat cells plays a key role in the development of obesity and adipocytes differentiation is a complex process involving the coordinated interplay of numerous transcriptional regulators and genes. Discovering natural compounds able to regulate size, number and function of adipocytes could greatly contribute to obesity prevention and treatment; particularly natural compounds could represent a potential novel strategy already exploited for preventing metabolic disorders. The current study had two aims: (1) to evaluate changes in the expression of adipogenic markers at four stages of the differentiation process; and (2) to investigate the anti-obesity effectiveness of the anti-adipogenic ability of three bioactives, docosahexaenoic acid (DHA), genistein (GEN), and sulforaphane (SFN). Using murine 3T3-L1 pre-adipocytes changes in the expression of adipocyte marker genes C/EBP $\alpha$ , PPAR $\gamma$  variant1 and variant 2, and GLUT 4 at growing, postconfluent, differentiating and mature adipocyte cell stages were evaluated by RT-qPCR. The ability of SFN, GEN and DHA to inhibit 3T3-L1 differentiation was assessed by both lipid accumulation and modulation of the above mentioned genes expression in mature adipocytes. Expression of the four marker genes was low and similar at the early stages of pre-adipocytes development, whereas a prominent increase was observed in mature adipocytes. The bioactive compounds were shown to suppress adipocytes differentiation and to decrease the expression of the adipogenic markers and lipid accumulation to the levels of pre-adipocytes. These results set the stage for further studies considering natural food constituents as important tools in preventing or treating obesity. The authors participate in the FP7 EU Project PATHWAY-27 'Pivotal Assessment of the Effects of Bioactives on the Health and Wellbeing, from Human Genome to Food Industry' (grant agreement no. 311876).

**Secretome analysis using RNA sequencing following physical exercise**

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Subcutaneous adipose tissue (WAT) and skeletal muscle (SM) are known to mediate biological effects through secreted proteins. No study so far has used RNA sequencing (RNA-seq) to profile both tissues in relation to dysglycemia and during short- as well as long-term physical exercise. Next-generation RNA sequencing technologies have revolutionized the world of transcriptomics. It enables quantification of the complete set of RNA isoforms as well as other nucleotide sequence variants, and RNA editing analyses. We hypothesized that this approach would give us novel insights on both known and novel myokines and adipokines. Our methods involved recruiting subjects who underwent combined resistance and endurance exercise for 12 w, including two endurance bicycle sessions (60 min) and two whole-body resistance-training sessions (60 min) per week. We sampled muscle biopsies and serum before, immediately after, and 2 hours after 45 min of ergometer cycling. Sampling was done before and after the 12 week training period in controls and dysglycemic subjects. Moreover, subcutaneous adipose tissue biopsies were taken at one time point before and after 12 weeks of training. WAT and SM samples underwent RNA isolation and cDNA synthesis before deep-sequencing using RNA-seq. Our RNA-seq results showed coherent results compared to classical RT-PCR. Differential expression analysis using three bioinformatical pipelines, Cuffdiff2, DESeq2 and edgeR, revealed many regulated genes coding for adipokines and myokines. Time-series analyses of the SM transcriptome around an acute bout of exercise showed 426 down and 564 up regulated predicted myokines during exercise in the controls. The corresponding numbers in dysglycemic individuals were 448 down and 536 up regulated. In the 2 h resting period after exercise, 541 predicted myokines were down and 356 up regulated the controls, while 521 were down and 336 were up regulated in dysglycemic individuals. Some genes showed a different behavior in dysglycemic individuals compared to control. 12 w training resulted in regulation of 3670 predicted myokines compared to 131 predicted adipokines in WAT. Profound changes in the WAT transcriptome were only seen in dysglycemic individuals, including down-regulated inflammation, which is associated with insulin resistance, overweight and diabetes. One potentially important regulated adipokine was the secreted frizzled related protein 4 (SFRP4). SFRP4 plasma levels are influenced by WAT production and may be linked to pancreatic beta-cell dysfunction. We conclude that both short-, and long-term physical exercise greatly impacts the expression of genes coding for secretory proteins in SM and WAT. The regulation in SM is larger than in WAT and the alterations depend on metabolic status. Many of the identified secreted proteins may serve as biomarkers and therapeutic targets.

**Untargeted metabolomics reveals urinary exposure biomarkers for intake of berries**

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In an era where nutritional science is complicated by the difficulties in determining conclusively the association between specific foods and their biological effects, metabolomics emerged as a technique that is increasingly applied to overcome the well-known limitations of the traditional dietary assessment methods. By exploring the perturbation of the metabolome following a dietary intervention, novel and objective biomarkers of food intake can be discovered, validated and thereafter applied to assess compliance in human intervention trials. Sea buckthorn and strawberry are two representative and compositionally different berries included in the New Nordic Diet (NND) and therefore good candidates for an investigation of berry biomarkers. We therefore aimed to identify urinary exposure markers following intake of a single-dose of sugar-sweetened strawberry and sea buckthorn meals, in humans. A randomized controlled single-blinded 3-way cross over meal study has been conducted in 16 overweight men. Each subject had a randomized sequence of the three intervention meals: strawberry, sea buckthorn berry, 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 analysed by UPLC-qTOF-MS. Principal component analysis (PCA) has been applied to explore the dietary exposures and partial least squares discriminant analysis (PLS-DA) to extract the most important features responsible for the separation of the three meals, at each time point. Subsequent MS/MS fragmentation has been performed to ease the identification process. The two berries showed different excretion kinetics for their markers. Strawberry exposure gave rise to early excreted markers, with a peak in excretion at 1-2 h, whereas sea buckthorn mainly gave discriminant features in the 2-24 h and pooled 24 h samples. Only few common markers appeared for the two berries, and all except one were in very low concentration and, thereby, not identified. 10 and 11 biomarkers have been (tentatively) identified for strawberry and sea buckthorn, respectively, mainly as glycine conjugates, sulphates and glucuronides. Three of the identified compounds validate already proposed exposure biomarkers for the NND. The step-wise aromatization of quinic to hippuric acid has been pointed out, after identifying all the intermediate compounds as late-excreted markers for sea buckthorn. Potential characteristic exposure markers have been distinguished in human urine after consumption of sea buckthorn or strawberries. Different combinations of markers are proposed for further validation, in larger and less-controlled study settings. Untargeted metabolomics has the potential to reveal novel explanations that give insights into mechanisms and bioavailability of specific food constituents.

**Applied metabolomics approaches to discover food-derived metabolites in human bio-fluids**

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An understanding of causal relations between diet and health is hindered by the lack of robust biological markers of food exposure. Most dietary biomarkers currently have been identified on the basis of knowledge of food composition by using hypothesis-driven approaches. However, the rapid development of metabolomics resulting from the development of highly sensitive OMICS analytical instruments, the availability of metabolite databases and progress in bioinformatics has aided in the identification of novel biomarkers for the intake of a range of foods including fruit, vegetables, beverages, meats and complex diets. We have previously used data-driven approaches using non-targeted metabolomic techniques coupled with semi-automated machine learning data mining to understand the limitations in discovering food-derived biomarkers in human urine. Our current study, M.A.I.N (Metabolomics at Aberystwyth, Imperial and Newcastle) builds on these preliminary data to discover and validate potential dietary biomarkers in both controlled clinical and epidemiological contexts. The project brings together researchers from internationally recognized UK teams who already have a track record of collaborating in research to develop novel approaches for estimating dietary exposure using metabolomics. In Aberystwyth, biofluids are being investigated with a range of metabolomic techniques, starting with Flow Infusion-High Resolution Fingerprinting (FIE-HRMS) using Orbitrap Mass Spectrometry (MS) coupled with multivariate classification and feature selection, to evaluate the likelihood of biomarker discovery for around 20 important dietary components. Where probable success is indicated we will use Ultra High Performance Liquid Chromatography-High Resolution MS (UHPLC-HRMS), using both Reverse Phase C<sub>18</sub> and Hydrophilic Interaction Chromatography (HILIC), to cover a range of metabolite chemistry and identify potential biomarkers. The combination of chromatography, accurate mass and tandem mass spectrometry (MS<sup>n</sup>) means that we can accurately elucidate potential biomarkers after modelling, without the need for extensive targeted studies. Pre-processing and multivariate analysis of high mass resolution data (both LC and FIE) is computationally intensive, therefore all metabolomics workflows are fully integrated with a High Performance Computer which provides the ability for more in-depth modelling, quicker processing times and robust validation of processing/model parameters. Potential food biomarkers will be validated by quantification (using chemical standards where possible) in biofluid samples obtained from controlled clinical studies and free-living individuals taking part in several independent cohort studies for which good quality dietary information exists.



**Proteomics responses to oral dietary challenges**

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Response to metabolic challenges represents a fundamental aspect of overall nutritional health. The Metabolic Challenge Study (MECHE) has enrolled 214 subjects aged 18-60 years to undergo a dietary challenge with either an oral lipid tolerance test (OLTT) or oral glucose tolerance test (OGTT). In addition to laboratory/clinical measures (measured at multiple time points following each challenge) and assessment of body composition and dietary habits, genotypic (HumanOmni5-Quad Omni5 BeadChip with 4,301,331 genetic markers) and plasma proteomic (1,129 proteins, Somamer™ platform) data were obtained at baseline and at 60 min post-OGTT and 240 minutes post-OLTT. The goal of these analyses is to find markers predictive of OGTT and OLTT response and to develop a systems view of the processes involved in the response to these metabolic challenges. We used robust regression to assess the proteomic response to both metabolic challenges. We identified 19 proteins that varied significantly following the OGTT and 273 proteins following the OLTT when taking gender, age, and BMI as covariates and correcting for multiple testing. Robust sparse k-means clustering on the proteomic data at baseline enabled the identification of 65 proteins that distinguished between two groups of subjects. Anthropometric measures were significantly different between both clusters ( $P < 0.01$ ). The cluster of individuals with higher measures of site-specific adiposity also had significantly higher plasma lipid response measures following OLTT. Glucose iAUC following OGTT was not different between both clusters. Functional analysis of the 65-protein signature revealed evidence of functional and physical interactions, with the most enriched GO term being Axon guidance. Furthermore, 6 of those proteins were found to contain significant pQTL suggesting a genetic basis for the proteomic group clustering. In summary, a 65-protein signature was associated with an anthropometric profile known to increase cardiovascular disease risk and was predictive of an increased plasma lipid changes following OLTT. Further work is needed to validate the pathways in which those proteins are involved but this could help identify mechanisms linked to impaired postprandial response to nutritional challenge.



**Breath analysis: potential applications in dietary interventions**

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Analysis of volatile organic compounds (VOCs) in exhaled air gives an insight into the physiological state of the body. VOCs measured in a breath originate, for instance, from internal metabolic (anabolic and catabolic) production or from the intestinal microbiota. Due to non-invasive nature of exhaled air analysis it has become a promising and rapidly developing tool in disease diagnosis and monitoring. In the current study we investigated wheatear dietary intervention would impact exhaled air composition. For that purpose, twenty healthy individuals were enrolled into the study. Excreted VOCs were monitored while adhering to a gluten-free diet for four weeks, followed by nine weeks of a normal diet. Exhaled air samples were collected at baseline and at the end of each of intervention week. The samples were analysed by thermal desorption-gas chromatography combined with time-of-flight mass spectrometry, methodology previously established by our team. Additionally, standard dietary intake was assessed to verify adherence to the diet and to get an insight into macronutrient intake during the intervention period. To identify VOCs that significantly change due to the gluten-free diet, analysis of variance and principal component analysis was employed. A set of 12 volatile compounds distinguished the samples obtained during the gluten-free diet from those obtained during a normal diet. Seven compounds could be chemically identified (2-butanol, octane, 2-propyl-1-pentanol, nonanal, dihydro-4-methyl-2(3H)-furanone, nonanoic acid and dodecanal). The availability of breath samples at consecutive time points of gluten-free and normal diet allowed following the individual response to the intervention. The trajectories of individuals reflected high level of variations and various respond speeds to the diet alternations. Interestingly, it was observed that after finishing the gluten-free diet period the VOCs profile goes back to the profile measured before the intervention. To conclude, we demonstrated that VOCs profile in exhaled air changes significantly due to a gluten-free diet. If the differences in response trajectories go beyond normal inter-individual variations need to be established further in the larger cohort.

**The role of age in the transcriptional response to a short period of caloric restriction**

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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. Results of these studies put CR as a beneficial tool to decrease risk factors in several age-related diseases in humans, contributing to a healthy ageing. The question remains at what age CR should be implemented to be most effective with respect to healthy ageing. The aim of our study was to elucidate the role of age in the transcriptional response to a 30% CR diet in immune cells, as immune response is affected during ageing. Ten healthy young men, aged 20-34, and nine healthy old men, aged 64-85, were subjected to a two week weight maintenance diet, followed by three weeks of 30% CR. Total RNA from peripheral blood mononuclear cells (PBMCs) was collected, isolated before and after the 30% CR diet, and used to evaluate gene expression on human whole-genome microarrays. Expression of 554 genes showed a different response between young and old men upon CR. Gene set enrichment analysis revealed a downregulation of gene sets involved in immune response in young men, but not in old men. As ageing is known to be accompanied by an increased expression of genes involved in immune function, we determined if there was a difference in immune related gene expression at baseline, before CR. Indeed, immune response related genes were higher expressed in old men compared to young men at baseline. Analysis to identify upstream regulators showed that most discovered upstream regulators were involved in the immune response, and were inhibited in young men after CR, and activated in old men at baseline. Based on the gene expression data, we conclude that a short period of CR is more effective in young men compared to old men regarding immune related pathways.

