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MOLECULAR NUTRITION – UNDERSTANDING HOW FOOD INFLUENCES HEALTH

BOOK OF ABSTRACTS

NuGO week 2017
Varna, Bulgaria
28-31 August 2017

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WELCOME TO NUGOWEEK 2017

It is a great pleasure and honour for me, as a chair of the Organising Committee to invite you to the 14th annual Nutrigenomics conference held this year in Varna, Bulgaria. NuGO Week 2017 will be held for the first time in Eastern Europe and will be hosted by the Medical University of Varna. Eastern Europe is a region known for its millennial history, rich culture, traditional scientific and engineering excellence that are most recently combined with irrepressible spirit to pursue freedom, democracy and economic development.

The focus of this year's meeting is on *Molecular Nutrition – Understanding How Food Influences Health.* The scientific programme will include plenary lectures from leading experts and scientific reports on themes that are relevant to the nutrigenomics field and to the fast changing European research landscape, such as model systems in nutrition research and personalised medicine. The parallel programme of the conference will include a pre-NuGO week course on *Advances in the Use of New Technologies in Molecular Nutrition Studies* for young researchers, PhD students and post-docs.

You have all chosen to be a part of our event because of our mutual passion for development of the research areas of molecular nutrition, personalised nutrition, nutrigenomics and nutritional systems biology. Our passions help us all to come together as one and the energy we create as one allows us to achieve our individual as well as group goals.

I am very confident the conference will bring the best of the participants and will foster future collaborations between partners.

I would like to cordially invite you to Varna to join your colleagues for mutual sharing of information and to experience the hospitality of our country.

May you have a very successful conference!

Prof. Diana Ivanova, PhD, DSc
On behalf of the Organising and Scientific Committee

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PRECISION NUTRIGENOMICS VIA MECHANISM-ANCHORED BIG DATA SCIENCE: ANALYTICS FOR SINGLE-SUBJECT EPISTASIS AND PERSONAL GENOME X ENVIRONMENT INTERACTIONS

Yves A. Lussier

University of Arizona, USA

With the advent of precision health, the view of healthy living is shifting from a population-based to a more individual one. While tailoring nutrition isn't new, the convergence of Big Data Science and multiple 'Omics have provided substantial evidence to the fact that one's response to diet is determined by complex genome x environment interactions and genomic interactions. Indeed, at equal caloric intake, distinct mice strain grow fat while others under specific macronutrient distribution of the diet (standard mouse chow, Western, traditional Japanese, traditional Mediterranean, high-fat, and low carb ketogenic) do not. Simply stated: mammalian diet response is highly personal and involves genetic x sex interactions as well as gene x diet interactions.

While individualizing nutrition according to age, gender, exercise, ancestry, and endocrine/growth patterns isn't new, precision nutrition seeks to incorporate genome x environment assays. However, conventional genomic studies are conducted using cohort-based statistics that are designed to discover average patterns rather than individual response.

Our group has been developing two novel approaches applicable for individualizing nutrigenomics:

(i) Single-subject epistasis: personal risks of metabolic conditions from genetic synergy and paradoxical antagony discovered using integrative and multi 'omics analyses of coding as well as non-coding polymorphisms. These results present the mitigating risks via environmental response of the metabolome.

(ii) Individualized transcriptome-level stimulation assays paired with single subject analytics. These results democratize the cost of 'omics, as the summative effect of one's unique 3.5 billion base-pairs interacting with a well-designed metabolism stimulation assay can be read through a few dozen transcript by affordable RT-PCR.

LINKING OF OMICS-BASED BIOMARKERS WITH NUTRITION AND METABOLIC OUTCOMES IN CHINESE

Xu Lin, Huaixing Li, Liang Sun

Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (CAS), Key Laboratory of Nutrition and Metabolism, CAS, China

With the rapid development of advanced technology, omics-based biomarkers are expected to provide a more precise tool to predict and control disease and also to evaluate nutrition status. However, limited prospective studies have systematically investigated major nutritional and genetic factors for metabolic disease in Chinese with the world largest populations with obesity and type 2 diabetes (T2D). Thus, by adopting multiple omics approaches in a 6-yr population-based cohort study and also leading a national genome-wide association study (GWAS) for T2D in Chinese Hans, we identified or confirmed over 100 genetic variants associated with obesity, T2D and hypertension. Moreover, we found about 50 genetic variants involving fatty acids, vitamin D and iron metabolic pathways. Meanwhile, multiple nutritional biomarkers (high carb or dairy intake and iron, vitamin D, vitamin B1 status) were found to be associated with prevalence or incident MetS, T2D and other health outcomes. A panel of acylcarnitines, especially long-chain acylcarnitines, substantially improved the predictive ability for incident diabetes beyond conventional risks. By conducting two randomized, double-blinded controlled trials, we found that health participants administered 0, 400 (recommend dietary allowance, RDA), 800, 1200 or 2000 (the upper intake level, UL) IU/d of vitamin D3 showed a curvilinear dose-response relationship with a plateau at around 6 weeks in the 16-week trial. In the second trial, we further confirmed that daily supplementation with 2000 IU vitamin D3 for 20 weeks significantly raised total and bioavailable 25(OH)D concentrations, but still left 25% participants with uncorrected deficiency. A PTH-based threshold of 25(OH)D (50.8 nmol/L) was suggested. Genetic factors exerted stronger impact than non-genetic factors (baseline value, BMI and gender) on both 25(OH)D responses. Overall, our studies provide important insights regarding the roles of genetic and nutrient-related biomarkers on metabolic diseases and nutritional status in Chinese and these findings are useful for establishing strategies of precision nutrition.

Keywords: *omics, metabolic disease, nutrition, biomarker*

DEVELOPMENT OF AN ASSAY FOR DIETARY AND EXPOSOME MEASUREMENTS FOR PRECISION MEDICINE

David S. Wishart

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Precision medicine is an emerging field of healthcare that uses multi-omics techniques (genomics, proteomics, transcriptomics, metabolomics) to precisely diagnose, monitor and treat individuals. The promise of precision medicine is that it will allow physicians to more precisely diagnose, treat or prevent disease. In this presentation I will try to make the case that precision medicine offers some of the best opportunities for metabolomics to successfully move out from the lab and into the clinic. I will also describe some of our laboratory's efforts in using metabolomics for several "real world" precision medicine applications. These include the development of metabolic disorder screening assays, the creation of software tools to help integrate quantitative metabolomics with other omics data as well as the development of graphical interfaces to facilitate the clinical interpretation and visualization of multi-omics data for disease detection and wellness monitoring. Through our interactions with both patients and phyisicans we also discovered that there were several shortcomings with our initial approach to metabolite analysis. This led to the development of a number of new, lower-cost and higher throughput metabolomic assays that could facilitate improved diagnosis and patient monitoring. One of these includes a quantitative MS-based dietary and exoposome measurements.

APPLICATION OF NON-TARGETED METABOLIC PROFILING TO REVEAL POTENTIAL BIOMARKERS LINKING EGG INTAKE AND PREVIOUSLY OBSERVED LOWER RISK OF DEVELOPING TYPE 2 DIABETES IN MIDDLE-AGED MEN

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Dietary cholesterol has been suggested to impair glucose metabolism and promote inflammation, thereby potentially increasing the risk of type 2 diabetes (T2D). Due to its high cholesterol content, high consumption of eggs has traditionally been discouraged. In middle-aged men from the prospective, population-based cohort Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) from Eastern Finland, higher egg intake was associated with a lower risk of T2D though the mechanism remains unclear. Here we present the application of non-targeted metabolite profiling to reveal potential biomarkers linking egg intake and the risk of T2D. From the KIHD study we collected 239 baseline plasma samples in 4 groups: 1) subjects with high egg intake who developed T2D within the average follow-up of 19.3 years (cases), 2) cases with low egg intake, 3) subjects with high egg intake who remained free of T2D within the follow-up period (controls), and 4) controls with low egg intake. Non-targeted metabolite profiling was performed with high performance liquid chromatography coupled with tandem mass spectrometry. The comparison of metabolite profile between cases and controls showed that some lysophosphatidylcholine species were higher in controls, whereas phosphatidylcholine species were mostly more abundant in cases. Although eggs are a major dietary source of choline, plasma choline level was not significantly different between high egg and low egg intake groups (p-value 0.389). Interestingly, plasma choline (odds ratio (OR) 0.69; 95% CI 0.53, 0.90; p-value 0.006) and phosphocholine (OR 0.74; 95% CI 0.56, 0.95; p-value 0.021) indicated lower OR of T2D. These results suggest that the inverse association of high egg intake with the risk of T2D in this study population of middle-aged men may be mediated by alterations of choline and its phospholipid-bound metabolites. Further studies are required to investigate the molecular and cellular mechanisms linking egg intake with the development of T2D.

Keywords: *metabolomics, eggs, HPLC-MS, type 2 diabetes, plasma*

METABOLOMICS APPROACH TO IDENTIFY BIOMARKERS OF PEAS INTAKE

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Legumes are known to exert potential health benefits with evidence stemming from epidemiological studies. Furthermore, a significant body of evidence demonstrates that isoflavone metabolites are good markers of soy intake, while research is lacking on specific biomarkers from other leguminous sources eg. peas. In this context, a randomised cross-over acute intervention study was conducted on healthy volunteers (n=11) with the objective to detect urinary biomarkers of peas intake using untargeted metabolomics. Participants consumed peas and couscous (control food) in a random order and urine samples were collected postprandially and analysed by liquid chromatography-mass spectrometry (UPLC-QTOF-MS). The data obtained was reduced to 819 features in positive mode and 1004 features in negative mode applying filtering procedures, such as data alignment and sample occurrence frequency. Following data pre-processing, multivariate statistical analysis was applied to further reduce the data dimensionality. Robust models were obtained for comparison of fasting against the postprandial time points. For example, fasting vs 4h and fasting vs 6h revealed robust PLS-DA models [(R2X=0.41, Q2=0.4) and (R2X=0.517, Q2=0.495) (-ve mode), respectively]. Subsequently, the models were validated by permutation testing. The variable importance for the projection (VIP) list was created from the PLS-DA plot and variables with scores ≥1.5 were considered discriminant between the two time points. Time series plots of these features revealed at least 26 features (14 in –ve and 12 in +ve) showing a time response following peas consumption. Overall, 8 features displayed a differential time course as compared to the control food. Further analysis will focus on identification of these discriminating features of interest by metabolite annotation using databases, such as Metlin, HMDB and confirmation by LC-MS/MS which should result in identification of specific pea markers.

Keywords: *metabolomics, biomarkers, legumes*

DNA METHYLATION-BASED CARDIOVASCULAR RISK ASSESSMENT WITH APPLICATIONS TO NUTRITION

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DNA methylation is an ideal biomarker for many applications, because it has both the stability for prognostic use and the plasticity to be altered by environmental variables including diet. Methylation risk scores (MRS) can be constructed to take advantage of epigenetic information in predicting disease incidence, and have notably been used in "biological age" measurements that robustly predict mortality risk. Thus, we hypothesized that DNA methylation could be used in a similar way to construct a specific predictor of cardiovascular disease (CVD) risk. We performed an analysis of methylation data, previously collected using the Illumina Infinium HumanMethylation450k platform, on samples from the longitudinal Framingham Heart Study Offspring Cohort. After preprocessing, methylation M-values were used to conduct a probe-wise epigenome-wide association scan employing Cox models to predict incident cardiovascular events. Significant loci from this step were taken forward into a combined regression whose fit defined the MRS as a weighted sum of methylation levels at these loci. Pending datasets will allow the validation of this MRS in an independent cohort, including assessments of the predictive performance of the model, both independently and with respect to established CVD predictors including the Framingham Risk Score. Additionally, component loci will be examined for biological significance in relation to known diet-responsive loci across the genome. The resulting MRS has potential for use in both clinical risk prediction and evaluation of the effectiveness of personalized dietary patterns.

Keywords: *epigenetics, methylation, cardiovascular, CVD*

EFFECTS OF KRILL OIL AND LEAN AND FATTY FISH ON LIPOPROTEIN SUBCLASSES AND LOW MOLECULAR-WEIGHT METABOLITES: A RANDOMIZED CONTROLLED TRIAL

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Fish consumption and supplementation with omega-3 fatty acids have beneficial health effects. Krill oil is an alternative source of marine omega-3 fatty acids and few studies have investigated its effect on human health. Thus, we aimed at comparing the effects on lipid metabolism of krill oil supplementation with intake of fish containing similar amounts of omega-3 fatty acids and a control group not receiving omega-3 fatty acids. In an eight-week randomized parallel study, 36 healthy subjects aged 18-70 years with fasting serum triglycerides between 1.3 and 4.0 mmol/L were randomized to receive either fish, krill oil or a control oil. In the fish group, subjects consumed lean and fatty fish in accordance with dietary guidelines. The krill group and control group received 8 capsules/day containing 4 g oil/day. The weekly intake of marine omega-3 fatty acids were 4103 mg in the fish group and 4654 mg in the krill group. We analyzed changes in fasting serum triglycerides and plasma fatty acids at routine laboratories as well as lipoprotein subclasses and low-molecular-weight metabolites using a high-throughput NMR metabolomics platform. The plasma levels of EPA and DHA increased significantly in the krill group and the fish group and DPA increased in the krill group. Fasting serum triglycerides decreased by 17% in the krill group and 7% in the fish group, however, the change was nonsignificant due to large individual differences. Lipoprotein subclasses were altered towards less of the largest VLDL subclasses in the krill group and the fish group. There were no changes in low-molecular-weight metabolites. To further understand the effects on lipid metabolism, changes in gene expression of lipid metabolism genes in peripheral blood mononuclear cells (PBMC) will be analyzed. In addition, we are performing a global profiling of miRNA expression in PBMC with validation of significant results using RT-qPCR.

Keywords: *fish, krill oil, EPA, DHA, lipoprotein subclasses, gene expression, miRNA*

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NUTRIGENETIC TESTS FOR LIFE-STYLE OPTIMIZATION IN THE BULGARIAN POPULATION – DIFFICULTIES, ADVANCES AND FUTURE PERSPECTIVES

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Introduction: **Nutrigenetics is a relatively new, but intensively developing scientific field. It aims to improve the quality of life by customizing dietary intake, physical activity and environmental exposures according to the personal genetic variantions.**

Aim: **The aim of the study is to examine the attitude of the Bulgarian society towards nutrigenetic tests and how knowledge of personal genetic data influences current lifestyle choices.**

Materials and Methods: **Seventy-eight Bulgarian probands were tested for the 56 single nucleotide polimorphisms (SNPs) in 52 genes, related to the lipid, carbohydrate, vitamin B and D metabolism, inflammation, oxidative stress, osteoporosis detoxification, PUFA, caffeine and salt responsiveness, predisposition to tendon injuries, as well as genes associated with power and endurance in athletes (DNA Life, Nordic Laboratories). Each proband provided a written informed consent before testing, as well as filled in questionnaires as part of pre-test counseling and then a few months later. Each patient was consulted by a trained genetic counselor. Probands were provided with detailed information about the test, detected polymorphic variants and personalized recommendations for diet, sport and lifestyle changes.**

Results: **The preliminarily results reveal that large portion of the respondents could directly follow the received personal recommendations. In a small number of the patients a chronic disease during the counseling was detected and they were directed to the gastroenterologist for treatment. A significant share of our cohort, found to be predisposed to obesity, insulin resistance and osteoporosis, has already manifested some symptoms and the individuals were referred to a dietitian and endocrinologist, respectively. Patient follow-up, as well as data collection from the questionnaires are ongoing.**

Conclusion: **This study shows that Bulgarians are inclined to do nutrigenetic tests for prevention of socially significant disorders and stresses the need for a trained physician to analyze test results and offer the most suitable lifestyle modifications in order to minimize morbidity risk.**

Keywords: *nutrigenetic, genetic counseling*

TISSUE-SPECIFIC EPIGENETIC AGE ACCELERATION: CONSTRUCTING ESTIMATORS USING PUBLICLY AVAILABLE DATA

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The plasticity of DNA methylation is manifested when persistent environmental factors, like chronic stress, change the cytosine methylation at specific DNA sites, potentially affecting chromatin state and gene expression. Tissue-specific epigenetic age, measuring deviations from the average age-related DNA methylation trajectory in a population, may be affected by such persistent environmental factors. We have developed a novel method that integrates existing information to construct tissue-specific epigenetic clocks for whole blood, and other tissues and cell-types. We train and validate the tissue-specific epigenetic clocks' performance in different publicly-available datasets.

Keywords: *DNA methylation, epigenetic age acceleration*

USING DRIED URINE SAMPLES FOR BIOMARKER MEASUREMENTS

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We tested 181 dried urine samples spotted on regular cosmetic cotton swabs for quantitative analysis of various potentially alcoholic beverage related metabolites across wide polarity and structural ranges. The samples were collected in a 3-week cross-over intervention trial with alcoholic beverages and abstention. The agreement of measurements between conventional 24-hour urines and dried urine spots made from them in situ corrected with creatinine ratio was evaluated by Passing–Bablok regression and Bland–Altman analysis. Determinations were performed by a recently developed and fully validated UPLC–MSMS multiplex method. There was full agreement in qualitative results making dried urine spots suitable for metabolomics profiling and screening analysis useful for compliance control in clinical trials. Quantitative analysis exposed certain limitations of dried spotting. Based on the slopes calculated from Passing–Bablok regression, results were underestimated in the range of 11–23% in case of tartaric acid, indoxyl sulfate, pyroglutamyl proline and dihydroepiandrosterone sulphate. Agreement was found in case of the alcohol intake related metabolites, ethyl sulfate and ethyl glucuronide. Partial overestimation ranging from 10–20% was observed for cresol sulfate. As a secondary objective, we examined variance of individual total daily creatinine excretion which was found to be up to 16%, comparing seven 24-hour urine samples collected from each of the 26 subjects. This finding suggests that a creatinine correction factor could be calculated as an average individual value obtained from several 24 hour urine samples. A creatinine correction factor could be useful in some long–term clinical trials for correction of measurements in dried urine spots provided that the resulting analytical error is acceptable for the research purpose.

MATERNAL-FETAL VITAMIN D RECEPTOR POLYMORPHISMS SIGNIFICANTLY ASSOCIATED WITH PRETERM BIRTH

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Aim: **Preterm birth (PTB) is a complex trait with strong genetic background, whose etiology is not fully understood. It was recently suggested that pregnancy duration is affected by fetal genetic variation even more than by the maternal genome. Vitamin D receptor (VDR) is involved in embryonic implantation and fertility. We studied the association between both maternal and neonatal vitamin D receptor (VDR) genetic variation and PTB.**

Materials and Methods: **Maternal and fetal (umbilical cord) DNA was isolated from Jewish Israeli idiopathic preterm newborns (24-36 weeks, n=146) and control term newborns (>37 weeks, n=229). Maternal and fetal VDR polymorphisms (FokI, ApaI, BsmI, TaqI) were analyzed by restriction fragment length polymorphism analysis. We have used SPSS analysis in order to determine the correlation between VDR genotypes and phenotypic variation: pregnancy duration, preterm birth and spontaneous miscarriages, adjusted for gravidity, parity and gender of newborn.**

Results: **Women homozygous to VDR ApaI (AA) genotype had significant twofold increase risk for PTB (OR=1.973, [CI] 1.183-3.289, p=0.009) compared to heterozygous women. Male newborns had significant ($p<0.05$) 1.73 fold increase of PTB. Women with history of previous (≥1) spontaneous miscarriage had a significantly increased risk for PTB if their newborn carried either of the VDR BsmI homozygous (BB or bb) genotypes compared to the heterozygous (Bb) genotype (OR=6.857,[CI] 1.273-36.934, p=0.018 and OR=9.231,[CI] 1.753-48.618, p= 0.008, respectively), or VDR ApaI homozygous (AA or aa) genotype compared to heterozygous (Aa) genotype (OR=4.33, [CI] 1.029-18.257, p= 0.046 and OR=7.2, [CI] 1.34-38.917, p=0.021, respectively).**