**Rat thyroid cells FRTL5 as a model for proteomic analysis of the effect of zinc in hormone secretion**

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Zinc (Zn) is an important micronutrient acquired from the diet through protein-rich foods. Being a structural, catalytic or regulatory component of hundreds of proteins, it is required by a wide spectrum of biological processes including cell division, growth and differentiation. Overall, the Zn proteome was reported to represent about 10% of human proteins, highlighting the molecular basis for the pleiotropic effects of a Zn deficient status. Zn homeostasis is tightly controlled by the coordinated regulation of uptake, efflux, distribution and storage of the metal ion, mostly through tissue and cell-specific expression of specific transport proteins, such as the Zn uptake transporters of the Slc39 (Zip) family and the intracellular compartmentalization transporters of the Slc30 (ZnT) family. Alteration in Zn homeostasis affects several biochemical processes and even mild Zn deficiency is linked to increased risk of developing a number of chronic/metabolic diseases. We have focused our recent work on the ZnT8 protein, abundantly expressed in pancreatic islets where it is involved in insulin secretion by  $\beta$ -cells, with a specific genetic variant associated to increased risk of developing type 2 diabetes. We have previously shown ZnT8 expression in specific endocrine cell types of pituitary, adrenal glands and thyroid, suggesting a more general role in regulating hormone secretion. Experimental evidence has been provided for a role of Zn in normal thyroid homeostasis, which might involve synthesis of thyroid hormones. However, 'classical' approaches in humans as well as in animal models are still inconclusive, mainly due to the lack of reliable biomarkers of Zn status. Omic-approaches, especially proteomics, can be very useful to clarify the molecular basis of the role of Zn and its specific transporters in thyroid hormone metabolism. To this aim, we turned to an *in vitro* model represented by the FRTL-5 cells, derived from a Fischer rat primary thyroid culture and displaying a primary thyroid cell phenotype in terms of growth properties, thyroid-stimulating hormone (TSH) dependence, expression of TSH receptor and ability to synthesize and secrete thyroglobulin. In these cells we could detect expression of the main Zn transporters associated with the secretory pathway. To identify the optimal conditions for proteomic analysis we exposed FRTL-5 cells to increasing concentrations of TPEN, a Zn-specific chelator that induces an intracellular Zn-deficient status. At 25  $\mu$ M TPEN for 2 h we identified mild Zn deficiency conditions leading to undetectable intracellular free Zn pools, decreased thyroglobulin secretion and increased apoptosis, corresponding to decreased levels of the apoptosis inhibitor protein XIAP and presence of caspase cleavage products. All these phenotypes were reversed by addition of Zn ions to the culture medium. We are presently using these conditions for proteomic analysis in the FRTL-5 cell line, which has shown to represent a useful model to investigate the effects of intracellular Zn levels in thyroid functionality.

**Brazil micronutrient project: preliminary clinical data**

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Micronutrient deficiencies are linked to risk of non-transmissible diseases, such as heart disease, obesity and diabetes. The objective was to analyse status and 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 and correlate the metabolic response to genes and proteomic groups. The experimental design was an intervention followed by a washout. The participants (aged 9 to 13) attended public school in a periurban area of Ribeirão Preto (São Paulo, Brazil). The study design is based on measuring metabolic and nutritional parameters at baseline (time point 1), after six weeks of a micronutrient daily supplement (time point 2) and following 6 weeks without the daily supplement (washout). Food intake and physical activity were monitored. Genotype data were generated using HumanOmni5-Quad Omni5 BeadChip with 4,301,331 genetic markers and proteomic analyses of 1,129 proteins (Somamer™ platform). Two metabolic groups were found based on cholesterol, LDL, triglycerides, and glycemic level. Cluster 1 (n=116) had a better metabolites profile (cholesterol, LDL, triglycerides, and glycaemia levels at baseline and following supplementation when compared to Cluster 2 (n=23). ANCOVA analyses comparing variables between both clusters and adjusting for age, pubertal status, gender, and energy intake showed cluster 1 with better baseline anthropometric and metabolic profiles and nutrient intake. A longitudinal analysis (correcting for age, gender, pubertal stage, and energy intake, carbohydrate, lipid and C-reactive protein) in cluster 1 showed that total and LDL cholesterol and glucose decreased throughout the study. In cluster 2, total and LDL cholesterol decreased throughout the study. Proteins that were increased during supplementation were involved in biological processes involved in acute inflammatory response, regulation of complement activation; while proteins that were decreased during supplementation were involved in negative regulation of B cell differentiation (related to activation of immune response). When C-reactive protein values are taken into account, 107 proteins were significantly influenced by intervention. Statistically significant proteins were correlated with glucose, cholesterol and LDL-cholesterol. Analysis of genetic association with and metabolic parameter response to supplementation are underway. The preliminary results described here indicate that inter-individual variation in physiology may be identified by short-term dietary challenges.

**Serum metabolic signatures are highly influenced by diet and physical exercise**

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The prevalence of metabolic syndrome (MetS) is increasing ever more in our society. Considering that this disorder may result in obesity, cardiovascular disease and/or diabetes, great efforts are being made to prevent its development. To avoid the overuse of drugs, it is recommended to follow more healthy dietary patterns and lifestyles. For instance, it is known that physical exercise provides numerous salutary effects. A good experimental model to study diet-induced MetS is the cafeteria (CAF) fed rats. Therefore, the aims of the present study are to investigate the impact of CAF diet and physical exercise on the overall serum metabolome by a non-targeted metabolomics approach and to find novel biomarkers of exercise through the identification of differentially expressed metabolites. In this experiment, female Sprague-Dawley rats were fed standard (ST) chow or CAF diet for 8 weeks. Additionally, both groups of animals were subdivided into 3 groups based on exercise intensity: sedentary (SED, n=9), mild exercise (TML, n=11) and intense exercise (TMH, n=9). The training groups followed a daily program of 30 minutes of exercise in a treadmill at low (TML, 12 m/min) or high (TMH, 17 m/min) speed. To identify differences in the metabolic profile between the 6 groups, serum samples were analysed by liquid chromatography coupled to mass spectrometry (LC-MS) in both positive and negative ionisation mode. Non-targeted metabolomics analyses were carried out using Mass Profiler Professional Software (Agilent Technologies) and multivariate statistical analysis based on a combination of principal component analysis (PCA), partial least squares for discriminant analysis (PLS-DA) and hierarchical clustering. Differences in the amounts of molecules were evaluated using two-way ANOVA ( $P < 0.05$ ) with Benjamini Hochberg correction. Finally, significant features were tentatively identified by LC-MS/MS through comparison of exact mass, retention time, and fragmentation information with spectral databases. The analysis of serum metabolome (6,594 aligned compounds in negative mode and 1207 molecular features in at least 75% of the samples within the same group; 5,973 aligned compounds in positive mode and 1,479 common features) revealed significant differences mainly due to diet and minor differences between training groups. Focusing on exercise, the results showed changes in the training groups of animals fed both diets in bile acids, carnitines, glycerophospholipids, retinoid and arachidonic acids derivatives, dipeptide Phe-Phe and androgens in comparison with sedentary animals. PCAs, PLS-DAs and clustering analysis showed a clear influence of diet and exercise in the metabolome. The influence of diet is far greater than the exercise. The use of comparative untargeted metabolomics allows discovering new potential serum biomarkers and characteristic metabolic signatures which could permit to predict the healthy status of the animals.

**New and vintage solutions to enhance the plasma metabolome coverage by single UHPLC-ESI-MS analysis**

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In LC-ESI-MS-driven untargeted metabolomics, unadequate preparation of complex biomatrices (i.e. blood fluids) and suboptimal data acquisition procedures are major sources of ion suppression phenomena, responsible for poor metabolome coverage and poor analytical reproducibility. Although the current initiatives focused on solving these methodological issues and setting objective criteria for method performance optimization, efforts have not converged yet into the definition of unbiased, standardized and globally accepted analytical protocols. This hinders field's progress and delays the necessary clear-cut change that would make large-scale studies possible, simple, fast and highly reproducible in inter-laboratory tests. We modularly investigated the response of the plasma metabolome coverage to specific variations in sample preparation (two SPE technologies, three sample-to-solvent extraction ratios) chromatographic separation (four RP columns, four elution systems) and ESI process enhancement procedures (two solvent quality grades, post-column modification of the mobile phase), while considering method compatibility to large-scale studies. Inter-method comparisons were carried out by using both commercial reference plasma (spiked vs unspiked) and plasma samples from an acute cocoa intervention study. Uni- and multivariate data analysis approaches were independently applied. The highest throughput metabolic fingerprinting was obtained by combining plasma hybrid extraction by OSTRO™ plate using a 1:12 sample to solvent ratio (v/v), an improved metabolite chromatographic separation through an RP column compatible with 100% aqueous polar phase (Atlantis T3, Waters), and to ESI enhancement by using UHPLC-MS purity grade methanol as both organic phase and post-column modifier. The method performance evaluation led to the following recommendations: submit plasma samples to hybrid extraction for removal of both proteins and phospholipids to minimize the major sample-dependent matrix effects, avoid sample extract evaporation and reconstitution if no peak shape distortion of the early-eluting metabolites is noticed, opt for a RP column designed to superior retention of highly polar species when no analysis fractionation is feasible, and enhance ESI efficiency by using UHPLC-MS quality grade solvents and postcolumn organic enrichment.

**Developing a metabolomics approach for characterising dietary intake in a free-living population**

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Effective 'healthy eating' strategies and improvements in population health will require the development of evidence-based interventions to increase consumption of specific food groups to lower chronic disease burden. These aspirations are predicated on the availability of robust tools for measuring dietary exposure. The M.A.I.N. (Metabolomics at Aberystwyth, Imperial and Newcastle) Study is using metabolomics-based approaches to discover food-derived metabolites in bio-fluids which could provide an objective method of measuring food intake. This PRE study aimed to measure the levels of compliance in a free-living population with a 3-day dietary intervention protocol, as well as test whether a standardised evening meal improves biomarker discovery. This PRE study also investigated urination behaviour and compliance with urine sampling procedures. Fifteen healthy adults (8 females) aged 22-63 years with a BMI < 30 kg/m<sup>2</sup> were recruited and provided with identical foods and drinks for three days on two separate weeks. They were randomised to receiving a standardised evening meal prior to commencing the intervention, or not, in the first week. Compliance was calculated as a percentage of total items eaten per day and percentage of participants collecting urine samples at the requested time points. Perceptions of hunger were determined using 10 point Likert scales with 10 being maximum. There was a high level of compliance with the intervention (e.g. day 1=93.8%). The standardised evening meal was eaten in all instances. 93% participants provided at least one urine sample post-dinner and collected a first morning void on each day of the study, whereas 67% provided a fasting sample each day. Post-breakfast and post-lunch urine samples were provided each day by 90% and 83% of people respectively. Males reported feeling more hungry than females and that there was not enough food (P=0.016 and P<0.001 respectively), whereas females reported feeling fuller than males and that there was too much food (P=0.008 and P=0.001 respectively). Overall, participants described the study as 'easy to follow', 'enjoyable' and 'interesting' and gave the experience a score of 8.7±1.3 out of 10. The analysis completed so far indicates that compliance with both the intervention and urine sampling regimen was high, suggesting the study design is suitable for purpose. We will now apply untargeted Flow Infusion fingerprinting and Liquid Chromatography-Mass Spectrometry profiling to the urine samples collected to identify novel metabolites of key foods relevant to public health which have been incorporated into the intervention. We will also identify which urine type is the most data rich and easily collected.



**Personalized food: science and visions**

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Like other sectors, food markets demonstrate also the thrive for diversification and individualization. Currently this is mainly based on food/taste preferences and enjoyment as visible in sectors such as coffee, chocolate or beverages. The highest level of personalization is achieved by internet offers to compose your 'own' food item such as a breakfast cereal (mymüsli) or chocolate. HEALTH is considered as the key driver of the 6<sup>th</sup> Kontradieff1 that defines the great economic cycles linked to societal changes. If health is taken into the food and nutrition sector, the key question is of course of how health-promotion can be achieved at the level of the individual and the foods consumed. The interrelationship between healthy life styles including diet and disease is traditionally explored by epidemiology and more recently by GWAS but new technologies such as whole genome sequencing pave the road for the GWAS II phase. Since this all is population based, the challenge is to translate the findings back onto the level of the individual. It remains to be seen of whether addressing a consumer as a 'genome' is helpful and increases for example the compliance to dietary counselling. Whereas genotyping is easy to perform, easily standardized and on high throughput level, human phenotyping is the bottleneck. If we want to link genotype to phenotypic outcome, we need to provide more comprehensive measures of the individual's phenotype, follow it over time and perform genotype/genome-based dietary intervention studies. This is much harder to do than genotyping and needs a highly standardized approach defined by the international science community with well-defined SOP's. Personalized nutrition thus needs proper phenotyping and similarly much better methods for food intake assessment. What can be predicted for the future is that there is a wide range of web-based health services which may include dietary counselling in combination with other lifestyle (i.e. exercise) interventions as well as 'personalized' food items that take individual preferences into account. It seems more feasible to assume that the entire supply of foods becomes personalized as consumers outsource this entirely by transferring the responsibility for food supply and for maintaining health to a service provider including a food delivery service. However, this will not be affordable for everyone. Health insurances may be part of such services and measures of compliance may be used in adjusting individual health insurance plans. Such a system of course challenges some fundamental principles of liberal societies and it remains to be seen how societies scope with this. Personalization can not only be the highest level of possible services but will clearly also be the highest level of personal responsibility. More difficult is to judge how technology developments in other areas will change food production and distribution. However, it can be envisaged that for example 3D-printers will rapidly develop and that they will be offered for producing foods in the private kitchen and by composing them based on the raw materials it can be personalized at a level or all essential nutrients and taking into account impairments in sensory or chewing/swallowing for example in elderly.