Conclusion: **We have shown the association between maternal and fetal VDR genotype variants with PTB.**

DIFFERENCES IN SKIN CAROTENOID LEVEL WITH REGARD TO SUBCUTANEOUS ADIPOSE TISSUE THICKNESS IN PATIENTS WITH ANOREXIA NERVOSA

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Introduction and Aim: **Besides the reduction of body structures, anorexia nervosa (AN) is accompanied by severe metabolic disturbances. Hypercarotenemia, the accumulation of carotenoids in the skin presumably due to disturbed lipid metabolism, is frequently observed in AN patients. However, the pathophysiology of hypercarotenemia is not yet fully understood. Moreover, differences in the amount of subcutaneous adipose tissue (SAT) in AN patients with the same BMI have been observed recently. Hence, we analyzed carotenoid levels in AN patients of different body composition.**

Materials and Methods: **SAT was determined at eight sites by a standardized, reliable and accurate ultrasound technique in AN patients (n=18). The sum of these sites (DINCL) represents the SAT amount. Because of the observed differences in DINCL, the group was divided by the median of DINCL for further analysis (G1, G2). Skin carotenoids were measured by resonance Raman spectroscopy, a non-invasive method that measures skin carotenoid concentrations via the light absorbing characteristics of carotenoids.**

Results: **G1 had a mean DINCL of 13.7±9.6mm and mean skin carotenoid level of 43889±12211 counts, while G2 had mean DINCL values of 44.6±10.6mm and skin carotenoid level of 26333±12797 counts. Group comparison revealed significant differences in SAT thickness ($p<0.001$) and skin carotenoid level ($p=0.009$), respectively.**

Conclusion: **AN patients with lower SAT thicknesses had significantly higher dermal carotenoid levels compared to AN patients with higher remaining SAT, indicating that the disturbances in carotenoid metabolism that led to the skin accumulation of carotenoids might go along with reduction in body fat mass. Thus, body composition may play an important role in the occurrence of hypercarotenemia in AN.**

Keywords: *eating disorders, hypercarotenemia, body composition, ultrasound measurement technique, resonance Raman spectroscopy*

Acknowledgement: *Josef Smolle (Initiator of the ESAN-Study IC3490), Medical University Graz*

DIETARY FIBRES REGULATE BILE ACID METABOLISM INDEPENDENT OF THE MICROBIOTA BY SUPPRESSING THE PRODUCTION OF SIGNALLING MOLECULE FGF15 IN THE ILEUM

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Dietary fibres alter the microbiota and are linked to lower cholesterol levels. In the liver, bile acids are produced from cholesterol and excreted into the duodenum, where they enable lipid digestion. After reaching the ileum most bile acids are re-absorbed, and induce the expression of signalling molecule FGF19 (Fgf15 in mice). FGF15/19 travels to the liver where it inhibits CYP7A1, the rate-limiting enzyme in de novo bile acid synthesis. Here we investigated the effects of dietary fibres on bile acid metabolism in the small intestine and liver. Mice were fed a chow or a fibre-free diet (FFD) for up to 6 months. Some mice were treated with broad-spectrum antibiotics and a subset of mice on the FFD were supplemented with fructooligosaccharides (FOS) with or without antibiotics. Bile acids were measured using HPLC, gene expression was measured using Q-PCR and the microbiota was analysed using 16S sequencing.

The expression of Fgf15 was increased on the FFD at all time points (3 days to 6 months), while the absorbance of bile acids, as measured by gene expression and bile acid secretion, was not changed during the early (up to 2 weeks) timepoints. Although we observed large differences in small intestinal microbiota between the diets, this did not cause the change in Fgf15 expression. Depleting the microbiota with antibiotics did not change the effect of the FFD on Fgf15. Supplementing mice on the FFD with FOS in their drinking water led to a decrease in Fgf15 expression, in both conventional and antibiotic treated mice. Cyp7a1 was consequently induced by the fibre-rich diets (chow and added FOS in drinking water).

In conclusion, dietary fibres modulate the expression of Fgf15 independent of the microbiota and via this mechanism play an important role in bile acid and cholesterol homeostasis.

Keywords: *bile acids, fibres, cholesterol homeostasis*

IN SILICO PREDICTION OF METABOLISM AS A TOOL TO IDENTIFY NEW METABOLITES OF DIETARY MONOTERPENES IN RATS

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Dietary terpenes have not been studied enough, despite the fact that they are absorbed well and display a range of biological properties. Better knowledge about their metabolism will help understanding the health effects of plant foods, herbs and spices and may provide new biomarkers of food intake. As part of the FoodBAll project we are investigating the metabolism of terpenes, identifying metabolites and biotransformations involved in their metabolism. PhytoHub (database that compiles all known metabolites of dietary phytochemicals, including terpenes) and Nexus Meteor (in silico prediction of metabolism), were used to identify biotransformations involved in the metabolism of monoterpenoids. Twenty-two selected biotransformations were used to predict the metabolism of camphene, camphor, carvacrol, carvone, 1,4-cineole, 1,8-cineole, citral, citronellal, cuminaldehyde, p-cymene, fenchone, geraniol, limonene, linalool, menthol, myrcene, nootkatone, perillyl alcohol, pinene, pulegone, terpinen-4-ol and thymol. In parallel, Wistar rats received a chemically defined diet with or without 0.05% of the referred compounds. Before and after 5 days of the exposure to the dietary monoterpenes, urine was collected and untargeted metabolomics analysis performed using high-resolution mass spectrometry (UPLC-QToF).

On average, 10 metabolites per compound were identified in rat urine, including new and known ones. Identification of metabolites was based on monoisotopic mass and formula match, presence of adducts and specific mass losses indicative of glucuronidation and conjugation to amino acids. Validation of identification is being done using orbitrap MS/MS and hydrogen-deuterium exchange experiments.

The combination of in silico prediction and in vivo experiment allowed the identification of known and new metabolites of different dietary terpenoids. Predicted metabolites of terpenes will be added in PhytoHub to complement the database of known metabolites.

Keywords: *monoterpenoids, insilico prediction of metabolism, metabolism of phytochemicals*

Acknowledgement: *Agence Nationale de la Recherche - FoodBAll project (JPI HDHL)*

RELATIVE GENE EXPRESSION OF SIRT6, FOXO4 AND HIF3A IN PATIENTS DIAGNOSED WITH COLORECTAL CANCER

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In recent years, there has been an expanding interest in understanding metabolic regulation in cancer cells. Since cancer cells are characterized by accelerated rate of proliferation, their metabolic demands are increased. The latter forces cancer cells to facilitate metabolic pathways, which are not normally active in healthy cells. Cancer cells comprising a tumor belong to two major metabolic groups: a) the glucose-dependent cells and b) the lactate-utilizing tumor cells. Taken into consideration the unorganized vasculature and shunts creating this regional hypoxia, tumor cells develop symbiotic metabolic behavior to utilize the lactate produced through aerobic glycolysis (Warburg effect) and anaerobic glycolysis (Pasteur effect). Our focus was on three regulatory genes SIRT6, FOXO4, HIF3A, which orchestrate basic metabolic pathways, such as energy metabolism, and regulate proliferation. Their expression is altered in conjunction with cancer type and stage. Our results of RAS mutant colorectal cancer (CRC) patients showed only slight increase of HIF3A level, whereas in RAS wild type (wt) patients all of the investigated genes have increased their expression, when the results are normalized against RPL37A endogenous control. By comparing the mutant with wt patients, we identified increased expression of all three protein-coding genes in the mutant and decreased levels in all of them in wt patients. When comparing the gene expression in the different stages of cancer progression, we detected decreased levels of SIRT6 in the earlier stages and increased levels of SIRT6 in the later stages of CRC. SIRT6 functions as an ADP-ribosylase and NAD+- dependent deacetylase and is able to regulate DNA repair, telomere maintenance and glucose and lipid metabolism, therefore affecting metabolic and age-related diseases, such as cancer. Our results are in agreement with the fact that the high levels of SIRT6 in later stages of CRC lead to survival of the cancer cells by decreasing the accumulation rate of mutations. FOXO is decreased at first, then is upregulated in the middle stage and in later stages it doesn't show any significant change in its gene expression levels. FOXO4 functions as tumor suppressor by inhibiting cell proliferation, inducing apoptosis and protecting cells from DNA damage and oxidative stress. HIF3A has increased accumulation rate in the early and middle stages, and later on its levels drop. HIF3A is induced by hypoxia. Overexpression of the related HIF3A increases colorectal cancer tumor growth by activating pro-survival STAT3 signaling pathways. Our analyses showed average correlation between SIRT6 and FOXO4 in RAS mutant patients and between SIRT6 and HIF3A in RAS wt CRC patients. We observed strong correlation between SIRT6 and HIF3A in stage IV of CRC progression. Our results showed an active relationship between the SIRT 6 and both FOXO4 and HIF3A, which is in agreement with the earlier suggested interactions between all these protein coding genes.

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NUTRITION AND GASTROINTESTINAL CANCER: AN UPDATE OF THE EPIDEMIOLOGICAL EVIDENCE

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Despite years of intensive research, cancer incidence is projected to rise over the next decades reaching 24 million new cases by 2035. According to authoritative reports by leading cancer prevention organizations such as the World Cancer Research Fund and the American Institute for Cancer Research, about one third of cancer cases can be prevented by adopting healthy nutrition and lifestyle. Among different cancers, those arising in the gastrointestinal system have been most commonly associated with nutritional exposures; however the recent accumulating evidence from human studies has not been summarized. We have reviewed the latest epidemiological evidence on the role of nutrition on the risk and development of gastrointestinal tumors: colorectal, stomach, liver, esophagial and pancreatic. A meta-epidemiological approach was used to identify and summarize the most recently published reviews and meta-analyses (up to June 2017). Existing research highlighted the importance of body fat as a major risk factor for the most common gastrointestinal cancers. Body fat distribution, particularly in the abdominal area, poses a higher risk for colorectal, liver and pancreatic cancer. Additional risk factors include high alcohol consumption and high intakes of processed meat and salted foods. Potentially protective role has been suggested for whole grain intake, dairy products and citrus fruits. The data revealed chemopreventive potential of optimizing micronutrient intakes, i.e. calcium, vitamin D, vitamin B6 and B12, and trace elements – i.e. zinc and selenium. However, having the observational nature of published research, data from randomized control trials is needed to prove the preventive role of these nutrients. Further research focus should be put on the evaluation of complex dietary patterns and assessment of life-course nutritional exposures. From a prevention perspective, concerted actions should be taken for the establishment of complex lifestyle recommendations, patient education about nutritional risks and benefits (i.e. salt, processed meat, aflatoxin exposure) and development of targeted nutritional interventions.

Keywords: *nutrition, gastrointestinal cancer, meta-epidemiological analysis, prevention*

ADIPOKINES AND MYOKINES ARE IMPORTANT FOR SEVERAL BIOLOGICAL EFFECTS OF ADIPOSE TISSUE AND SKELETAL MUSCLE AS WELL AS HEALTH

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White adipose tissue and skeletal muscle are essential in energy metabolism and they represent the largest organs in the human body. Both tissues have endocrine functions and are able to secrete hundreds of bioactive molecules, many of which are called adipokines and myokines. These terms should be reserved for proteins and molecules secreted from adipocytes, whereas corresponding molecules from skeletal muscle should be derived from myocytes.

During the last decades it has been observed that very few proteins are exclusively derived from either adipose tissue or skeletal muscle but rather produced and released from both cell types; these bioactive molecules expressed in both adipose tissue and skeletal muscle should be named adipo-myokines.

It is well known that diet may extensively influence adipokine expression, transcription and secretion, as exemplified with leptin. Indirectly and directly, exercise can also affect adipokine expression for leptin and myostatin, respectively. It is likely that many adipo-myokines may be influenced by exercise and dietary changes, and provide mechanistic explanations for benefits of exercise and healthy diets.

So far we have little knowledge of how diet may influence expression of myokines in skeletal muscle although it is likely that diet has smaller effect on myokine expression in skeletal muscle as compared to the effect of exercise on adipokines.

Recently, we discovered that physical activity can reduce plasma concentration of the hepatokine fetuin A (protein mainly secreted from the liver). The change in plasma fetuin A and free fatty acids (FFAs) interacted to predict change in glucose infusion rate (GIR) after exercise.

With another approach we discovered 161 candidate secretory transcripts in skeletal muscle biopsies that were upregulated after acute exercise, and 99 that where increased after 12 weeks exercise training. Furthermore, we focused on 17 exercise-responsive transcripts encoding new secretory proteins from skeletal muscle. One of these candidate myokines were identified as colony stimulating factor 1 (CSF1), which was secreted from cultured muscle cells and upregulated in vitro as well as in plasma after exercise. Thus, plasma CSF1 might be a marker of physical activity for short- and long-term although this has to be examined in much larger studies.

ACYLCARNITINE AND AMINO ACID PROFILING IN PLASMA AND TISSUES OF NZO MICE AS A MODEL FOR OBESITY-INDUCED TYPE 2 DIABETES

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Metabolomics has identified biomarkers in human plasma with a predictive quality for type 2 diabetes (T2D) development. Amongst these markers are various acylcarnitine species, most prominently those derived from branched-chain amino acid (BCAA) breakdown. The metabolic perturbations in tissues underlying these changes in plasma are often unknown and hard to assess in humans. Thus, animal models are used to have access to the different tissues. In contrast to diet-induced obesity models or monogenetic mouse models of obesity and diabetes, the New Zealand Obese (NZO) mouse is a prototypical polygenic model for obesity, with a male-specific susceptibility to developing diabetes. We have used this model to study the metabolic alterations in obesity-induced diabetes for changes in tissue-specific acylcarnitine and amino acid profiles. Twelve male and 11 female NZO mice at an age of 8 weeks were fed a chemically-defined high carbohydrate diet for 12 weeks. Acylcarnitine and amino acid profiles were obtained from plasma, skeletal muscle, heart muscle, liver, kidney and adipose tissues, using a high-resolution LC-MS/MS method. This method covered around 35 amino acids and 43 acylcarnitine species originating from fatty acids and amino acids, as well as odd-numbered and dicarboxylic acylcarnitines. Furthermore, blood glucose, plasma insulin, non-esterified fatty acids (NEFA), triglyceride and urea concentrations were measured. Hierarchical cluster analysis of principle components derived from plasma samples revealed a grouping of normoglycemic females, normoglycemic males and hyperglycemic males and this originated mainly from differences in concentrations of acylcarnitines derived from BCAAs and odd-numbered short- and medium-chain fatty acids. Hyperglycemic males were characterized by high insulin levels and low levels of NEFAs. These findings largely match with findings in humans. Comparison of metabolite profiles in diabetic and non-diabetic male mice revealed the largest overlap of significant metabolite changes between plasma and liver, including longer-chain odd-numbered acylcarnitine species. In conclusion, the alterations in acylcarnitine and amino acid profiles in NZO mice largely overlap with findings in humans, suggesting similar underlying metabolic perturbations in this diabetes model as compared to humans. The metabolite profiling of individual tissues in this model suggests a specific role of the liver in the generation of odd-numbered fatty acylcarnitines.

Keywords: *type 2 diabetes, metabolomics, LC-MS/MS, obesity, acylcarnitines*

IMPACT OF SHORT- AND LONG-TERM EXERCISE TRAINING ON BRANCHED-CHAIN AMINO ACID TURNOVER AND INSULIN SENSITIVITY IN PRE-DIABETIC AND NORMAL WEIGHT HUMANS

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Introduction: **Obesity is associated with reduced insulin sensitivity (IS), reduced branched-chain amino acids (BCAA) catabolism, and elevated plasma BCAA. Physical exercise is known to enhance IS and influence BCAA metabolism.**

Aim: **To investigate the relationship between BCAA metabolism and IS in overweight/dysglycemic (pT2D) and normal weight (control) individuals during short- and long-term exercise.**

Materials and Methods: **We measured IS by hyper-insulinemic–euglycemic clamp, transcriptomics by mRNA sequencing of skeletal muscle and adipose tissue biopsies, and plasma concentration of BCAA by HPLC in 13 control men and 13 men with pT2D. Samples were obtained at rest, directly after and 2h after a bicycle challenge, before as well as after 12 weeks of combined endurance- and strength-exercise intervention. The effect of BCAA on glucose metabolism was analyzed in cultured human myotubes.**

Results: **IS was lower in pT2D vs. controls and increased significantly following exercise in both groups. Plasma concentration of BCAA was higher, whereas BCAA catabolism based on pathway analyses was lower in both skeletal muscle and adipose tissue in pT2D vs. control men. Plasma concentration of BCAA did not change on average with exercise, whereas BCAA catabolism based on transcriptomics increased in both skeletal muscle and adipose tissue. The increase in BCAA catabolism was higher in pT2D vs. control men in both tissues. Increased skeletal muscle catabolism predicted increased IS, the decrease in molar sum of circulating BCAA predicted improvement in HOMA-IR, and the increase in adipose tissue BCAA catabolism predicted increased expression of adipose tissue citric acids cycle enzyme mRNA. The addition of BCAA to cultured myotubes reduced the fractional glucose oxidation.**

Conclusion: **A mechanism involving increased BCAA catabolism in adipose tissue and skeletal muscle, may mediate interactions between exercise, BCAA metabolism and IS.**

EFFECT OF LOW CARBOHYDRATE / LOW FAT DIETARY INTERVENTION ON GENE EXPRESSION OF GLUT IN ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE OF TYPE 2 DIABETES PATIENTS

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Type 2 diabetes mellitus (T2DM) global prevalence is increasing rapidly along with obesity marking it as a major public health problem. The causes of its epidemic are embedded in a complex interaction between genes, metabolites and external factors, such as diet. To investigate the short termed effects of a moderate low fat diet (<30% fat, 50% carbohydrate, 20% protein, 1000-1200 kcal/d) and a low-carbohydrate diet (60-70% fat, 5-10% carbohydrate, 20-30% protein, 1200-1500 kcal/d) on metabolic parameters and focusing on diet-induced regulation of glucose transporters (GLUT) in subcutaneous adipose tissue (SCAT) of recently diagnosed T2DM patients. Seventeen T2DM patients were randomly assigned to the low-carbohydrate (LC) diet or to the moderate low-fat (LF) diet. SCAT biopsies and clinical parameters were obtained at baseline and after the 3 weeks dietary intervention. Reverse transcriptase polymerase chain reaction (RT-PCR) was employed to quantify the expression of GLUT in human SCAT. Both interventions led to significant reduction of weight (LC: p=0.004, LF: p<0.000), BMI (LC: p=0.008, LF: p<0.000), LDL/HDL ratio (LC: p=0.004, LF: p=0.002), total cholesterol/HDL ratio (LC: p=0.008, LF: p=0.020) and HbA1c levels (LC: p=0.025, LF: p=0.001). Whereas LDL (p=0.001) and total cholesterol (p=0.003) levels were only significantly reduced after the LF diet, waist-to-hip ratio (WHR) (p=0.013) and triglyceride (TAG) levels (p=0.013) were significantly reduced in the LC group only. After the LF diet, GLUT3 (1.19 ±0.6 to 1.78 ±0.5, p=0.019), mRNA levels increased significantly and, on the contrary, GLUT5 (1.65 ±1.5 to 0.58 ±0.44, p=0.045) mRNA levels showed a significant reduction. No significant differences were observed between diet groups after 3 weeks of diet. This short-term dietary intervention separates the improvements on WHR, TAG, total cholesterol and LDL from dietary composition-related effects. Furthermore, moderate low-fat, calorie-restricted diet may significantly upregulate GLUT3 expression and down-regulate GLUT5 expression in SCAT of T2DM patients. However, future studies on its influence on GLUT transports are needed.