**Personalised nutrition: opportunities and challenges**

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Personalised nutrition offers many attractive options for the improvement of public health nutrition. However, there are still many challenges which need to be addressed. Personalised nutrition can operate at either of three levels or any combination thereof: personalised dietary analysis, personalised phenotype analysis and personalised genomic analysis. Personalised dietary analysis is the bedrock of clinical nutrition where patients receive one-on-one counselling developing a personalised dietary plan. However, this is now possible using the Internet and many such examples exist. A key challenge to this area of personalised nutrition is the need to balance convenience for the user and accuracy for the provider. The greater the level of accuracy sought, the lower the convenience to the user. A second challenge is the translation of optimal food choices into optimal meal planning and this will require moving from individual food codes to individual meal codes. A third challenge in this area is the need to validate any algorithms which might generate personalised dietary advice. Personalised phenotypic analysis using anthropometric variables is easily achieved on the internet type model of personalised nutrition but blood-based phenotypic data will not succeed on a mass level if attendance at a clinic is necessary. Thus there is growing interest in the use of dried blood spot technology to provide such data with a postal-based system operating. Phenotypic data can be extended to a very large number of variables but this creates the problem that as the number of variables grows, the number of untenable solutions grows. This has led to the concept of metabotypes or nutritypes where individuals who share a common metabolic profile will share a common base of personalised or group dietary advice. Finally, genomic analysis can be added to personalised nutrition but the use of association/correlation studies based on large samples of individuals will create ethical and legal issues and ultimately will require dietary intervention studies to verify the true association between diet, genotype and a diet related health phenotype. Food4Me is a large multi-centre study funded under FP7 and the presentation will draw on some of the findings of that study to illustrate the above comments.

**Organizing and integrating diverse data to improve decision making in health and nutrition research**

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Life today is becoming increasingly data intense. Raised consciousness about self-empowerment in health maintenance, accompanied by growing technological advances in molecular profiling assays and self-monitoring devices stimulates innovative approaches to our daily health management. The resulting availability of digital information on our lifestyle and health status (e.g. body weight and composition, energy expenditure, dietary habits, genetics information, clinical and physiological readouts, questionnaires outcomes, food purchase records etc.), provides an opportunity to integrate multiple aspects of health and behaviour into a unified, systems, and person-specific health profile. On a personal level, this will allow us to make better informed decisions to stay healthy. On a population level, this enables discovery of patterns which improve decisions on lifestyle intervention strategies. The challenge that remains is translation of this complex and diverse data into relevant knowledge to empower such decision making. We develop and apply network-based platforms for integration and mining of diverse data and knowledge to facilitate development of systems nutrition strategies for health improvement and maintenance. For consumers, this means organizing readouts of diverse assays and devices and integration of this information with the growing body of knowledge on molecular and physiological food-health interactions. This enables translation of existing but scattered information into intuitive profiles to guide, monitor and benchmark behavioural or lifestyle changes. For food producers, this approach facilitates assessment of health benefits of novel and existing products – often targeting specific consumer’s subpopulations (e.g. with certain genotype, phenotype or gut microbiome composition). Together, the ability to organize, integrate and exploit diverse and abundant information on food-health interactions supports development of novel healthy products targeted to optimally fit consumer needs, and contributes to a self-conscious, motivated, healthier and happier community.

**Baseline characteristics of the Food4Me study: a Pan-European web-based personalised nutrition trial**

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Interest in internet-based nutrition and lifestyle research is growing but there is little information on the profile of the European population interested in personalised nutrition (PN) delivered via the Internet. The Food4Me Proof of Principle Study is the largest pan-European Web-based intervention trial designed to investigate the effectiveness of PN on health-related behavioural change. This study aimed to describe the baseline characteristics of the European participants recruited into a Web-based PN intervention trial. Potential participants from seven European countries (Ireland, Germany, Greece, Spain, Poland, the Netherlands and the UK) were recruited via the Internet to emulate a Web-based PN service. Data on socio-demographic characteristics, health profiles, anthropometrics (weight, height and waist circumference) and lifestyle factors were self-reported via the Internet. 1,609 volunteers were randomised to the study. Participants had a mean age of 39.8 years (range 18-79 years) and 61% were female. The mean BMI was 25.5 kg/m<sup>2</sup> and 45% of the participants had a BMI ≥ 25 kg/m<sup>2</sup>. 25% of the participants had a waist circumference of ≥ 88 cm for women and ≥ 102 cm for men. 32% of participants reported a good level of physical activity, while 12% were current smokers. The present results confirm that women are more likely to volunteer to participate in nutrition-related studies including those delivered via the Internet. Our results show that the people interested in PN are broadly representative of the adult population and are not skewed towards either already very healthy people (the 'worried well') or individuals wishing to lose weight. Just under half (46%) of participants had a BMI ≥ 25 kg/m<sup>2</sup> and 32% were physically inactive, which is in line with the prevalence of these characteristics in European adults. This study shows that the European population participating in Internet-delivered PN is similar to that of the adult population, most of whom would benefit from improving diet and other lifestyle behaviours.

**The genetic predisposition for obesity and the consumption of sweetened beverages**

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Obesity is increasing side by side with the changes in our environment towards an imbalance between energy intake and consumption, and a more sedentary lifestyle. The consumption of both sugar- and artificially sweetened beverages (SSB, ASB) has increased during the last decades and this has been strongly associated with the risk of obesity and weight gain. Recently, high consumption of SSB was shown to accelerate the genetic susceptibility for future risk of obesity in an American population. Herein, we investigated if SSB consumption modifies the genetic risk of obesity in a Swedish cohort studies. For this study we included 21 999 healthy individuals from the Malmö Diet and Cancer Study cohort (MDCS) who had complete information about beverage intake, genotype and anthropometric measures. A genetic risk score (GRS) of 31 in genome wide association studies identified BMI SNP's were constructed. We performed cross sectional analyses of association between SSB and ASB use and BMI, and of interaction between of SSB and ASB and the GRS on BMI. Consumption of both sugar sweetened and artificially sweetened beverages was associated with a higher BMI ( $P > 0.0001$ ). We also observed a nominally significant interaction between the consumption of SSB and the GRS on BMI ( $P = 0.064$ ). In line with findings in the American prospective studies, we observed that high consumption of SSB accelerated the genetic risk of a higher BMI in a Swedish population.

**Ability of the online Food4Me food frequency questionnaire to estimate dietary intake**

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Online dietary assessment tools have the potential to become invaluable methods of assessing dietary intake for personalised nutrition services. Compared with traditional methods they have many advantages including the automatic storage of input data and the immediate generation of nutritional outputs. The aim of this study was to compare the recently developed online Food4Me food frequency questionnaire (FFQ) with the validated European Prospective Investigation of Cancer (EPIC-Norfolk) paper based FFQ. The Food4Me FFQ consists of 157 food items and was designed to include a range of portion size options and standardised colour photographs to quantify portion size for each food item. Participants completed the FFQ online and for most food items, participants were requested to choose their usual serving size among seven possibilities. Participants were recruited in two centres (Dublin and Reading) and each received the Food4Me FFQ and EPIC-Norfolk FFQ in random order. Participants with more than 4 weeks between completing both FFQ's were excluded from the analyses. The level of agreement between both methods was evaluated for both nutrient and food group intakes using the Bland and Altman method and cross-classification into quartiles of daily intake. 113 participants were recruited with a mean age 30±10 years (41% males, 59% females). Mean energy intakes were significantly higher for the Food4Me FFQ in comparison with the EPIC-Norfolk FFQ. Cross-classification into exact plus adjacent quartiles ranged from 77% to 97% at the nutrient level. Agreement was highest for alcohol (97%) and lowest for % energy from polyunsaturated fatty acids (77%). Crude unadjusted correlations for nutrients were moderate to high ranging from 0.43 to 0.86. Bland and Altman plots demonstrated an acceptable level of agreement between the two methods for energy, total fat and carbohydrate intakes as a percentage of total energy. Good agreement was also observed at the food group level, with more than 75% of participants correctly classified into the exact plus adjacent quartiles for each of the 35 food groups analysed. Food group correlations ranged between 0.41 for savouries (lasagne, pizza) and 0.90 for other fruits (apples, pears, citrus fruits). The results demonstrate that the online Food4Me FFQ has good agreement with the EPIC-Norfolk FFQ, for assessing both nutrient and food group intakes. This combined with its ease of use make the online Food4Me FFQ a useful tool for ranking individuals based on their nutrient intakes and could be potentially valuable for use in epidemiological studies. Food4Me was funded under the European Union 7<sup>th</sup> Framework Programme (KBBE.2010.2.3-02, Project no. 265494).

**COST Action POSITIVE: interindividual variation in response to consumption of plant food bioactives**

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To combat the burden of cardiometabolic disease, which constitutes a major public health issue in Europe, it is of crucial importance to develop efficient strategies that target the dietary behaviours of European consumers and improve the food supply. Plant foods are rich sources of a large range of bioactive compounds (polyphenols, carotenoids, phytosterols, glucosinolates) that beneficially affect our health, particularly by decreasing the risk of cardiometabolic diseases. However, heterogeneity in individuals' responsiveness to plant food bioactives can obscure associations between dietary intakes and health, hinder the identification of health benefits for specific population groups and limit our understanding of the exact role of the different bioactives. Interindividual variation in response to consumption of plant foods bioactives may come from differences in bioavailability or in biological responsiveness related to cardiometabolic health. Several determinants such as genetic background, gut microbiota composition, age or gender may explain these interindividual variations. However to date, limited and scattered data are available on the topic. The main objective of the COST Action POSITIVE is to create a European multidisciplinary and inter-sectorial network to tackle the question of the inter-individual variation in response to plant food bioactives consumption in relation to cardiometabolic health. POSITIVE will gather experts in human nutrition and plant food bioactives, in cutting edge omics technologies and in forefront research fields (such as human gut metagenome or personalised nutrition), together with representatives from regulatory agencies and Agrofood industry. The activities of this network will be focused on the identification of the main determinants involved in between subject variation regarding bioavailability and physiological responses to plant food bioactives consumption and on the integration of these findings to identify those with the greatest interest for translation into applications. The COST Action POSITIVE will constitute an ideal tool to promote European research in this active research field, provide scientific knowledge to regulatory authorities for a new generation of nutritional recommendations targeted to large population subgroups and foster the competitiveness of the European food industry by underpinning the development of new functional/customized foods. POSITIVE will start by the end of 2014 with more than sixty partners from 19 European countries.

**Perceived barriers to the uptake of personalised nutrition: a comparison between European countries**

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Personalised nutrition is a relatively new field of research aiming to provide personalised dietary advice, which can be based on an individual's genotype, phenotype, dietary and lifestyle data. According to this approach, dietary recommendations are tailored to meet personal nutritional needs. The main advantage is that genetic differences between individuals, which may interact with phenotype and co-determine health impacts of dietary choices, are explicitly taken into account. The success of a personalised nutrition approach will depend upon consumer acceptance and the barriers for the adoption of personalised nutrition may vary between different socio-demographic and cultural contexts across Europe. The goal of this analysis is to explore differences in perceived barriers to the uptake of personalised nutrition between consumers in different European countries. Data for this research were collected in February and March 2013, using on-line survey methodology. A total of 9,381 participants from 9 European countries (Germany, Greece, Ireland, Poland, Portugal, Spain, the Netherlands, the UK, and Norway) were quota sampled from an existing panel of a social research agency to be nationally representative for each country, on sex, age (18-29, 30-39, 40-54, 55-65 years) and education level. The questions were derived from prior qualitative research and formed part of a larger survey. Perceived barriers for the adoption of personalised nutrition were measured using 18 items for which responses were on a five-point scale where respondents had to indicate their level of concern regarding various circumstances that could potentially prevent them from taking up personalised nutrition. Factor analysis indicated the existence of three factors: trust; family; and, social barriers. Trust was related to confidence in the safety of personal data, while family and social barriers were related to concerns about the impact personalised nutrition could have on their social functioning. One-way ANOVA showed significant differences between the 9 European countries in perceptions of barriers to the uptake of personalised nutrition. In some countries, like Greece, Spain and Germany, trust barrier was dominant while in other (e.g. Poland and Ireland) family and social barriers were deemed more important. This implies that policies targeted at promoting adoption of personalised nutrition need to be adapted for each country. The results presented here are a part of Food4Me project that has received funding from the European Union's Seventh Framework Programme under grant agreement n°265494.