Keywords: *GLUT, adipose tissue, type 2 diabetes mellitus, low-fat diet, low-carbohydrate diet*

SKELETAL MUSCLE PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE ARE RESPONSIVE TO EXERCISE AND RELATED TO INSULIN SENSITIVITY IN HUMANS

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Introduction: **Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) composition in skeletal muscle has been linked to insulin sensitivity.**

Aim: **To evaluate relationships between skeletal muscle PC:PE ratio, physical exercise and insulin sensitivity in humans.**

Materials and Methods: **PC and PE were measured in m. vastus lateralis biopsies obtained from subjects examined in different studies with different degrees of insulin sensitivity: type 2 diabetes (n=13), impaired glucose metabolism and untrained (n=13), impaired glucose metabolism and trained (n=13), obesity (n=13), untrained normal weight controls (n=13), trained normal weight controls (n=13) and endurance trained athletes (n=6). From a subset of the subjects (MyoGlu; n=26) biopsies were obtained at rest, immediately after 45 min of cycle ergometer at 70% maximum oxygen uptake, and 2 h post-exercise, before as well as after 12 weeks of combined endurance- and strength-exercise intervention. Insulin sensitivity was measured by euglycemic hyperinsulinemic clamp. In addition, several measures of physical fitness were obtained, global RNA-sequencing was performed on the biopsies, and mitochondria and lipid droplets were quantified using point counting on electron microscopic images.**

Results: **Skeletal muscle PC:PE ratio was highest in the type 2 diabetic group and gradually lower in groups with increasing insulin sensitivity. The lowest ratio was observed among endurance athletes. Twelve weeks of exercise intervention enhanced skeletal muscle levels of PC by ~21% and PE by ~42%, and reduced the PC:PE ratio by ~16%; a reduction in the PC:PE ratio predicted increased insulin sensitivity (β=-1.6, P<0.001). Transcriptomic analyses indicated that enzymes involved in PC and PE synthesis may explain the increased levels of PC and PE and the reduction in the PC:PE ratio after 12 weeks of exercise. The PC:PE ratio also correlated with mRNA expression of genes related to insulin receptor, Akt/PkB and mTORC1 signaling, and to the fraction of lipid droplets and mitochondria within skeletal muscle cells. Mitochondrial volume was increased from 3.4-5.7% after 12 weeks of exercise intervention.**

Conclusion: **Skeletal muscle PC:PE ratio is inversely related to insulin sensitivity. The molecular link between skeletal muscle PC:PE ratio and insulin sensitivity probably involves several mechanisms including, but not limited to, mitochondrial function.**

CHEMERIN AS A NOVEL BIOMARKER OF IMMUNITY AND METABOLISM: ASSESSMENT OF CROSS-SECTIONAL ASSOCIATIONS AND METHODOLOGICAL UTILITY

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Introduction: **The interplay of low-grade chronic inflammation and metabolism has recently gained much interest regarding its potential involvement in chronic disease development. Based on findings from mainly mechanistic studies the novel biomarker chemerin shows great potential in this regard. However, studies investigating chemerin in humans are scarce.**

Aim: **The aim of our study was therefore to investigate chemerin in relation to adiposity and inflammation-related biomarkers. In addition, we assessed the reliability of chemerin measurements to enable future studies relying on single time-point measurements.**

Materials and Methods: **The study comprised 207 apparently healthy participants (124 women and 83 men) which provided blood samples on two occasions over a 4-month period. Plasma chemerin concentrations were measured using sandwich ELISA.**

Results: **At baseline, median chemerin concentration was 159 (IQR: 137 - 186) ng/mL. Age- and sex-adjusted Spearman correlation analyses revealed positive correlations of chemerin with body mass index (BMI) [Rho=0.35 (95%-CI: 0.22, 0.47)] and waist circumference [0.37 (0.24, 0.48)]. Chemerin was further correlated with C-reactive protein [0.26 (0.10, 0.41)], as well as the adipokines fatty acid-binding protein-4 [0.28 (0.12, 0.42)] and progranulin [0.23 (0.07, 0.38)], even after adjusting for body mass index. No correlation was observed with monocyte chemoattractant protein-1, omentin-1 and vaspin. In multivariable linear regression analysis, a combination of factors including body mass index, waist circumference, C-reactive protein, progranulin and fatty acid-binding protein-4 explained 28.0% of variance in chemerin concentrations. The intraclass correlation coefficient (ICC) as a measure of reliability suggested a good level of agreement of chemerin measurements over time [ICC: 0.72 (95%-CI 0.65, 0.78)].**

Conclusion: **Overall, the results of our cross-sectional analyses highlighted the implication of chemerin as a biomarker linking immunity and metabolism. To investigate the relation of chemerin with chronic disease development, additional prospective studies are highly warranted.**

Keywords: *chemerin, adipokine, reproducibility*

CIRCULATING BIOMARKERS OF LINOLEIC ACID INTAKE

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Linoleic acid (LA) is a major polyunsaturated fatty acid in western diets. Circulating LA levels have been used as a biomarker of LA intake in diet-outcome studies, especially in recent studies of type 2 diabetes. Different plasma fractions for the assessment of circulating LA levels are available. Whether ranking of individuals based on their LA intake depends on the plasma fraction used is currently unknown. In the present study we classified Dutch cardiac patients from the Alpha Omega Cohort based on habitual LA intake. Patients filled in a 203-item food frequency questionnaire (FFQ) which was linked to the Dutch food composition table (NEVO 2006) to estimated daily LA intake (energy percentage, en%). Non-fasting blood samples were collected in which LA and other circulating fatty acids were determined in plasma cholesterol esters (CE; n=4746) and in phospholipids (PL; n=1019) by means of gas chromatography. LA in CE and PL were expressed relative to total fatty acid composition (weight percentage, wt%). Fatty acids were also assessed in total plasma using nuclear magnetic resonance metabolomics (NMR; n=873) (molar percentage, mol%). Patients with an intake of <4 en%, 4-8 en%, and >8 en% from LA showed corresponding plasma levels of LA, especially when measured using CE and NMR. Meanwhile, when patients were classified into quintiles of intake, only plasma levels of patients in the lowest and highest quintiles of intake differed from each other. Plasma CE agreed better with NMR than with PL. To conclude, plasma levels showed good correspondence to intake. Within our cohort, evaluation of LA as a biomarker of intake will be further explored in relation to disease outcomes.

Keywords: *linoleic acid, polyunsaturated fatty acids, dietary intake, blood, biomarker*

Acknowledgement: *VLAG-NWO graduate programme*

EFFECT OF FERRUM PHOSPHORICUM D12 (IRON PHOSPHATE) ON GENE EXPRESSION IN J744A.1 MACROPHAGE CELL LINE

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Introduction: **Regardless of their extensive application and the huge amount of empirical data, scientific research on biological activities of homeopathic remedies is still missing. Ferrum phosphoricum D12 (iron phosphate) (FP) Schussler salt is applied in the initial phase of infections and inflammations, fever, as well as fresh wounds, cuts, scratches, and first-degree burns.**

Aim: **The aim of the present study was to examine the effect of FP-containing tablets on cell viability and the expression of genes associated with antioxidant protection and inflammatory status in J744A.1 macrophage cells.**

Materials and Methods: **Commercially available PF-containing tablets and placebo tablets, provided by the manufacturer, were used for cell treatment. Tablets were dissolved in nutrient medium to concentrations from 0.0125 mg/mL to 10 mg/mL. MTT reduction assay was performed to assess cell viability. Expression of antioxidant defense enzymes glutamine-cysteine ligase (GCL) and glutathione peroxidase (GPx) and the inflammatory cytokines interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and TNF-alpha, and the phagocytic enzyme NADPH oxidase was measured using real-time qPCR.**

Results: **FP–containing tablets of 0.0125 mg/mL and 0.025 mg/mL exerted significant proliferative activity in the medium. Concentrations from 2.5 mg/mL to 10 mg/mL appeared to have increasing cytotoxic effect up to 30%. Treatment of the cells with FP tablets had no significant effect on GPx expression as compared to non-treated cells. However, when compared to placebo control, 2.5 mg/mL and 0.025 mg/mL concentrations were established to increase the expression by 24% and 27%, respectively. Expression of IL-1β gene, interestingly, was significantly affected by placebo 0.0125 mg/mL tablets (67% decrease, $p<0.05$) when compared to non-treated control. At the same time, the slight nonsignificant stimulatory effect of the 0.0125 mg/mL FP tablets resulted in a 3.5 fold difference ($p<0.05$) to its respective placebo ones. Obviously, placebo tablets content should be taken into account when evaluating the effects of Schussler salts incorporated into the commercial drug forms. No significant changes in other genes expression levels were established.**

Conclusion: **This pilot study showed that even in very high dilutions FP preparations may exert biological activity in cell cultures.**

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TECHNOLOGY OF BREAD, ENHANCED THE BODY RESISTANCE TO THE ACTION OF STRESSORS

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Introduction: Nowadays, the frequent and prolonged stress has a negative influence on metabolism, resulting in free radicals accumulation. Oxidative stress launches a metabolic cascade, irreversibly damaging the cell.

Aim: The aim of this paper is to study the possible involvement of soluble carbohydrates in antioxidant mechanisms as molecular interceptors of active forms of oxygen and the possible influence of fucoidan on the hormonal status.

Materials and Methods: We uesd bread wheat flour (gluten 28.5%, ash content 0.55), enriched with the herbal supplement fucoidan. The preparation of raw material was carried out according to the technological instructions for the production of bread. Hormones were determined by ELISA (total T3-vector Best RU; T4 total-vector Best, RU; cortisol-Best vector, RU).

The experiment: We selected two groups of subjects. One group received regular bread and the other - bread with fucoidan for 28 days. Blood was collected twice before and after the consumption of bread to study the levels of cortisol, T3, T4.

We used the recommended dose of fucoidan for humans – 100 mg per day and daily intake of bread of 325-345 g.

The effect of herbal supplements on the sensory and chemical indicators of bread quality was determined. Optical microscope Altami-136T (Altami, Russia) was used to study the activity of the yeast cells with fucoidan. Microscopic studies were performed on Jeol JEM-2100 (Jeol Ltd., Japan).

The obtained results confirmed the possibility of using fucoidan to improve the quality of fresh bread. The residual content of fucoidan in the finished product was 0.196±0.0015 mg/g. This demonstrates the thermal stability of fucoidan in the composition of bread.

This study showed that after eating bread with fucoidan there was a tendency to normalise the levels of T3 and T4, and the levels of cortisol remained unchanged.

THE NEW FACE OF VITAMIN K - MORE THAN BLOOD CLOTTING FACTOR

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Introduction: **Twenty years ago, the metabolism of vitamin K was connected with its role in hemostasis. Since that time, it has been shown that vitamin K exerts multiple functions mediated by the Gla-proteins, having vitamin K as a cofactor. Numerous publications affirm that these Gla-proteins are related to physiological processes beyond coagulations, such as bone metabolism, vascular health and energy homeostasis.**

Aim: **The aim of this research is to provide new data for the role of vitamin K in a myriad of physiological processes beyond blood clotting. Additionally, it aims to assess the potential new applications of vitamin K as a supplement for the prevention of bone and vascular diseases.**

Materials and Methods: **Using the online databases Scopus, PubMed and Google Scholar, a search with the keywords: "vitamin K2", "bone metabolism", "cardiovascular diseases", "osteocalcin" and "MGP" was conducted. Information regarding the effects of vitamin K on bone and vascular health was referred to in this work.**

Results: **Vitamin K and vitamin K-dependent proteins play pivotal roles in the physiology of bone mineralization and in preventing ectopic calcification. Osteocalcin, a Gla protein located in bone and dentin, is important for bone mineralization. Following the posttranslational carboxylation of Glu-residues with a cofactor vitamin K2 (menaquinone), rather than vitamin K1 (phylloquinone), osteocalcin shows increased affinity for calcium. Osteocalcin is believed to be involved in osteoblast regulation and hydroxyapatite crystal growth. Matrix GLa-protein (MGP), sharing some sequences with osteocalcin, is a local inhibitor of arterial calcification. Vitamin K deficiency impairs the function of osteocalcin and MGP and, therefore, presumably contributes to bone demineralization and vascular calcification, the so-called calcium paradox.**

Conclusions: **Vitamin K deficiencies, traditionally regarded as a cause for internal hemorrhages and blood clotting disorders, apparently can be linked to cardiovascular calcification and abnormal bone modelling. Appropriate treatment of vitamin K deficiency may improve bone and arterial health.**

IDENTIFYING METABOTYPES: THE CHALLENGES FROM NUTRITECH

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Human metabolic health may be defined as the organism´s capability to maintain or regain homeostasis in an everchanging environment, and thus also in response to dietary conditions. To maintain long-term homeostasis, a proper "buffering capacity" with the ability to counteract quickly on metabolic disturbances is needed. I define a metabotype as the observable (and quantifiable) property of the organism to respond to such metabolic disturbance (challenge) in a distinct and reproducible manner. I shall use data from EU-project NutriTech to demonstrate the existence of metabotypes and how robust they are. In NutriTech, 72 healthy volunteers (aged 50-65 years with BMI of 25-35kg/m2) underwent a baseline characterization followed by the assessment of their metabolic responses to a standardized oral glucose tolerance test (OGTT), a mixed-meal tolerance test (MMTT) and a mixed-meal tolerance test with built-in exercise (MMTT+PA). Time-dependent sampling of venous blood before and during the challenges allowed clinical chemistry, and plasma metabolite and protein profiling. Volunteers were then randomized into a weight loss (n=40) or weight maintenance group (n=32) and their metabolic responses to the same challenges were followed a second time after 12 weeks. In addition to blood analysis of around 600 entities (metabolites, hormones, cytokines, etc.), body composition was assessed using MRI at the beginning and the end of the intervention period. Exome sequencing, as well as fecal microbiome analysis by 16S sequencing, were performed as well. Metabolomics employed NMR, GC- and LC-MS/MS to cover a wide range of metabolites. Metabotypes were identified based on the time-courses in a subset of around 20 plasma metabolites as representative indicators of lipolysis, fatty acid ß-oxidation and hepatic ketogenesis that all respond drastically to rising insulin levels during the MMTT or OGTT. Volunteers were clearly separated into 2 clusters with almost equal distribution of men and women within each cluster. Most interestingly, when the effects of the weight loss intervention (mean weight loss: 5.6 kg) were assessed, only one of the clusters revealed a significant improvement, for example, of the glucose profile during the OGTT. This suggests that the two subgroups display different "sensitivity" to metabolic improvements upon a modest weight loss. Amongst the metabolite panel, bile acids (BA) were the most responsive metabolites in the postprandial state and most interesting also when only glucose was provided in the OGTT. However, they also displayed a high level of variability. In analyzing the causes of the diversity of the responses we explored genetic heterogeneity in a subset of around 30 genes/proteins involved in BA synthesis and enterohepatic circulation and also in the gut microbiome sequences. Although the latter did not contribute too much to plasma BA diversity, SNPs in genes were affiliated with BA metabolism and, in particular, absorption in the small intestine showed significant effects. NutriTech provides a wealth of information on human metabolic diversity in response to standardized challenges which asks for more efforts in exploitation. I shall however also address current limitations in the approaches to do better metabolic phenotyping and show why nutrition science needs better standardization.

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DATA INTEGRATION AND PHENOTYPIC FLEXIBILITY

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Health is characterized by the ability to continuously adapt in varying circumstances where multiple mechanisms of system flexibility are involved. Their ability to adapt can act as an indicator for maintenance or improvement of physiological function. The term 'phenotypic flexibility' expresses the cumulative ability of overarching physiological processes (e.g. metabolism, inflammation, oxidation) to return to homeostatic levels after short-term perturbations.

The EU NutriTech project aimed to demonstrate and validate that phenotypic flexibility is a sensitive biomarker of metabolic resilience and that it can serve as a measure of health. A key factor is determining the physiological relevance of specific changes in phenotypic flexibility in a healthy (sub) population, for example in response to a dietary intervention. Ideally, this is pursued by validation against established markers of health, but these do not exist. Alternatively, modifications in challenge responses can be correlated to established markers of disease or to long-term health outcomes (disease risk, longevity) in cohort studies for validation purposes which is not easy.

Therefore, new data integration methodology was introduced based on network-biology with the objective to allow for validation of biomarkers across studies to enhance scientific evidence for effects of dietary interventions on identified markers of phenotypic flexibility. Network analysis enables integration and visualization of multi-layered experimental datasets and prior knowledge. Data from 4 human studies focusing on phenotypic flexibility were available in the phenotype database and used for a multi-study analysis providing a metabolic syndrome reference network. When this reference network is combined with the NutriTech human study data, the resulting visualization shows how the caloric restriction diet has impacted biomarkers of phenotypic flexibility related to metabolic syndrome. The NutriTech data integration pipeline offers a sustainable knowledge resource and toolbox for assessment of health effects of nutritional interventions.

Keywords: *phenotypic flexibility, biomarker, validation, multi study analysis, nutritional intervention*

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EFFECTS OF A DIET HIGH IN MUFA AND A MEDITERRANEAN DIET ON SERUM METABOLIC PROFILE: A RANDOMIZED FULLY CONTROLLED TRIAL

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We studied the effects of partly replacing saturated fatty acids (SFA) by monounsaturated FA (MUFA) in a Western-type diet and the effects of a full Mediterranean (MED) diet high in MUFA on serum metabolic measurements in overweight subjects or such with abdominal obesity. In total, 60 subjects, both men and women, were allocated to an eight-week completely controlled SFA-rich diet, a MUFA-rich diet, or a MED diet. Serum metabolic profiles were assessed by using a nuclear magnetic resonance (NMR)-based platform providing information on lipids, lipoprotein subclasses, and low-molecular-weight metabolites, resulting in 162 measurements. Serum metabolic profiles were measured in 47 subjects. Compared to the SFA diet, 32 metabolites were affected upon consumption of the MUFA diet, and 52 were affected upon consumption of the MED diet. Both the MUFA and MED diet decreased all subclasses of LDL, and the MED diet additionally decreased the larger subclasses of very low-density lipoprotein (VLDL). Within the MUFA and MED diet groups the smaller VLDL subclasses and all low-density lipoprotein (LDL) subclasses were decreased, the MED diet additionally decreased the larger subclasses of VLDL, and increased several subclasses of high-density lipoproteins (HDL). Within the SFA diet group no effects were found on lipoprotein subclasses. Lastly, the exposure markers CLA, CLA.FA, DHA, and Faw3.FA were important metabolites for the discrimination between the three diets. A decrease in subclasses of LDL and VLDL, and an increase in subclasses of HDL have been associated with a decrease in future cardiovascular events. Therefore, replacing SFA with MUFA has positive health effects, and the consumption of a MED diet has additional positive health effects, which might be partly explained by the higher fish intake. Comprehensive metabolic profiling in this fully controlled trial elucidated part of the metabolic influences of replacing SFA by MUFA, and the additional effect of a full MED diet.

Keywords: *metabolomics, MUFA, Mediterranean diet, NMR spectroscopy*

Acknowledgements: *JPI-HDHL*

EMBRACING THE UNCORRELATED: DYNAMIC TIME WARPING FOR THE EXPLORATION OF HIGH DIMENSIONAL TIME-SERIES METABOLOMICS DATA FROM THE HUMET STUDY

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The data analyzed has been collected in the framework of the HuMet study via different metabolomics approaches to assess normal metabolic variation within 15 healthy young men selected to be as homogenous as possible. After comprehensive phenotyping (clinical chemistry, DEXA-scanning, indirect calorimetry) all volunteers underwent four distinct nutritional challenges (with intermittent fasting) over a time period of 4 days in a controlled environment. For the present analysis, metabolites within blood samples collected at 0, 15, 30, 45, 60, 90, and 120 minutes following a 75 g oral glucose tolerance test, were identified and quantified using liquid chromatography tandem-mass spectrometry (LC-MS/MS) as performed by Metabolon, Inc.