**Delivering anthocyanins in the gastrointestinal tract: processing conditions and food matrix effect**

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Over the last decade, many studies have demonstrated the potential benefits of using anthocyanins (ACs) in the prevention of metabolic syndrome. Nevertheless, most of these studies have only considered ACs as pure compounds rather than ingredients of bioactive-enriched foods. However, the AC-food matrix interaction could deeply impact on its digestibility and bioaccessibility, and hence, effectiveness. The objective of this study was to evaluate the effectiveness of different dairy and egg-based matrices on the delivering of ACs in the gastrointestinal tract under physiological relevant conditions. Two foods per matrix –milkshake and cream dessert for dairy matrices, and omelette and pancake for egg-based matrices – were produced under industrial conditions and fortified using a highly enriched AC powder obtained from red grapes (*Vitis vinifera*). After assessing the AC content in the foods by RP- HPLC and pH differential method, simulated gastro-intestinal digestions were carried out following the guidelines developed by INFOGEST (COST action FA 1005). This physiologically relevant model comprises an oral, gastric and intestinal phases, and takes into account among other factors: the presence of digestive enzymes at their physiological concentrations, electrolyte composition of each digestive compartment, temperature, calcium and bile salts concentrations, pH and digestion time. AC present in the soluble fraction at the end of the gastrointestinal digestion was quantified and bioaccessibility % of AC calculated for each food. The heat treatment applied during omelette and pancake production degraded around half of the AC added (47.39 and 58.58% of recovery respectively). However these products presented the best bioaccessibility values after *in vitro* digestion (44.14 and 40.91%). The solid texture of omelette and pancake could have protected AC from their degradation during the *in vitro* digestion process. In the non-cooked products, the low bioaccessibility values of AC observed after digestion (36.76% for milkshake and 15.83% for dessert) was compensated with the no losses of AC during its production. Although more experiment have to be done, it seems that non- cooked solid foods are the best option to maximize the delivering of AC in the gastrointestinal tract. Acknowledgements: The authors participate in the FP7 EU Project PATHWAY-27 'Pivotal Assessment of the Effects of Bioactives on the Health and Wellbeing, from Human Genome to Food Industry' (grant agreement no. 311876)



**Food4Me: Food choice motives and intention to adopt personalised nutrition**

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Motives underlying the selection of food are hypothesised to serve as barriers to, and facilitators of adopting personalised nutrition. The research presented here aimed to explore food choice motives in relation to the intended uptake of personalised nutrition among the general public. Findings of prior qualitative research published by the Food4Me group have been used to develop a questionnaire with which to probe consumer acceptance of personalised nutrition. Questionnaire items included Steptoe's Food Choice Questionnaire (FCQ) and an item enquiring as to an individuals' intention to take up personalised nutrition. Volunteers from the UK (n=1,061) and Ireland (n=1,020) were quota sampled based on age, sex, education level and region and were surveyed on-line. Multi-group confirmatory factor analysis was used to check the factor structure of the 36-items from the FCQ. Consistent with previous factor analyses the 36-items of the FCQ converged into 9-factors with good model fit (TLI=0.952, CFI=0.957, RMSEA=0.037, SRMR=0.041). Food choice motives were then entered into multiple regression analysis as predictors of intention to take up personalised nutrition, controlling for country, age, sex, and education level. Results indicated that food choice motives explained 21.9% of the variance in intention to adopt personalised nutrition, after controlling for country, age, sex, and education level ( $R^2$  change=0.219, F change (9, 2,064)=68.78,  $P<0.001$ ). The model indicated that 8 of the 9 food choice motives significantly predicted intention to adopt personalised nutrition. Individuals scoring higher on health ( $\beta=0.18$ ,  $P<0.001$ ), mood ( $\beta=0.10$ ,  $P<0.001$ ), convenience ( $\beta=0.06$ ,  $P=0.01$ ), natural content ( $\beta=0.07$ ,  $P=0.01$ ), weight control ( $\beta=0.19$ ,  $P<0.001$ ) and ethical concern ( $\beta=0.07$ ,  $P<0.001$ ) may be more likely to indicate intention to take up personalised nutrition. Those who scored higher on sensory appeal ( $\beta=-0.07$ ,  $P=0.01$ ) and price ( $\beta=-0.05$ ,  $P=0.02$ ) were less likely to indicate intention to take up personalised nutrition. Familiarity was not associated with intention to take up personalised nutrition ( $\beta=0.02$ ,  $P=0.48$ ). Personalised nutrition needs to take into consideration food choice of potential consumers, including those relating to convenience and ethical aspects of food choices whilst providing a variety of choices. This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 265494.

**The effect of low carbohydrate high fat (LCHF) diet**

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Use of low carbohydrate high fat (LCHF) diet is the most popular slimming diet at present in Norway. As a result, thousands of non-obese people use LCHF as well. The aim was to study the effect of a typical Atkins LCHF diet for 3 weeks affects risk factors for atherosclerosis in healthy young non-obese subject. Thirty healthy young subjects participated in a randomized controlled crossover (2×3 weeks) intervention study. Fasting blood samples were taken at baseline and after 3 and 6 weeks. Compliance with the diet was assessed by weight dietary record for 3 days and detection ketone bodies in urine and serum. Mean values of total cholesterol, Apolipoprotein B, LDL cholesterol, and HDL cholesterol increased by 27%, 28%, 37%, and 20% respectively,  $P < 0.001$  for all. Fasting triglyceride remained unchanged. Free fatty acid and urea increased with 80% and 42%, respectively. Most interestingly, marked individual difference in the response to LCHF was observed for all parameters. For example, plasma LDL cholesterol remained unchanged in two subjects, while increasing with more than 130% in two others. SREBP-1 mRNA was increased after 3 weeks on LCHF as measured in peripheral white blood ( $P = 0.009$ ). Three weeks on a LCHF diet resulted in a significant increase in all plasma lipids, except triglycerides. Atherogenic lipids, like LDL cholesterol and apolipoprotein B increased most. Despite individual differences, short-term use of LCHF diet in healthy non-obese subjects increased the level of atherogenic lipids and thus the calculated risk for developing atherosclerotic disease.

**Validation of web-based self-reported socio-demographic and anthropometric data: the Food4Me study**

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With the growing numbers of e-health intervention studies, concerns have arisen regarding the validity and reliability of Web-based self-reported (SR) data. The aim of this study was to assess the validity of Web-based SR anthropometric and socio-demographic data compared with standardized measurements performed face-to-face in a validation study (VS). A total of 140 participants from seven European countries, participating in the Food4Me Proof of Principle Study designed to investigate the utility of personalised nutrition, were invited to take part in the VS. Participants visited a research centre in each country within two weeks of self-reporting their data via a Web-based questionnaire. For SR data, participants were provided with detailed instructions, including photographs and online videos, on how to take each measurement. Differences between SR and VS were investigated using paired t-test, Intraclass Correlation Coefficient (ICC) and Bland-Altman limits of agreement for continuous variables (height, weight and BMI). The results demonstrate a strong ICC between SR and VS for SR anthropometric data (height 0.990 [95% CI 0.986 to 0.993],  $P < 0.0001$ ; weight 0.993 [0.991 to 0.995],  $P < 0.0001$  and for the derived estimates of BMI 0.983 [0.977 to 0.988],  $P < 0.0001$ ). However, the Web-based SR for height ( $\Delta$  0.2 cm [95% limits of agreement -2.3 to 2.7],  $P = 0.046$ ) was slightly higher than the VS measurements but lower for weight ( $\Delta$  -0.7 kg [-3.6 to 2.1],  $P < 0.0001$ ) and, therefore, for estimated BMI ( $\Delta$  -0.3 kg/m<sup>2</sup> [-1.6 to 1.0]  $P < 0.0001$ ). In addition, the Bland-Altman analyses show that just 5.7, 7.1 and 5.0% of the total number of participants fall outside 95% limits of agreements for height, weight and BMI respectively. A perfect concordance was found for age and sex between SR and VS data. Key findings: Our findings confirm the reliability of Web-based SR anthropometric and socio-demographic data collected in the Food4Me study. This reliability of SR data may have been aided by the provision of standardised instructions, including photographs and videos, to facilitate self-measurement by the participants.

**The nutritional research cohort: a new paradigm in nutritional research?**

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The use of quantified-self devices and self-measurement services is growing exponential. These tools and services enable everyone to measure their health with increasing accuracy. These devices and measurements, although primarily developed for consumer use, unlock new research and healthcare possibilities. Last year a research initiative based on standardized self-quantification data, the Nutrition Researcher Cohort was set up. Within the cohort, an initiation study started, aimed at the personal Nutritype focused on food intake and metabolomics. After the NRC initiation study, the NRC has shifted gear, and a new n=250 study is initiated. This study will collect information varying from personal genetics, metabolomics, microbiome, diet, phenotypic and health and lifestyle questionnaires. The primary, but not exclusive, focus of the research will be to explore the connection between diet, health, cognition, lifestyle, genotype and phenotype. This presentation will be about the organization of the n=250 study and building of the NRC infrastructure. Issues like ethics, type of measurements and data sharing will be addressed. More than 20 universities, institutes and SMEs joined the initiative. 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, in the future, the whole world could become a self-quantifying cohort with respect to personal healthcare.

**Health data cooperatives: towards the realization of P4 health**

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Healthcare is often ineffective and costs are steadily rising. This is in a large part due to the inaccessibility of medical and health data stored in multiple silos. Furthermore, in most cases molecular differences between individuals that result in different susceptibilities to drugs and diseases as well as targeted interventions cannot be taken into account. Technological advances in genome sequencing and the interaction of 'omics' data with environmental data on one hand and mobile health on the other, promise to generate the longitudinal health data that will form the basis for a more personalized, precision medicine. For this new medicine to become a reality, however, millions of personal health data sets have to be aggregated. The value of such aggregated personal data has been recognized as a new asset class and many commercial entities are competing for this new asset (e.g. Google, Facebook, 23andMe, PatientsLikeMe). The primary source and beneficiary of personal health data is the individual. As a collective, society should be the beneficiary of both the economic and health value of these aggregated data and (health) information. We posit that empowering citizens by providing them with a platform to safely store, manage and share their health related data will be a necessary element in the transformation towards a more effective and efficient precision medicine. Such health data platforms should be organized as co-operatives that are solely owned and controlled by their members and not by shareholders. Members determine which data they want to share for example with doctors or to contribute to research for the benefit of their health and that of society. Members will also decide how the revenues generated by granting third parties access to the anonymized data that they agreed to share, should be invested in research, information or education. Currently no functional Health Data Cooperatives exist yet. The relative success of health data repositories such as 23andme and PatientsLikeMe indicates that citizens are willing to participate in research even if – and in contrast to the cooperative model – the commercial value of these data does not go back to the collective of users. In the Health Data Cooperative model, the citizens with their data would take the centre stage in the healthcare system and society would benefit from the health-related and financial benefits that aggregation of these data brings.

**Ranges of phenotypic flexibility in 100 healthy subjects**

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In this study, we aimed to assess the ranges of phenotypic flexibility as a measurement of health within the apparently healthy population. Therefore, a total of 100 healthy subjects were enrolled (50 males, 50 females). We included males and females with a range in age (from 20 to 70 years) and in body fat percentage (low, medium, high), to ensure variation in phenotypic flexibility. Phenotypic flexibility was quantified by measuring in each volunteer the response of 160 markers to the PhenFlex challenge test (PC). The PC is a drink containing high amounts of fat and glucose. We have shown previously that this challenge test is able to quantify the adaptive capacities of most relevant metabolic processes for diet-related health. The markers were selected to monitor the response of the following 4 processes relevant for phenotypic flexibility: glucose metabolism, lipid metabolism, protein metabolism, and low grade chronic stress. At  $t=0$  (fasting) and 6 time-points ( $t=0.5, 1, 2, 4, 6$  and  $8$  h) after PC, blood was sampled from each subject to measure the total of 160 markers related to glucose metabolism, lipid metabolism, protein metabolism and low grade chronic stress. Next, the range in phenotypic flexibility of the study population was analysed in the 'health-space', a tool developed at TNO that shows individual phenotypic flexibility in a 4 dimensional space defined by the 4 metabolic processes. The health space showed a different adaptation to PC in the extremes of the recruited population: persons of young age with low to normal fat percentage had a significant different response to PC compared to persons of old age with normal to high fat percentage (both genders). Furthermore, the health space allowed the quantification of the individual metabolic health state. For example, specific cases became apparent where persons had a higher 'metabolic age' according to the health space result than would be expected based on solely their age and fat percentage. Ultimately, visualizing phenotypic flexibility in the health space before and after nutritional interventions will allow the evaluation of individual efficacy of the intervention. TNO has embraced this approach as a new road towards obtaining qualified health claims.

**Regulation of angiotensin-like protein 4 production during and after exercise**

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Angiotensin-like protein 4 (ANGPTL4) may regulate lipoprotein lipase-dependent plasma clearance of triacylglycerol from skeletal muscle during exercise. The aim of this study was to examine the importance of muscle in regulating ANGPTL4 in response to exercise. We sampled muscle biopsies and serum before, immediately after, and 2 h after 45 min of ergometer cycling. Sampling was done before and after a 12 weeks training intervention in controls and dysglycemic subjects. Moreover, adipose tissue biopsies were taken before and after the training intervention. The regulation of ANGPTL4 was also investigated in several tissues of exercising mice, and in cultured myotubes. ANGPTL4 levels in serum and expression in muscle were highest 2 h after acute exercise in both groups. Whereas ANGPTL4 was higher in muscle of exercising controls as compared to dysglycemic subjects, the opposite was observed in serum. In exercising mice, Angptl4 mRNA showed both higher basal expression and induction in liver as compared to muscle. Angptl4 mRNA level was much higher in adipose tissue than muscle and was also induced by exercise. We observed two mRNA isoforms of ANGPTL4 in muscle and adipose tissue in humans. Both were induced by exercise in muscle; one isoform was expressed 5-10-fold higher than the other. Studies in mice and cultured myotubes showed that both fatty acids and cortisol have the potential to increase ANGPTL4 expression in muscle during exercise. In conclusion, ANGPTL4 is markedly induced in muscle in response to exercise. However, liver and adipose tissue may contribute more than muscle to the exercise-induced increase in circulating ANGPTL4.

**Do tailored e-health interventions achieve weight loss and reduce central obesity: a meta-analysis**

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The numbers of overweight people continue rising globally, and more than one billion adults have a body mass index (BMI) greater than 25 kg/m<sup>2</sup>. Face-to-face intervention programs for treatment of obesity are known to result in significant weight loss but can be expensive and limited in reach. Personalised (or tailored) eHealth lifestyle-based interventions offer potentially attractive and scalable approaches for obesity management and prevention, but their effectiveness remains unclear. The objective of the present study was to conduct a systematic review and meta-analysis of randomised controlled trials (RCTs) that tested the effect of personalised, eHealth lifestyle-based interventions on weight loss and in reduction of central obesity in adults. Seven databases (ASSIA, CAB Abstracts, IBSS, Medline, Psych Info, Scopus and Embase) were searched using the following criteria: (1) RCTs; (2) personalised vs non-personalised advice; (3) Web-based interventions; (4) dietary-related outcomes; (5) weight or obesity-related outcomes, (6) adult participants ≥18 years. Data were pooled as weighted mean difference (WMD) and analysed using a random effects model. The full protocol has been registered in PROSPERO (CRD42014010275). We screened 1,152 abstracts of which 24 studies met our inclusion criteria and focused on body weight (n=4,375, mean age 45.2±9.4 years, 69% females) and 15 studies focused on waist circumference (n=2,908, mean age 43.9±8.1 years, 47% females) as intervention outcomes. These studies were conducted in the United States, the Netherlands, Australia and Japan. Pooled analysis of the results showed that personalised Web-based interventions were more effective in reducing body weight (WMD: -2.0 kg [95% CI: -2.4 to -1.7]; P<0.0001) and waist circumference (WMD: -2.3 cm [95% CI: -3.3 to -1.3]; P<0.0001) than non-personalised Web-based interventions in the short- to medium-term (12 to 48 weeks). These results provide strong evidence that personalised interventions delivered digitally are more effective in promoting weight loss and reducing central obesity, than non-personalised interventions at least in the short- to medium-term.



**Effect of tailored web-based interventions on fruit and vegetable consumption: a meta-analysis**

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Although the health benefits of greater fruit and vegetable consumption are well known, a large proportion of the population fails to meet the recommended intake of at least 5 portions per day. Tailored Web-based nutritional interventions are becoming a popular strategy for improving fruit and vegetable intake but the effectiveness of such Web-based interventions in increasing fruit and vegetable intake remains uncertain. The objective of the present study was to conduct a systematic review and meta-analysis of randomised controlled trials (RCTs), which tested the effect of personalised, Web-based nutritional interventions on fruit and vegetable intake. Seven databases (ASSIA, CAB Abstracts, IBSS, Medline, Psych Info, Scopus and Embase) were searched using the following criteria: (1) RCTs; (2) tailored vs non-tailored advice; (3) Web-based interventions; (4) dietary-related outcomes; (5) adult participants  $\geq 18$  years. Data were pooled as weighted mean difference (WMD) and analysed using a random effects model. The full protocol has been registered in PROSPERO (CRD42014010275). Nine studies were conducted in the United States and four in the Netherlands, Australia and Belgium. The mean age of participants in the studies ranged from 27.4-61.2 years. Pooled analysis of 13 studies (n=5,465 participants) showed that Web-based personalised interventions were more effective in increasing fruit and vegetable consumption (WMD: 0.41 serving portion per day [95% CI: 0.22 to 0.61];  $P < 0.0001$ ) than non-personalised advice. These results suggest that the use of Web-based, personalised interventions could be a more effective strategy for increasing fruit and vegetable consumption in the adult population, than non-personalised advice.

**Integrated and predictive approach for identifying determinants of health changes: role of nutrition**

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The overall objective of the project is to develop accurate and robust markers of the evolution of health status toward metabolic syndrome (MetS), and to determine to what extent nutrition is a major determinant, by using a multidisciplinary approach, putting together sociology, epidemiology, nutrition, statistics, and computer science. The project uses the French population-based cohort GAZEL, an on-going epidemiological study set up in 1989 (around 20,000 volunteers) among employees of the French national Gas and Electricity Company. The study consists in integrating demographic, socioeconomic, clinical, and biological data (from annual questionnaires, including food frequency questionnaires (FFQs)) to analyse food trajectories between 1998 and 2009. A case-control approach is used within a sub-cohort to characterize metabolic signatures and identify early discriminant factors predictive of MetS development. Male subjects between 52 and 64 years old, with high BMI ( $25 \leq \text{BMI} < 30$ ) who developed MetS (NCEP criteria) after the follow-up ('Case' group) were selected and compared for several parameters (socio-demographic, collected clinical, biochemical parameters, and food habits) with those who did not but still having some risk factors ('Control' group, matched for BMI, age and sex). Metabolomic analyses of serum samples collected between 2000 and 2003 will be performed using a mass spectrometry-based untargeted approach. Analyses of correlations between social characteristics, food habits and metabolic signatures will be done to build predictive models and determine whether integration of multidimensional parameters improves prediction. Among the 9,000 individuals having responded to FFQs in 1998, three different dietary patterns were identified, Western Diet (WD), Healthy, and Traditional French, after Multiple Correspondence Analyses (MCA) based on 22 food items. These patterns were confirmed from FFQs in 2004 and 2009. Food trajectories of individuals were found to be different according to their dietary patterns: strong decrease of 'WD' dietary pattern with time, in opposition with an increase in 'Healthy' and 'Traditional' behaviours. The at-risk sub-cohort appears to be representative of the whole cohort after MCA analyses on the 22 identified food variables, the nature of the dietary patterns, and the trend of changes. However, the evolution of behaviours for the Cases (MetS) and Controls is different in its amplitude: a deeper decrease in 'WD' pattern in the Cases in favour of an increase in 'Traditional' behaviour. These data will be correlated with metabolic changes identified from metabolomic profiles. This approach should provide new tools to better stratify at-risk populations. Furthermore, these analyses will allow identifying the role of nutrition as determinant and modulator in MetS etiology. Finally, this study should contribute to develop a more personalized nutritional prevention.

**The perceived impact of the National Health Service on personalised nutrition delivery in the UK**

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Personalised nutrition (PN) has the potential to reduce disease risk, and optimise health and performance. Whilst research has shown good acceptance of the concept of PN in the United Kingdom (UK), preferences regarding the delivery of a PN service (e.g. online vs face-to-face) are not fully understood. It is anticipated that the presence of a free at point of delivery healthcare system, the National Health Service (NHS), in the UK may have an impact on end-user preferences for deliverances. To determine this, supplementary analysis of qualitative focus group data relating to PN service delivery collected as part of the Food4Me project in the UK and Ireland (IE) was undertaken. IE data provided comparative analysis of a healthcare system that is not provided free at the point of delivery. A total of eight focus groups were conducted, four at each site (Reading, UK and Dublin, IE), between October and December 2011 using standardised semi-structured discussion protocols. In total 73 participants were recruited. Two focus group discussion guides: 'Consumer Perceptions of PN' and 'PN business models' were used in this research. Data were transcribed verbatim, verified by an independent researcher and analysed using a 'framework approach'. Overall, both countries preferred for PN services to be provided by the government and delivered face-to-face, which was perceived to increase trust, transparency and add value. Both countries associated paying for nutritional advice with increased commitment and motivation to follow guidelines. However despite the perceived benefit of paying, and contrary to IE, UK discussants still expected PN services to be delivered free at the point of delivery by the NHS. Consideration of this unique challenge of free healthcare that is embedded in the NHS culture will be crucial when introducing PN to the UK. This work is supported by the EU funded 7<sup>th</sup> 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>.

**Nutrition researcher cohort: integration of metabolomics into a self-quantification cohort**

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The Nutrition Researcher Cohort (NRC) was launched at the 10<sup>th</sup> NuGOweek 2013 and represents a new generation research platform. Thereby, each individual provides and owns her/his self-quantification data on her/his health data using various gadgets and/or clinical analysis. The NRC approach is thus in a stepping-stone 'development phase' for a new way to merge observational research, personal health empowerment, (nutritional) intervention studies and preventive healthcare with the goal to become a globally accepted standard. Thereby, the NRC aims to renew the relationship between research and healthcare. Participants actively take part in research studies, by monitoring their health status themselves, instead of being passive data-and-blood-donating humans. In addition, participants will have a direct health benefit from participating in research by personalized health information and advice based on their personal data. We here present the first results of the initiation study, which included metabolomics and metabolite profiling of Dried Blood Spots (DBS) of finger prick blood, accomplished by five participating laboratories. Furthermore, all participants were asked to enter self-quantification data on food intake, anthropometrics, age, blood pressure, glucose response and several more. In total, about 300 metabolites were identified and relatively or absolutely quantified using LC-MS/MS and GC-MS. In addition, four trace elements were determined using total-reflection X-ray fluorescence analysis. Metabolite profiles will be connected to self-quantification data, anthropometrics and food intake. Thereof, first examples of visualization will be presented. The final aim is to provide personal advice on health, lifestyle and diet. As the NRC is a 'crowd or citizen science' initiative, any lab is invited to participate in optimizing and supplying DiY sampling (e.g. DBS, mucosal swaps) and analytics, metabolomics, data visualization and interpretation or other relevant methods. If you like to become a contributing participant entering your own health data, please check our website <http://nrc.dbnp.org>.

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

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

**Associations between FTO variants and macronutrient intake: a systematic review and meta-analysis**

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Risk variants of the fat-mass and obesity (FTO) gene have been associated with increased obesity. However, the evidence for an association between the FTO gene and increased macronutrient intake has not been assessed systematically. Our aim was to evaluate whether variants in the FTO gene are associated with macronutrient intake. A systematic literature search in Medline, Scopus, EMBASE and Cochrane identified 21 studies that reported the association between macronutrient intake (total energy, total fats, carbohydrate and/or protein) and FTO genotype in adults. Individual study mean intakes and standard deviations, using per-allele comparisons, were evaluated and differences in energy and macronutrient intake per kilogram body weight were calculated. Random-effects models were used to assess the pooled effect sizes. The studies identified were published between 2008 and 2013 and included a total of 95,732 participants; with mean age of 52.0±9.2 years and BMI of 27.5±3.4 kg/m<sup>2</sup>. Weight (P<0.001) and BMI (P<0.001) were significantly higher in individuals carrying two copies of the FTO risk allele than in those with no copies. Preliminary findings show that carriage of the FTO risk allele is associated with 97.7 kJ/day (95% CI: -175.5, -20.0; P=0.014) and 4.8 kJ/day/kg body weight (95% CI: -5.63, -3.87; P<0.001) lower energy intake compared with non-carriers of the risk allele. Similarly, fat (-0.03 kJ, 95% CI: -0.05, -0.01; P=0.005), carbohydrate (-0.14 kJ, 95% CI: -0.16, -0.13; P<0.001) and protein (-0.03 kJ, 95% CI: -0.05, -0.02; P<0.001) intakes per kilogram body weight were lower in individuals carrying the FTO risk allele. Perhaps surprisingly, carriage of the FTO risk allele is associated with 1.3% lower energy intake compared to those with no risk. Further research is needed to determine whether the inverse relationship between carriage of the FTO risk allele and energy intake is independent of mis-reporting dietary intake and of changes in energy metabolism and physical activity.

**Profile of European adults interested in Internet-based personalised nutrition: the Food4Me study**

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Personalised nutrition (PN) advice, especially when delivered via the Internet, may promote greater dietary and lifestyle changes, but the characteristics of individuals interested in such a service are unclear. Therefore, the aim of this paper is to describe the characteristics of individuals interested in Internet-based PN advice in the Food4Me study. Participants in seven European centres (UK, Ireland, Germany, The Netherlands, Spain, Greece and Poland) were invited to participate in the study via the Food4Me website (<http://www.food4me.org>) and were screened using online questionnaires to collect data on socio-demographic, anthropometric and health characteristics. 5,562 individuals (age range 15-87 (mean age 41.6±13.1) years) expressed an interest in the study, of whom 65% were female. 6.6% were following a therapeutic diet and 12% reported a food allergy or intolerance. Germany had the lowest proportion of screenees following a therapeutic diet (2.4%) but the highest proportion of those reporting a food allergy or intolerance (17%). Of the 3,811 individuals who were eligible for the intervention study, 97% were white, 47% were overweight or obese and 29% were sedentary during work and leisure time. 13% of individuals screened were smokers, 45% were on medication and 47% reported the presence of a disease (such as cancer, diabetes or blood disorders). Individuals from Greece were more likely to be sedentary (42%) and overweight or obese (56%), whereas subjects from Spain showed the lowest use of medication (39%) and individuals from the UK had the lowest disease burden (38%). European individuals interested in participating in an online PN study were characterised as predominantly female, under the age of 45 years and with a normal or overweight BMI. Furthermore, high proportions of subjects were taking medication or suffering from a disease. Our data indicates that individuals volunteering to participate in an Internet-based PN study are representative of the wider European adult population, most of whom would benefit from improved diet and/ or greater physical activity.



**Nutritional genomics and personalized nutrition**

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Nutritional Genomics: is the study of how foods affect gene expression and how individual genetic variation affects the way an individual responds to nutrients in food. It can be divided into two disciplines: (1) Nutrigenomics: analyses the effects of bioactive food components (nutrients and non-nutrients) on gene expression. ex. To tested the effects of diets enriched with natural compounds, such as olive oil and salmon oil, in mice that spontaneously develop intestinal polyps. the researcher found the salmon oil-enriched diet, containing a high percentage of omega-3 polyunsaturated fatty acids, and, to a lesser extent, olive oil-enriched diet reduced polyp number and volume through a reduction of proliferation and a marked proapoptotic effect. These biological effects were mediated by an inhibition of fatty acid synthase and HMGCoA reductase gene expression and activity and an increase of ER $\beta$ /ER $\alpha$  ratio. these findings suggest that a proper dietary lifestyle could contribute to primary cancer prevention. (2) Nutrigenetics: studies how variations in genes among individuals can affect the reactions of an individual to specific dietary ingredients and even eating behaviour. in study to examined whether SNPs (AHR: rs6968865 and rs4410790; CYP1A1-CYP1A2: rs2472297 and rs2470893) and 6 additional tag SNPs in the AHR gene were associated with habitual caffeine consumption in a Costa Rican population, that already studied in populations of European descent were associated with habitual caffeine and coffee consumption. They found subjects who consumed >400 mg caffeine/d Compared with subjects who consumed <100 mg caffeine/d, were more likely to be carriers of the T, C, or T allele for rs6968865, rs4410790, and rs2472297. Personalized Nutrition: The goal of personalized nutrition is to identify individuals who benefit from a particular nutritional intervention (responders), and identify alternatives for those who do not (non-responders). Individuals should no longer be subjected to unnecessary diets they find unpleasant and ineffective when there may be an alternate dietary approach that is more effective. Currently, the RDA for a particular nutrient is defined by the development of deficiency diseases, but with advances in the field of genomics, there is a view that we should be more sophisticated and use biomarkers to define inadequacy and safe upper limits of intake. An example of this would be the epigenetic effects of nutrients. Epigenetics refers to the inheritance of traits that are not linked to DNA sequence, but rather to modifications of DNA, and among these modifications is DNA methylation, which affects gene expression.