Time-series metabolomics data, though rare, is often plagued by the 'curse of dimensionality'. Low observation occurrence coupled with high variable number has restricted the applicability of many temporal analytic methods and increased reliance on feature selection to cope. Non-correlated metabolites naturally are the first to fall victim to aggressive dimension-reducing techniques despite an unexplored translational potential. Interestingly, when utilizing temporal alignment algorithms to correct for lag asynchrony between uncorrelated metabolite series, significant latent trend-shape similarities are uncovered.

Within this present project, dynamic time warping (DTW), a temporal alignment measure popular within automated speech recognition, was used to establish a metabolite alignment dissimilarity index with glucose monotonicity. The index was used to evaluate the shape-dependent temporal relationships of 189 metabolites as possible glucose-response variables within a 2 lag parameter. Metabolites with high initial correlation coefficients to glucose ($rs > 0.50$) were removed, and the remaining indexed metabolites scoring above the median were classified into various combinations to assess their capability to produce subject clusters with high correlation to subject clusters predicated by glucose alone (cophenetic coefficient: $c > 0.60$). The results identified promising targets, as 3-metabolite and 4-metabolite combinations, all with an average $rs \approx -0.10$, displayed pronounced efficacy at replicating subject clusters of the glucose-response curves ($c \approx 0.70$). This new approach thus defines novel metabolites and clusters in the multi-metabolite dynamics of the glucose response.

Keywords: *metabolomics, dynamic time warping, time series, trend-shape, non-correlated*

Acknowledgements: *Metabolon, Inc.*

MONOSACCHARIDES IN POST-WEANING DIET OF YOUNG MICE PROGRAM BODY COMPOSITION AND FEEDING BEHAVIOUR IN ADULTHOOD

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Nutrition in early life has permanent consequences affecting development and later-life health. In this study we investigated the potential of monosaccharides in the post-weaning diet to program adult metabolic health. Female and male C57BL/6JRccHsd mice were time-mated and fed a low-fat diet ad libitum. Litters were culled to six pups per nest and randomly assigned to foster dams. Mice were weaned onto one of three experimental diets containing 32 energy% as glucose (GLU), fructose (FRU) or an equimolar mixture of glucose and galactose (GAL). At six weeks of age, all mice received the same high-fat diet (HFD). Food intake, body weight, and body composition were measured bi-weekly. Whole-body metabolism was analysed towards the end of the post-weaning diet and during the last stretch of the high-fat feeding period in GLU and FRU mice. Additionally, an oral glucose tolerance test and a fasting-refeeding challenge as an indicator of metabolic flexibility were performed. At the end of the post-weaning intervention period, no significant differences were found in body weight or lean mass across all dietary groups, and in 24-hour energy expenditure and substrate oxidation between GLU and FRU. At week 15, cumulative food intake, body weight, and fat mass were significantly lower in GAL females compared to GLU females. Glucose tolerance in all groups, and energy expenditure, fuel utilization, and metabolic flexibility between GLU and FRU did not reveal programmed differences in metabolic phenotype. None of the parameters studied indicated long-lasting effects in males. This study provides evidence of the potential of galactose in the post-weaning diet to program a reduction in food intake resulting in lower fat mass during adulthood. Our findings highlight the relevance of carbohydrates as a dietary intervention target for future clinical studies in the field of metabolic programming.

Keywords: *metabolic programming, monosaccharides, carbohydrates, metabolism, indirect calorimetry*

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GENOME-WIDE METHYLATION APPROACH IDENTIFIES GENES INVOLVED IN AMPK SIGNALING AND INSULIN RESISTANCE PATHWAYS IN OBESE WOMEN SUBJECTED TO GASTRIC BYPASS

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Introduction: **The approach of nutritional genomics allows analyses of the associations between the genome and food. Epigenetic patterns related to obesity may explain the risk of developing this disease and associated comorbidities. Knowledge of the DNA methylation pattern before and after interventions for weight loss may promote understanding of the molecular mechanisms that determine the treatment's efficacy.**

Aim: **We aimed to evaluate the whole blood DNA methylation changes 6-months after Roux-en-Y gastric bypass (RYGB) in a genome-wide methylation approach.**

Materials and Methods: **The genome-wide methylation pattern was measured in DNA samples isolated from circulating leukocytes and were hybridized in the Infinium HumanMethylation 450k BeadChip. Data were compared between samples from obese women (body mass index (BMI) 44.2±6.6 kg/m² and age of 36.4±10.7 years) (n= 12) before and 6 months after RYGB. The methylation level of each cytosine was expressed as a β-value (fluorescence intensity ratio of the methylated and the unmethylated alleles) that ranged between 0 (unmethylated) and 1 (completely methylated). Data normalization, statistical filtering, and raw data were transformed into β-values using the Genome Studio Illumina software (V2010.3).**

Results: **After adjustments, we found 473.999 final valid CpGs sites. Differentially methylation analysis identified 1,142 differentially methylated CpG sites (DMCpGs) after RYGB. Thereby, a hypermethylated profile was observed after RYGB with 977 CpGs sites. On the other hand, 165 CpGs sites became hypomethylated after surgery. Specifically, the methylation levels of AKT, PPARG, ADIPOR2, SREBF1, PTEN, IL-12B, genes involved in AMPK signaling and insulin resistance pathways exhibited statistically significant hypermethylation between periods ($q < 0.05$; $p < 0.001$).**

Conclusion: **With a genome-wide methylation approach, we showed that RYGB promotes methylation changes of blood DNA, mainly hypermethylation in genes involved in AMPK signaling and insulin resistance pathways. Therefore, this study provided new and valuable DNA methylation biomarkers of obesity through peripheral blood analysis, which is essential for personalized weight loss strategies**

Keywords: *methylation, obesity, insulin pathway, bariatric surgery*

Acknowledgements: *São Paulo Research Foundation (FAPESP) grant #2015/18669-0*

RESEARCH ON THE MOLECULAR MECHANISMS OF SULFUR-CONTAINING MINERAL WATERS FROM THE VARNA BASIN ON HUMAN METABOLISM IN REGARD TO THEIR USE AS A DRINKING REMEDY

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The mineral waters from the Bulgarian region of the Northern Black Sea comprise almost a quarter of the hydro-mineral resource of Bulgaria, the second richest country in the world in natural resources of mineral waters with balneologic and healing potential. The Varna Basin mineral waters belong to the category of the so-called sulphur waters, containing hydrogen sulfide and soluble sulfides. Experimental data from models, systems or interventional studies on the biological effects of these type of mineral waters is lacking. At the same time, thousands of people actively use them daily as drinking water.

The aim of the current research is to explore the molecular mechanisms of the impact of the sulfur-containing mineral waters from the Varna Basin on the human metabolism and thus elucidate their health effects as reported by the traditional medicine.

After a detailed physico-chemical analysis of the mineral water from the Varna Basin, up to 50 healthy volunteers are to be enrolled in a 12-week intervention study with the mineral water replacing their daily drinking water. Blood and urine samples collected before and after the intervention period will be analyzed for markers for oxidative status, inflammation, lipid metabolism and vascular tone. Gene expression analyses of factors involved in nuclear factor kappa-B (NF-kB) dependent signaling pathways will be focusing on the mechanisms of mineral water metabolic effects.

The research will provide new knowledge regarding the impact and the mechanisms of the effects of Varna Basin sulfur-containing mineral water on human metabolism.

Acknowledgements: *Bulgarian National Research Fund support, Project DN 01/12 - 16/12/2016*

METABOLOMIC AND CLINICAL BIOMARKERS ANALYSIS OF THE IMPACT OF SANGHUANG-DANSHEN ON PLATELET HYPERACTIVITY IN HUMANS

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The postprandial condition is recognized as a procoagulant state. Postprandial platelet hyperactivity plays a crucial role in the pathogenesis of metabolic syndrome. Botanical foods typically contain a multi-component composition derived from herbal practices. Therefore, it is difficult to determine which components regulate platelet hyperactivity. Metabolomic analysis is a valid and powerful tool with which to further define the mechanisms. We therefore aimed to assess the effect of Sanghuang-Danshen (SD) in the metabolome and clinical biomarkers on platelet hyperactivity after an acute challenge with high-lipid/glucose formula in healthy subjects. In a crossover randomized and placebo-controlled intervention study, fifty-six subjects received three doses of SD or placebo. Platelet aggregation and mRNA expression of cyclooxygenase-1 and vascular cell adhesion molecule-1 were all decreased in the SD group. SD administration appears to reduce the platelet hyperactivity associated with a postprandial condition. These changes are accompanied by alterations of the metabolomics. A total of 13 metabolites were found to be significantly different between placebo and the high-dose SD group, which were strongly involved in linoleic acid metabolism, alanine, aspartate and glutamate metabolism, arachidonic acid metabolism, and glycine, serine and threonine metabolism. By integrating components in SD, markers, and phenotype, we find that SD may modulate the platelet hyperactivity occurring in the postprandial state.