**Metabotyping towards personalised nutrition**

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The mapping of the human genome back in 2000 has led to the concept of personalised nutrition where individuals receive dietary advice based on their specific diet, lifestyle and genetic make-up. Concomitant to the concept of personalised dietary advice, the idea of 'targeted' dietary advice to groups of similar individuals has developed. 'Metabotyping' refers to the process of stratifying individuals based on their metabolic profiles. The objectives of this study were to: (1) investigate whether cluster analysis could be used to identify metabotypes; and (2) examine the influence of genotype on these metabotypes. Biochemical and genetic data was obtained from the National Adult Nutrition Survey (NANS), a nationally representative sample of 1,500 Irish participants. Clustering techniques were employed to identify metabotypes based on four biomarkers of metabolic health (triacylglycerol, total cholesterol, HDL cholesterol and glucose). Descriptive statistics were used to characterise the clusters. Distributions of single nucleotide polymorphisms (SNPs) relating to obesity and lipid metabolism were examined (n=48). Gender, prevalence of metabolic syndrome and allelic distribution were assessed using chi square distributions. General linear models and Bonferonni post hoc tests were used to examine the differences across the clusters controlling for age, gender, BMI and multiple comparisons. Two step cluster analysis revealed the presence of three metabotypes. The clustering variables were found to be significantly different across the three clusters. Cluster 1 subjects were defined as having the highest HDL ( $2.01 \pm 0.35$  mmol/l), lowest triacylglycerols ( $0.97 \pm 0.33$  mmol/l) and lowest glucose ( $5.07 \pm 0.65$  mmol/l). Subjects in cluster 2 were characterised by having the lowest total cholesterol levels ( $4.28 \pm 0.68$  mmol/l). Cluster 3 subjects had the highest values in relation to all four clustering variables. These subjects were also the oldest and heaviest, with an average age of  $47 \pm 16$  years and BMI of  $29.3 \pm 4.7$  kg/m<sup>2</sup>, and had the highest prevalence of the metabolic syndrome (35.5%). Parameters relating to the metabolic syndrome were found to be significantly different across the clusters such as NEFA ( $P=1.33 \times 10^{-03}$ ) and adiponectin ( $P=3.72 \times 10^{-14}$ ). Cluster 1 subjects had the lowest levels of insulin resistance and highest levels of insulin sensitivity whereas the opposite was true for subjects in cluster 3. In relation to cardiovascular health, cluster 2 subjects were identified as having the lowest levels of all the apolipoproteins investigated (ApoA1, ApoB, ApoC2, ApoC3 & Apo E). No differences were found in terms of allelic distribution for any of the SNPs investigated across the clusters. Cluster analysis can be successfully applied for the identification of metabotypes. Three distinctly different clusters were characterised in terms of metabolic health and related parameters. The metabotypes identified were not found to be influenced by genotype in relation to the SNPs investigated. Potentially, tailored dietary advice could be developed for each metabotype. This may have future applications in healthcare settings towards the delivery of personalised nutrition.

**Human variation in ketogenesis revealed through challenge tests**

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Challenge tests are a powerful method for uncovering metabolic variation between humans, especially when combined with metabolomic analysis. A recent publication showed that a fasting challenge can reveal large variation in ketone body levels between individuals. To better understand the causes of human variation in ketogenesis, we asked what phenotypic factors are associated with plasma ketone body levels. To address this question we utilized the Nutritional Phenotype Database (dbNP), analysing multiple studies that included a challenge test in healthy volunteers. After a 36-hour fasting challenge, ketone body levels negatively correlated with body mass index (BMI). In studies applying high-fat challenge tests we detected similar anti-correlation with BMI as early as six hours after the meal. Interestingly, a correlation with BMI was not apparent after an overnight fast, implying that a challenge test is necessary to link the metabolic response to a phenotypic characteristic. The relationship of BMI with plasma levels of ketone bodies suggests that leaner individuals are quicker to synthesize ketone bodies from stored fat, demonstrating higher metabolic flexibility. In addition to BMI, blood glucose levels were also anti-correlated with ketone body levels after 36-hour fasting, suggesting an individual balance between the two energy sources. These observations represent first steps towards a detailed picture of human variation in ketogenesis.

**Modelling gene expression network for chronic diseases based on DNA microarray data and nutrigenomics**

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The chronic diseases such as breast cancer, type 2 diabetes mellitus (T2DM) and obesity is a serious health problem worldwide and the most prevalent in Mexican people. Gene expression by microarray technology is an important topic in the field of nutrigenomics research to investigate the complex networks of genes and their relationship to nutrition. We propose a network of interacting genes (FT0, PPAR $\gamma$ , ER-a, ER-b, and BRC2 BRC1) for chronic diseases like breast cancer, obesity and T2DM. Furthermore, we analysed the gene expression levels in customized diets based on vitamin D. The analysis of expression levels in chronic diseases will be guide of future research to design personalized diets based on Nutrigenomics.

**An artificial neural network for investigating metabolic components in BMI**

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General speaking, artificial neural networks (ANNs) are mathematical models developed for tackle and solve Non Linear Chaotic Complex Systems; the interactions between the variables, of that kind of systems, are sometimes unknown or too complex to define in advance. ANNs belong to Artificial Intelligence field, in fact learning is the process by which adapt themselves for better improve the ability to understand the systems and its future behaviour. Weight loss can be considered as the result of complex interaction among many factors interacting in a non-always linear way, so it is mandatory to investigate the whole process utilizing this logical/mathematical model. 350 hospitalized obese subjects, males and females, were studied. All subjects underwent a 21 day dietary treatment and a physical activity training. Dietary treatment was the same for males (1,500 kcal/day) and females (1,200 kcal/day) with a similar repartitioning of total energy (54% CHO, 25% FAT, 21% PROT). At the beginning (T0) and in the end of the study (T21), 44 variables (anthropometric, metabolic and psychological data) were measured. The BMI at T21 was chosen as output of the study. An 'associative learning' ANN was built with 50 layers and with the Levenberg-Marquardt training algorithms; after some epochs the ANN reached a good confidence level ( $R > 0.96$ ), so we were able to extract the 'synaptic weights' associated with every variables that represents the level of importance in forecasting the final BMI. The ANN detected 5 positive (having an direct relationship with the outcome) and 5 negative (having a inverse relationship with the outcome) variables as main determinants of the final output (BMI at the end of the dietary treatment). Positive variables were: body weight (kg) at T0; waist circumference (cm) at T0; systolic blood pressure (mmHg) at T0; height (cm), while negative variables were blood total protein concentration (g/dl) at T0, blood triglyceride (mg/dl) at T0; blood iron concentration ( $\mu\text{g/dl}$ ) at T0; age of obesity onset; and Beck Depression inventory. Data herein presented, represents the first step in the interpretation of a very complex metabolic process, i.e. the counteraction of obesity through the reduction of energy intake, and could set the stage for the set-up of systems supporting decisional operation simulating different scenario even in the nutrigenomic field. In the future, it could allow to identify the best dietary treatment in each single person (personalized nutrition).

**The impact of urine sampling on metabolome recognition accuracy after standardized lifestyle**

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Different nutritional habits are reflected in the small-molecule composition of urine. Urine contains a clear individual metabolic signature although embedded within a large daily variability. Given the potential of metabolomics to monitor disease onset from deviations from the 'healthy' metabolic state, we have evaluated the effectiveness of a standardized lifestyle in reducing the 'metabolic' noise using daily multiple urine sampling. We analysed the impact of the total number of samples on the recognition accuracy and report on the extent to which the urine metabolome can be normalized by our standardized lifestyle/dietary protocols. Urine was collected from 24 (5 men, 19 women) healthy volunteers over a period of 10 days: phase I, day 1-7 in a real life situation; phase II, day 8-10 in a standardized diet and day 10 plus exercise program. Data on dietary intake and physical activity have been analysed by a nation specific software and monitored by published protocols. Urine samples have been analysed by <sup>1</sup>H NMR followed by multivariate statistics. The individual fingerprint emerged and consolidated with increasing the number of samples and reaches ~100% cross-validated accuracy for about 40 samples. Diet standardization reduced both the intra-individual and the inter-individual variability; the effect was due to a reduction in the dispersion of the concentration values of several metabolites. Under standardized diet, however, the individual phenotype was still clearly visible, indicating that the individual's signature was a strong feature of the metabolome. Consequently, cohort studies designed to investigate the relation of individual metabolic traits and nutrition require multiple samples from each participant even under highly standardized lifestyle conditions in order to exploit the analytical potential of metabolomics. The presence of a strong signature of the personal phenotype, which goes beyond the individual lifestyle, will permit to assign deviations from donor-specific metabolic traits to biochemical alterations of his/her 'healthy' status for an early disease diagnosis. We have established criteria to facilitate design of urine metabolomic studies aimed at monitoring the effects of drugs, lifestyle, dietary supplements and for accurate determination of signatures of diseases.

**A high-fat, high-caloric drink as standard to perturb homeostasis: the PhenFlex challenge**

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Nutritional health research has major difficulties in demonstrating nutritional health effects. This is partly due to the subtleties of the effects, but a more fundamental cause lies in the design of studies and in selecting sensitive biomarkers. In the project 'Phenotypic flexibility and diet-related health' (PhenFlex), a new health paradigm is adopted, based on the thesis that normal physiology acts as absorber of daily shocks to maintain healthy homeostasis. In this view, adequate nutrition reduces the impact of metabolic shocks and ensures rapid recovery of homeostatic values of biomarkers. Essentially, the ability of our body to cope with daily-life challenges has been proposed as a new definition of health, with restoration of homeostasis as target resultant of various physiological stress responses. Physiology acts as a well-orchestrated machinery to adapt to the continuously changing environment, of which food takes a major share. We term this adaptive capacity 'phenotypic flexibility', which is a general principle in biological systems and which can be assessed by applying a challenge test. An expert review was performed on 44 studies applying different types of metabolic challenge tests. To acknowledge the multi-target role of nutrition, a relevant subset of 35 processes that govern optimal health, with high relevance to diet, was used to define phenotypic flexibility. Subsequently, we assessed the response of biomarkers related to this subset of processes to the different challenge tests. Based on the outcomes, we designed the PhenFlex challenge: a high-fat, high-caloric drink, containing 60 g palm oil, 75 g glucose and 20 g dairy protein in a total volume of 500 ml. The use of the standardized PhenFlex challenge in nutritional intervention studies is expected to demonstrate subtle improvements of phenotypic flexibility, thereby enabling substantiation of new health claims.

**Biomarkers for phenotypic flexibility as evaluated in healthy and diabetic subjects**

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‘Optimal health’ can be defined as the ability to adapt 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 challenge test. This study aims to investigate whether the PhenFlex challenge (PC), a drink containing high amounts of fat and glucose, can quantify the adaptive capacities of most relevant metabolic processes for diet-related health. We selected the following 5 processes as being most relevant for phenotypic flexibility: glucose metabolism, lipid metabolism, amino acid metabolism, inflammation and oxidative stress. Requirements of the challenge are: the selected processes have to respond to the challenge in healthy individuals (adaptation) and the selected processes have to show a different adaptation in state of impaired health. Therefore, two groups of male participants aged 30-70 years participated in this randomized cross-over study: 20 healthy males vs 20 males with Type 2 Diabetes Mellitus (T2DM). Both groups were given the PC. At t=0 (fasting) and 6 time-points (t=0.5, 1, 2, 4, 6 and 8 h) after PC, blood was sampled from each subject to measure markers of glucose metabolism (including glucose, insulin, glucagon, C-peptide, GLP-1, GIP and fructosamine), lipid metabolism (including free fatty acids, triacylglycerol, cholesterol (HDL, LDL and total), adiponectin, leptin) inflammatory response (CRP, sAA, sICAM, sVCAM), oxidative stress (glutathione ratio) and sets of metabolites measured by metabolomics technology (including endogenous metabolites involved in oxidative stress, glucose, lipid and amino acid metabolism). The selected processes showed a different adaptation in state of impaired health compared to healthy in response to the PC. Furthermore, the PC allowed the quantification of the involvement of 8 different organs in state of T2DM. Ultimately, the PC could become a standard method in dietary intervention studies to substantiate health claims.

**Phytochemicals for personalized health**

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Chronic inflammation is often a major contributor to the onset and progression of cardiometabolic dysfunction. Whether through effects on the inflammatory response system or independent of inflammation, plant-derived polyphenols comprise a micro-nutrient class important in CVD and other cardiometabolic traits. Polyphenols and other phytochemicals produce effects that can be categorized into four types of interactions: physiological, polyphenolic-microbiotic, polyphenolic-epigenetic, and polyphenolic-genomic. Certainly, the best-known effects are physiological: reducing blood glucose, acting as a diuretic, or raising HDL cholesterol, for example. This is illustrated by the phytochemical cinnamic acid, which activates insulin-mediated glucose transport through SLC2A2 (GLUT4) and effectively lowers blood glucose. Polyphenolic-microbiotic interactions appear as recent emergences in the primary literature and are profoundly important in phytochemical metabolism. Metabolism of polyphenols by the gut microbiome contributes to absorption of phytochemicals. For example, the flavanol quercetin is degraded by *Eubacterium ramulus* to 3,4-dihydroxyphenylacetic acid. Variations in polyphenolic metabolism exist due to the composition of the gut microbiome, with different levels of metabolic enzymes that vary from person to person. Polyphenolic-epigenetic interactions produce their effects through an interaction with DNA: either by inhibiting or activating histone modification enzymes or by chromatin remodeling. Curcumin, for example, activates histone deacetylase and inhibits histone acetyltransferase. Both proteins are employed by curcumin in down-regulating inflammatory genes responsible for transcribing TNF and IL6. Polyphenolic-genomic effects are exemplified by variations in the apolipoprotein (apo) E genotype. Individuals with the APOE4 allele have been demonstrated to be at higher risk for coronary artery disease, where the beneficial effects of quercetin, a polyphenol found in onions and tea, were moderated by this genotype. It was found that quercetin exerted a greater effect on waist circumference and BMI reduction in individuals carrying the APOE3 genotype compared with the APOE4 genotype. These and other examples from the literature have been collected into a database for the purpose of constructing molecular networks that depict relationships between diet, cardiometabolic traits, aging and inflammation. The resulting nutrition-inflammation interactome is a repository for information pertinent to understanding the diet-disease-inflammation axis and a vehicle for generating testable hypotheses in the laboratory setting. In the case of polyphenols, this database on polyphenolic effects and the resulting networks provide a mechanism to begin to construct individualized recommendations for phytochemical intakes.



**Determinants of pancreatic  $\beta$ -cell function**

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The incidence of type 2 diabetes mellitus (T2DM) has increased rapidly on a global scale. Oral glucose tolerance tests (OGTTs) are widely used to diagnose impaired glucose homeostasis.  $\beta$ -cell dysfunction is often seen in T2DM and the metabolic syndrome, where compensation of the  $\beta$ -cells to produce insulin, often due to insulin resistance, leads to the gradual failure of  $\beta$ -cells. The objective of this study is to identify potential determinants of  $\beta$ -cell function using human data and to perform follow-up analysis *in vitro*. This research focuses on data obtained from the Metabolic Challenge Study (MECHE). 214 healthy participants aged between 18-60 years were recruited and randomised to one of three groups; 76 participants received an OGTT and an oral lipid tolerance test (OLTT) on two separate clinical visits, 69 participants received an OGTT on two separate clinical visits and 69 participants underwent an OLTT on two separate clinical visits. Three measures of  $\beta$ -cell function were assessed for 130 participants who completed an OGTT and had valid glucose and insulin data at time-points 0 and 30 minutes.  $\beta$ -cell function,  $\beta$ -cell function adjusted for homeostatic model assessment of insulin resistance (HOMA-IR) and the disposition index (D.I.) were calculated for each participant. Linear regression analysis was applied using various anthropometric and biochemistry parameters as potential modulators of  $\beta$ -cell function. Ceramide data was also examined. *In vitro* experiments were performed using BRIN-BD11 pancreatic  $\beta$ -cell line. Cells were treated for 24 hours with resistin, globular (g) adiponectin and a high or low resistin-to-adiponectin ratio (AR index). A high AR index was defined as 20 ng/ml resistin and 10 nmol/l g-adiponectin, and a low AR index was defined as 10 ng/ml resistin and 20 nmol/l g-adiponectin. Examination of the determinants of  $\beta$ -cell function revealed that waist-to-hip ratio was the strongest anthropometric modulator, with  $\beta$ -coefficients of -0.331 and -0.299 for  $\beta$ -cell function/HOMA-IR, and D.I. respectively ( $P < 0.002$ ). For the biochemical parameters the AR index was the strongest modulator of  $\beta$ -cell function, with  $\beta$ -coefficients of -0.238 and -0.249 for  $\beta$ -cell function/HOMA-IR, and D.I. respectively ( $P < 0.05$ ). Analysis of the ceramides revealed that C10:0(OH) was significantly related to  $\beta$ -cell function/HOMA-IR and D.I. ( $P < 0.0001$ ). *In vitro* experiments revealed that the AR index was a more potent regulator of insulin secretion compared to either adipokine alone. Exposure to a high AR index caused a significant reduction in insulin secretion compared to the low AR index ( $30.97 \pm 1.99$  vs  $41.54 \pm 2.60$  insulin(ng/mg protein) ( $P = 0.005$ ). In conclusion, waist-to-hip ratio and AR index were significant modulators of  $\beta$ -cell function. The *in vitro* data provided further evidence that supported the AR index as a determinant of  $\beta$ -cell function. Future work will validate these findings in another cohort and investigate methods for modulating the AR index.

**Altered serum metabolites of type 2 diabetes mellitus in a prospective, nested case-control study**

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Application of metabolite profiling is believed to expand our etiological knowledge of type 2 diabetes mellitus (T2D). However, prospective studies applying broad untargeted metabolite profiling to reveal the comprehensive metabolic alterations preceding the onset of T2D are currently limited in number. We applied untargeted metabolite profiling in serum samples obtained from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort comprising 300 individuals who developed T2D after a median follow-up time of six years and 300 matched controls. For that purpose, we used ultra-performance liquid chromatography-mass spectrometry with a protocol specifically designed for large-scale metabolomics studies regarding robustness and repeatability. Following multivariate classification to select metabolites contributing strongest to disease classification, we applied multivariable-adjusted conditional logistic regression to assess the association between these metabolites with T2D. Among several alterations in lipid metabolism, we detected an inverse association with T2D for metabolites chemically annotated as lysophosphatidylcholine (dm16:0) and phosphatidylcholine (0-20:0)/0-20:0). Hexose sugars were positively associated with T2D, whereas higher levels of a sugar alcohol and a deoxy-hexose sugar reduced the odds of diabetes by about 60 and 70%, respectively. Furthermore, our study provides first suggestive evidence for a positive association of the circulating purine nucleotide isopentenyladenosine-5'-monophosphate with incident T2D. This study constitutes one of the largest metabolite profiling approaches of T2D biomarkers in a prospective study population to date. Our findings could be used to generate new hypotheses about diabetes etiology and to develop further targeted studies of a smaller number of potentially important metabolites.

**Venus and the clover: news about a hidden affair of copper and a trefoil family factor**

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The past decades have seen considerable advances in understanding the food requirement of copper and the strictly surveyed molecular mechanisms regulating its cell uptake, trafficking and distribution, but many aspects of its biological roles still remain unclear. Humans are rarely affected by severe copper deficiencies, with the exception of genetic defects (Menkes disease, Occipital Horn Syndrome), but it has been postulated that the Western diet provides suboptimal level of copper (2 mg/d) so that some populations may experience marginal and chronic copper deficiency. On the other hand, the significance of mild-moderate copper deficit and chronic borderline copper intake is not well established, although many reports demonstrate that low levels of copper induce pathological cardiovascular abnormalities, activity decrease of antioxidant enzymes (SOD, CCO, Catalase, GPx), hypercholesterolemia, higher LDL oxidation and increased risk of atherosclerosis. Our former studies of the intestinal transcriptome of rats fed a copper deficient diet revealed that two members of the trefoil factor family proteins (TFFs), TFF1 and TFF3, were significantly up-regulated following two months of dietary treatment. TFFs are small peptides that modulate the repair of gastric epithelium and are characterized by a conserved domain containing six cysteine residues that form a cloverleaf structure. Among these, TFF1 plays a key role in the correct formation of the mucous layer, promotes the epithelial restitution after injury and protects the integrity of the epithelial barrier. TFF1 homodimer formed through its seventh cysteine is the most active form as motogenic factor, as well as it may act as docking site for *H. pylori* adhesion on gastric surface. Our studies pointed out that the peptide is able to bind copper *in vitro*, favouring its homodimerization. The expression of TFF1 is strongly induced after mucosal injury, it is frequently lost in gastric cancer and overexpressed in most cases of breast cancer. Our most recent results show that copper overload leads to a reduced secretion and an increased intracellular localization of TFF1, mainly into the trans-Golgi compartment. In addition, we demonstrate that TFF1 hyper-expressing AGS-AC1 cells store higher levels of copper if compared to non-induced cells. These findings suggest that TFF1 levels might play a role in copper transport and homeostasis. Furthermore we evaluated the influence of copper levels on the rheological properties of the mucus produced by HT29-E12 intestinal goblet cells, in order to verify possible alterations of the mucus where *H. pylori* localizes onto the gastrointestinal epithelia. Changes in dietary copper levels could then alter the gastrointestinal microenvironment thus modulating the colonization of the microorganism, as well as the pathological consequences related to the chronic inflammation of the gastrointestinal tract resulting in nutrient malabsorption and cancerogenesis.

**Metabolomic profile of muscle differs between young and old and between healthy and frail elderly**

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Populations around the world are rapidly ageing and concomitant loss of physiological functions compromises independency of the elderly. The major contributor to the frailty syndrome of ageing is skeletal muscle loss, which can lead to increased disability in the elderly population. Of all physiological changes that occur at an advanced age those that impact on muscle function are most susceptible to modulation by lifestyle. The underlying regulatory mechanisms are however only partly understood. Understanding the process of ageing at the metabolic level, might pave the way to grow old while maintaining quality of life. In this study we assessed the muscle biopsy metabolome of healthy young, healthy elderly and frail elderly subjects to determine the effect of ageing on the metabolic signature of muscle tissue. Moreover, effects of whole-body resistance exercise training on the muscle biopsy metabolome of elderly subjects was examined. Metabolite levels were measured in skeletal muscle tissue of 30 young male (Y), 65 healthy elderly men and women (HE) and 44 frail elderly men and women (FE). We also measured metabolites in plasma for 50 Y, 75 HE and 64 FE. In addition, we measured plasma (46 HE, 34 FE) and in tissue (38 HE, 24 FE) metabolite levels after 6 months of resistance-type exercise training, with either 3 (HE) or 2 (FE) exercise sessions per week. In total 96 metabolites including amine, acylcarnitines, organic acids, oxylipins and nucleotides were measured. For both healthy and frail elderly part of the subjects received 15 gram protein supplement, once a day for healthy elderly and twice a day for frail elderly. Primary differences in muscle metabolite levels between old and young were mostly amino acids. Similar differences were also observed when comparing frail elderly with healthy elderly subjects. The difference in amino acid levels could potentially be explained by a higher protein turnover, which would be in line with the observed induction of genes responsible for tissue remodelling. Within the three groups, the muscle metabolome correlated with muscle performance in a pattern that suggests enhanced vascularisation. Training affected muscle levels of acylcarnitines in both healthy and frail elderly. A difference in effects on short and long chain acylcarnitines suggest a different utilisation of branched chain amino acids and fatty acids in both groups.

**Strength training improves muscle health-related gene expression in frail and healthy elderly people**

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As the global population ages it is increasingly important to maintain the independence of our elderly population. The function of the skeletal muscle system plays an important role in the independence of elderly and decreased skeletal muscle mass and function is associated with frailty. Several strategies exist to increase skeletal muscle mass in elderly people, including resistance-type exercise training. In this study we examine the effects of resistance-type exercise training in frail and healthy elderly subjects on the skeletal muscle transcriptome. We have two goals for this study. The first goal is to better understand frailty by comparing whole genome gene expression in healthy elderly, (pre-)frail elderly and young subjects. The second goal is to determine the effect of resistance-type exercise training on the skeletal muscle transcriptome in elderly people. Transcriptome profiles were measured in muscle biopsies collected from 52 young subjects, 61 (pre-)frail elderly (FE) and 73 healthy elderly (HE) subjects. In addition, we measured the transcriptional response following 6 months training in 31 samples from the FE subjects and 41 samples from the HE subjects collected after the training period. Training consisted of progressive whole body resistance-type exercise performed twice weekly for FE and three times weekly for HE. Exercise training showed a significant effect on the gene expression profiles in both FE and HE (431 and 1395 significantly affected genes, respectively). At baseline 178 genes were differentially expressed between FE and HE subjects (q-value below 0.05). 306 genes were robustly changed in both groups after resistance type exercise training. Of these genes 237 genes were also significantly different between young and older subjects. Affected genes include genes involved in mitochondrial function, ribosomal function, vascularization, neural development, connective tissue development and energy metabolism. Genes belonging to gene cluster protocadherin gamma play a role in axonal guidance and were found differentially expressed after training, between young and old and between FE and HE. In our data protocadherin gamma genes are also associated with muscle strength. We hypothesize that this gene cluster plays a role in denervation-reinnervation cycles in ageing muscle. Resistance exercise improves muscle health accompanied by changes in expression levels of related genes in both frail and healthy elderly people. Some of the age-related differences we observed between young and older subjects appear to be reversed towards the younger phenotype after resistance-type exercise training. This suggests a significant remaining plasticity of senescent skeletal muscle to adapt to regular exercise.

**The regulation of myostatin in relation to training, obesity and pre-diabetes**

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Myostatin is a secreted peptide mostly known for its inhibiting effect on muscle growth. However, myostatin may also play a role in energy metabolism. It has been shown to inhibit Akt signalling and increase insulin resistance in skeletal muscle of mice, and it seems to modulate adipogenesis, by inhibiting adipocyte differentiation and counteract obesity. In this study we investigated the regulation of myostatin in response to acute and chronic physical activity and in relation to dysglycemia and overweight. We performed an extensive human training intervention study; healthy, normalweight (n=13) and pre-diabetic, overweight (n=13) male subjects exercised 4 times weekly for 12 weeks. Blood samples and muscle biopsies were collected before and after a 45 min acute exercise test, both before and after the 12 week training intervention. Subcutaneous adipose tissue biopsies were taken at one time point before and after the training intervention. The mRNA expression of myostatin and its receptor, ACVR2B (measured by global mRNA sequencing), were downregulated in m. vastus lateralis after 45 minutes training (fold change=0.54,  $P<0.0001$  and fold change=0.8,  $P=0.001$ , respectively) and myostatin was also downregulated after 12 weeks of chronic training (fold change=0.66,  $P<0.001$ ). In adipose tissue the regulation was in the opposite direction – a 22% increase after 12 weeks ( $P=0.04$ ). The plasma concentration of myostatin increased 13% after acute exercise ( $P<0.001$ ), and it did not change in response to chronic training, suggesting a more local effect of myostatin signalling. The plasma concentration did not differ between control and dysglycemic subjects. There was a tendency for the control subjects to have a lower expression of myostatin in muscle than dysglycemic subjects, but a higher expression in adipose tissue (31%,  $P=0.07$  and 54%,  $P=0.001$  at baseline and after 12 weeks, respectively). Interestingly, there was a negative correlation between myostatin mRNA in muscle and glucose infusion rate (GIR, mg/kg/min,  $r=-0.49$ ,  $P=0.01$ ), whereas in adipose tissue this correlation was in the opposite direction ( $r=0.4$ ,  $P=0.05$ ). There was also a good correlation between the change in muscle myostatin expression and the increase in fat free mass ( $r=0.54$ ,  $P=0.004$ ) or the reduction in fat percentage ( $r=-0.5$ ,  $P=0.009$ ) after 12 weeks of exercise. In conclusion, myostatin is differentially regulated in muscle and adipose tissue in relation to training, obesity and prediabetes. The expression of myostatin in muscle is negatively correlated with insulin sensitivity, whereas the expression in adipose tissue follows an opposite pattern.

**The myokine decorin is regulated by contraction and involved in muscle hypertrophy**

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The health-promoting effects of regular exercise are well known, and specific myokines released from contracting muscle might mediate some of these beneficial effects. One of these myokines is the small leucine-rich proteoglycan decorin, which is part of the extracellular matrix. Although it has been described as a myokine for some time, its regulation upon physical activity and impact on skeletal muscle has not been investigated in detail. Therefore, we aimed to study the release and expression of decorin upon skeletal muscle contraction using different approaches. Moreover, *in vivo* overexpression of decorin in murine skeletal muscle was used to study effects of enhanced expression of this myokine. We used electrical pulse stimulation to induce contraction in cultivated primary human skeletal muscle cells and observed a 1.3 fold enhanced release of decorin compared to non-contracting controls ( $n \geq 6$ ,  $P < 0.05$ ). Moreover, in response to acute resistance exercise in humans circulating decorin levels increased significantly immediately after the challenge (1.3 fold vs basal,  $n=10$ ) and returned to basal levels within 2 h of rest. Decorin expression in human skeletal muscle was significantly enhanced after 12 weeks of exercise intervention ( $n=13$ ). In mice, 4 weeks of endurance training resulted in a significant increase of decorin expression in skeletal muscle (1.3 fold vs sedentary mice,  $n=6$ ). Because decorin directly binds myostatin, a potent inhibitor of muscle growth, we investigated a potential function of decorin in the regulation of skeletal muscle growth. *In vivo* overexpression of decorin in murine skeletal muscle promoted expression of the pro-myogenic factor MyoD, which is negatively regulated by myostatin. We also found MyoD, one of the major regulators of myogenesis, and follistatin, a potent regulator of skeletal muscle growth, to be increased in response to decorin overexpression. Moreover, muscle-specific ubiquitin ligases atrogin1 and MuRF1, which are involved in atrophic pathways, were reduced by decorin overexpression. In conclusion, our study establishes decorin as an exercise-regulated myokine that is secreted in response to muscle contraction. Following *in vivo* overexpression of decorin we found increased expression of different genes, which are involved in pathways of skeletal muscle growth. This was accompanied by reduced catabolic processes often suppressed during muscle growth. Consistent with a putative function in muscle hypertrophy enhanced levels of plasma decorin were observed in response to hypertrophy-promoting strength exercise. Thus, we hypothesize that decorin secreted from skeletal muscle cells in response to exercise is involved in restructuring of muscle during hypertrophy.



**Subsarcolemmal lipid droplet responses to exercise training**

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Muscle lipid stores and insulin sensitivity have a recognized association although the mechanism is unclear. We investigated how a 12 week supervised exercise intervention influenced muscle lipid stores in sedentary overweight dysglycemic subjects and normal-weight control subjects (n=18). Muscle lipid stores were measured by magnetic resonance spectroscopy (MRS), electron microscopy (EM) point counting, and direct EM lipid droplet measurements of subsarcolemmal (SS) and intermyofibrillar (IMF) regions, and indirectly, by deep sequencing and real-time PCR of mRNA of lipid droplet-associated proteins. Insulin sensitivity and  $VO_2$  max increased significantly in both groups after 12 weeks of training. Muscle lipid stores were reduced according to MRS at baseline before and after the intervention, whereas EM point counting showed no change in LD stores post-exercise, indicating a reduction in muscle adipocytes. Large scale EM quantification of LD parameters of the sub-sarcolemmal LD population demonstrated reductions in LD density and LD diameters. Lipid droplet volume in the sub-sarcolemmal LD population was reduced by ~80%, in both groups, while IMF LD volume was unchanged. Interestingly, the lipid droplet diameter (n=10,958) distribution was skewed, with a lack of small diameter lipid droplets (smaller than ~200 nm), both in the SS and IMF regions. Our results show that the SS LD lipid store was sensitive to training, whereas the dominant IMF LD lipid store was not. Thus, net muscle lipid stores can be an insufficient measure for the effects of training.



**The effects of chronic hypothyroidism on ovarian follicular development in rats**

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In recent years it has become more and more clear that changes in thyroid hormone (TH) levels affect metabolism and impair female fertility. TH can directly influence oocyte maturation but also indirectly through its action on follicular granulosa and stromal cells. (Pre)pubertal hypothyroidism results in a reduced body weight, suggestive of an effect on growth and metabolism. Furthermore, antral follicle development is negatively affected. Our own initial data show that hypothyroidism in adulthood leads to premature ovarian failure, due to an accelerated depletion of the primordial follicle and primary follicle pool, respectively. Whether TH affects the recruitment of primordial follicles in the growing pool or influences follicular degeneration is at present not clear. The objective of this study was to investigate the effect of chronic hypothyroidism on rats ovarian follicle development, using RIA for hormone, (immuno)histology. Before mating, dams were put on a diet consisting of an iodide-poor feed supplemented with a low dose of perchlorate to inhibit the uptake of iodide by the thyroid and, with their offspring, were kept on this diet until death. In present study, 12-day-old, 64-day-old and 120-day-old offsprings of these dams were researched. In the pups from day 21 postpartum onwards, plasma thyroid-stimulating hormone levels were increased 20-fold, whereas thyroxine, tri-iodothyronine and free tri-iodothyronine levels were severely depressed, confirming a hypothyroid condition. Our preliminary results showed that a significant increase in TSH levels was measured for the first time in the offspring at the age of 12 days. However, there was no difference in ovarian primordial follicle numbers compared with age-matched controls, confirming that primordial follicle pool formation during foetal development was not affected. In 64-day-old rats, plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were significantly elevated compared with age-matched controls. The number of antral follicles was significantly decreased. The number of primordial follicles are being counted now. We are also investigating 120-day-old rats now. In conclusion, the results show that dietary-induced chronic hypothyroidism may lead to premature ovarian failure in the rat.

**Regulation of perilipin 4 in human skeletal muscle by long-term exercise**

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Perilipin family proteins (Plins) coat the surface of intracellular neutral lipid storage droplets in several cell types. Studies across many species demonstrate that Plins are important for the regulation of lipid turnover by interacting with lipases and other regulatory proteins associated with the lipid droplet surface. *In vitro* data on adipocytes reveal perilipin 4 (PLIN4) as an exchangeable Plin protein, existing in the cytosol and being recruited to the lipid droplet during lipid droplet formation or residing on nascent high-density droplets awaiting a lipid challenge. The exact role of PLIN4 in droplet synthesis is unknown. However, it has been suggested that it influences the size and shape of the droplet. Although it is known that PLIN4 is expressed in skeletal muscle, data on its regulation by chronic exercise is scarce, and the distribution of PLIN4 within skeletal muscle has not been investigated. We investigated the location and expression of PLIN4 in skeletal muscle, using human biopsies from m. vastus lateralis before and after 12 weeks of combined endurance and resistance exercise. The exercise intervention study was conducted on lean normoglycemic ( $23.5 \pm 2.0 \text{ kg/m}^2$ ,  $n=13$ ) and overweight dysglycemic subjects ( $29.0 \pm 2.4 \text{ kg/m}^2$ ,  $n=13$ ). Muscle biopsies were studied using immunohistochemistry, electron microscopy, quantitative RT-PCR and RNA sequencing. The mRNA expression of PLIN4 was down-regulated by 19.4% in response to 12 weeks of training. The change in expression was more pronounced in healthy controls (-24.3%) compared to dysglycemic subjects (-14.4%). Using two different PLIN4 antibodies, we observed PLIN4 staining close to the cell membrane, as well as on adipocytes in the intramuscular interstitium as confirmed by positive co-staining with PLIN1. PLIN4 mRNA was positively correlated with lipid droplets diameter in the subsarcolemmal region as monitored with electron microscopy ( $r=0.72$ ,  $P=0.019$ ). In conclusion, PLIN4 mRNA is down-regulated by long-term exercise and the protein is located both in muscle adipocytes and within muscle fibres. Our preliminary data; suggest that PLIN4 is specifically located to the subsarcolemmal region of muscle fibres.

**Meat intake unmasked by plasma and urinary 1-methyl-histidine in a 4-days human trial**

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Numerous epidemiological studies show that a high intake of red meat might be strongly associated not only with an increased incidence of type 2 diabetes but also with various forms of cancer. As current instruments to quantify food intake such as food frequency questionnaires are biased due to errors like under-reporting, alternative methods are needed to provide more reliable data on food consumption. We here report that 1-methyl-histidine might serve as a surrogate exposure marker reflecting meat intake. Data on plasma and urinary methyl-histidine levels were obtained from a pilot-study in which 15 healthy volunteers underwent five different metabolic challenge tests (fasting for 36 hrs., refeeding, OGTT, lipid tolerance test, physical exercise) over a total period of 4 days. This period was divided into two separate periods of two consecutive days interrupted by at least four weeks. On the evening before each study period all volunteers were invited into the study centre and were served a standardized convenience dish containing a standardized portion of chicken meat. During the test days volunteers received defined liquid diets without meat or meat-based products. Following, during the study a total of 56 blood samples was taken at defined time points. Furthermore, 20 urine samples were collected and were profiled by using different techniques e.g. a LC-MS/MS approach for a comprehensive analysis of metabolites including quantification of 40 amino acids and their derivatives. Whereas most amino acids displayed challenge-specific changes in plasma concentrations of 2 methyl-histidine metabolites did not show a dependence on any of the different metabolic conditions. However, plasma levels of 1-methyl-histidine declined over both two-day test periods with almost identical elimination rates whereas levels of 3-methyl-histidine remained constant. Urinary excretion mirrored those changes over time with similar kinetics. Our data strongly suggest that 1-methyl-histidine may serve as an exposure marker of previous meat intake and that its plasma levels are not affected by fasting, exercise or by any other metabolic condition.

**The development of a meal coding system and the examination of its impact on nutrient intake**

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Dietary pattern analysis is an emerging technique that explores the whole diet, rather than single nutrients; taking this approach, significant advancements have been made in understanding the interactions between diet and disease. However, to date, few studies have investigated meal patterns. Meal-pattern analysis is complex due to the number of potential variations of food combinations between individual meals. Meal coding attempts to describe these combinations in a numerical format, for use in meal-pattern analysis. The objectives of this study are to develop a meal coding system and model the impact of using such a coding system on estimated nutrient intakes, in a nationally representative population. Dietary data used in this analysis was acquired from the Irish National Adult Nutrition Survey (NANS), which contains detailed dietary intakes of a nationally representative sample of 1,500 healthy adults, based on 4-day food diaries. The dataset contains 2,552 individually-coded food items, and self-reported meal types for each eating occasion (Breakfast, Light meals, Main meals, Snacks and Beverages). All food items were grouped into 20 food groups e.g. Cereal, Bread. Combinations of these food groups in each meal was determined, and grouped into common generic meals. The nutrient content of generic meals was determined by calculating the average nutrient composition of the meals in each generic meal. Mean daily intakes were calculated for the substituted generic meal dataset and for the original dataset. Comparisons of these intakes were performed using statistical analysis including Mann Whitney U tests, Spearman correlations, cross-classifications, and Bland and Altman analysis. Original meals were reduced to generic meals: 5,588 Breakfasts were reduced to 14 generic Breakfasts, 4,279 Light meals were reduced to 18 generic Light meals, 5,653 Main meals were reduced to 14 generic Main meals, 4,293 Snacks were reduced to 9 generic Snacks, and 2,135 Beverages were reduced to 8 generic Beverages. Comparing mean daily intakes of nutrients between the original (O) and generic (G) datasets, significant differences were observed for energy (O:  $2,008.25 \pm 656.06$  kcal; G:  $1,893.23 \pm 328.30$  kcal,  $P < 0.05$ ). However, no significant differences were observed for macronutrients, such as fat (O:  $71.74 \pm 12.87$  g; G:  $75.70 \pm 29.36$  g) and carbohydrate (O:  $218.18 \pm 41.47$  g; G:  $228.29 \pm 78.94$  g) and for many micronutrients including iron, calcium, phosphorus, sodium, vitamin B6, thiamine and riboflavin. Bland and Altman plots showed good agreement (<5% outside limits of agreement) for energy, protein and carbohydrate. Significant differences were seen for some micronutrients including vitamin C, vitamin E, folate and vitamin B12, ( $P < 0.05$ ) and vitamin D ( $P < 0.001$ ). Cross-classification analysis for those placed into exact and adjacent quartiles ranged between 72-88%. Spearman correlations were in the range 0.27-0.66. This meal coding system revealed good agreement for many nutrients, implying that use of generic meals may be adequate when examining nutrient intakes in the population, giving promise its applications, in future nutritional analysis. This work was funded by Food4Me (KBBE.2010.2.3-02, Project no. 265494).

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