Keywords: *metabolome, postprandial, platelet, Sanghuang, Danshen*

A 48-HOUR, RANDOMIZED CROSSOVER, VEGAN DIET CHALLENGE IN HEALTHY WOMEN AND MEN IDENTIFIED A BRANCH-CHAIN AMINO ACID RELATED, HEALTH-ASSOCIATED, METABOLIC SIGNATURE

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Introduction: **Limited research has been conducted on short-term healthful diet challenges to optimize phenotypic flexiblity.**

Aim: **We hypothesized that a short-term, plant-based protein (vegan) diet regimen nutritionally balanced in macronutrient composition to an omnivorous (animal protein) diet would have measurable, beneficial effects on metabolic measurements of blood sugar regulation, blood lipids, and amino acid metabolism.**

Materials and Methods: **A randomized, cross-over, controlled vegan verses animal diet challenge study was conducted on 21 (11 female and 10 male) healthy participants. Fasting plasma was collected at baseline and before and after each 2-day diet intervention to measure clinical variables, metabonomics, and bile acids. Intervention diet meal plans were tailored to calculated individual caloric needs. All foods were provided and the majority of meals and snacks were supervised. Intervention diet compliance was monitored and actual intake was calculated.**

Results: **The vegan diet lowered fasting triglycerides, insulin and HOMA-IR, bile acids, elevated magnesium levels, and changed BCAA metabolism which may further potentiate insulin and blood sugar control after just 48 hours. Cholesterol control as assessed by a lower CHOL/HDL ratio improved significantly from the vegan diet. Significant differences seen in plasma amino acid and magnesium responses positively correlated with dietary amino acid composition changes between plant- and animal-based diets. Polyunsaturated fatty acids and dietary fiber were inversely correlated with insulin, HOMA-IR and triglycerides. The short-term dietary changes that improved measurements of health were impacted by sexual dimorphism; including, baseline nutritional biochemistries; BCAAs at baseline and post-intervention with males having higher plasma levels; and insulin and HOMA-IR post-intervention with females having higher plasma levels.**

Conclusions: **This study demonstrates that a health-promoting, BCAA-associated metabolic signature can be obtained from a short-term, healthy, vegan diet challenge. The long-term impact of periodic short-term vegan diet challenges is a promising research concept and an approach that may be used to promote sustainable intake of protein sources and improve phenotypic flexibility as a measure of health.**

Keywords: *animal diet, bile acid, branched chain amino acid, glucose, gender, metabonomics, insulin, vegan diet*

Acknowledgements: *Nestle Institute of Health Sciences, Metabolic Unit-Nestle Research Centre*

AMINO ACID-DERIVED ACYL-CARNITINE CONCENTRATIONS CHANGE DOSE-DEPENDENTLY UPON CHICKEN MEAT INTAKE

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Metabolic responses are highly individual upon ingestions of different types of foods. Within the JPI-funded FOODBALL-consortium, we performed a well-controlled human cross-over intervention in which 12 volunteers (6m/6f) consumed defined doses of chicken breast (0g, 100g and 200g) and were followed during the subsequent 24 hours. Using our targeted liquid chromatography tandem-mass spectrometry (LC-MS/MS) method, we measured plasma and urine samples and analysed a broad panel of acylcarnitine species, ranging from C2 to C18 including various isomeric compounds derived from different pathways of fatty acid and amino acid breakdown. In plasma, a dose-dependent increase in acylcarnitine species derived from amino acid breakdown (propionyl-, isovaleryl-, 2-methylbutyryl-, methylcrotonoyl-, hydroxyisovalerylcarnitine, derived from branched-chain amino acids) could be observed. In contrast, no changes in rates of appearance and disappearance between the different doses of meat were seen for fatty acid-derived acylcarnitine species. The collection and analysis of plasma concentrations at seven time points within the 24 hours after meat consumption allowed performing kinetic analysis of metabolite concentrations. For the highest meat dose, increased plasma concentrations of amino acid-derived acylcarnitines were observed up to 12 hours after chicken meat consumption. Moreover, the appearance and disappearance of acylcarnitine species more downstream in breakdown pathways (especially for tiglylcarnitine and 3-methylcrotonylcarnitine) seemed to be retarded compared to more upstream metabolites in the breakdown pathway. Analysis of urine showed similar dose-dependent increases of amino acid-derived acylcarnitines. Here, especially metabolites upstream of carboxylases (propionyl-CoA carboxylase and methylcrotonoyl-CoA carboxylase) seemed to accumulate.

In conclusion, the intake of meat caused a dose-dependent increase in amino acid-derived acylcarnitines. The use of different doses and the analysis of different time points allowed kinetic analysis. Increased plasma concentrations of amino acid-derived acylcarnitine species at high doses were seen 12 hours after consumption and changes in plasma concentration largely mirrored changes in urine.

Keywords: *food biomarker, acylcarnitines, amino acids, kinetics*

Acknowledgements: *JPI-funded FOODBALL-consortium*

PUERARIA LOBATA/REHMANNIA GLUTINOSA AND EXERCISE INTERACTIONS ON INSULIN RESISTANCE AND METABOLIC INFLEXIBILITY USING 1H-NMR-BASED METABOLOMICS IN ESTROGEN-DEPRIVED ANIMAL MODEL

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Lifestyle modifications, such as diet and exercise, are becoming increasingly recognized as important strategies to reduce the cardiometabolic risks during menopausal transition because the concern about safety issues in the use of hormone therapy. The present study aimed to investigate the effect and interaction of a combination supplement of Pueraria Lobata/Rehmannia Glutinosa (PR) and aerobic exercise on insulin resistance and metabolic inflexibility observed in an estrogen-deprived animal model. Ovariectomized (OVX) Sprague-Dawley rats fed with a high-fat diet (HFD) were randomly assigned to receive placebo, PR (400 mg/kg body weight), or estradiol (0.2 mg/kg body weight) by oral gavage with or without aerobic exercise for 8 weeks. Indices related to insulin resistance and cardiometabolism were determined in the blood, adipose tissue and muscle. Furthermore, 1H-NMR metabolites were measured in urine. The PR supplementation significantly improved insulin responsiveness in adipose tissue and muscle, leading to a restoration of metabolic flexibility. Adipose tissue became smaller, had more GLUT4 protein in membrane, less macrophage infiltration and higher hormone-sensitive lipase mRNA expression. The metabolic profiles before and after PR supplementation and aerobic exercise were separated based on multivariant analyses of principal component analysis (PCA) and orthogonal partial least square-discriminant analysis (OPLS-DA). Thirty-six metabolites from variable importance plots were screened before and after PR supplementation and aerobic exercise. The 23 metabolites had significantly increased the levels of choline and taurine but significantly decreased levels of ethylene glycol, fumarate and phenylalanine after PR supplementation. Taken together, the results suggest that PR supplement with aerobic exercise has a potential to relieve insulin resistance and its related metabolic defects in postmenopausal women, reflecting changes in metabolite composition.

Keywords: *ovariectomy, Pueraria Lobata/Rehmannia Glutinosa, aerobic exercise, insulin resistance, metabolic inflexibility*

Acknowledgements: *This work was supported by the Bio-Synergy Research Project (NRF-2012M3A9C4048761) of the Ministry of Science, ICT and Future Planning through the National Research Foundation*

AP-TO-BAS, AN INTESTINAL DISPOSITIVE TO MEASURE PIG MUCOSA ENTEROENDOCRINE SECRETORY ACTIVITY IN RESPONSE TO APICAL STIMULATION

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The enteroendocrine system helps to coordinate the processes occurring at the gastrointestinal (GI) tract and the functionality of the whole organism. However, the low abundance and scattered distribution of the enteroendocrine cells makes the study of the mechanisms underlying their function difficult. To overcome the ethical limitations of animal studies and the limitations of the available cell lines, ex vivo approaches seem to be the most simplified physiological alternative. In its turn, ex vivo models, such as Ussing Chambers still have the drawback of the limited number of samples that can be analyzed or the number of cells in each section. Here we provide an ex vivo system to assay vectorial transepithelial processes without the main limitations of the Ussing Chambers methodology.

We worked with different segments of the GI wall of a pig, from which muscular layers were removed, and mounted it in a dispositive that we called "Ap-to-Bas". We glued the samples to a silicon tube and placed it inside of a cell culture insert without bottom membrane. This allowed us to work with plenty of replicates of each intestinal segment. Tissue viability was checked by measuring lactate dehydrogenase activity in culture media, which after 60 minutes was negligible. The efficiency of the gluing process was evaluated using a 70-kDa fluorescein isothionate (FITC)-dextran. Barrier properties, measured by the crossing of Lucifer Yellow from apical to basolateral side, were maintained similar as in segments mounted in Ussing Chambers. Finally, to check the enteroendocrine functionality, tissues were apically treated with a Grape Seed Proanthocyanidin Extract (GSPE) and basolateral enterohormone secretions were measured. GSPE modulated entehormone (acyl-ghrelin, PYY and CCK) secretion at the duodenum. It also stimulated the secretion of total GLP-1 in the right colon.

In conclusion, the Ap-to-Bas dispositive allows performing entoerohormone studies while keeping the apical and basolateral zones of the intestine separated.

Keywords: *enteroendocrine system, enterohormone secretion, ex vivo model*

1H-NMR METABOLOMIC APPROACH REVEALS THAT CONJUGATED LINOLEIC ACID SUPPLEMENTATION IMPROVES THE LIVER METABOLOME PROFILE IN RATS WITH METABOLIC SYNDROME

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Introduction: **Metabolic syndrome (MS) is a combination of metabolic and medical disorders such as obesity, elevated fasting glucose, hypertension and dyslipidemia. Conjugated linoleic acid (CLA) has been reported to have beneficial effects on atherosclerosis and insulin resistance, as well as in obesity, all of them related to MS. The metabolome is directly related to the phenotype and NMR-based metabolomics is a potent approach to study the metabolic profile in the liver.**

Aim: **The aim of this study was to determine the effect of a chronic administration of CLA in the liver metabolome of rats with MS.**

Materials and Methods: **Five-week-old male Wistar rats (n=16) were divided into three groups. The first one was fed a normal standard diet (STD), the second one - a cafeteria diet (CAF) for 10 weeks and the third was fed a CAF for 10 weeks and treated with CLA (100mg/Kg) the last 3 weeks.**

Animals were sacrificed by decapitation and livers were stored at -80ºC. Aqueous and lipid extracts from each liver were analysed by 1H-NMR spectrometry.

Results: **The results showed different metabolic profiles in the three groups. CLA reverted the increase of lactate, alanine and glucose levels induced by the CAF diet. However, betaine and methionine levels remained low in CAF and CAF+CLA groups. CLA decreased diglyceride levels, while triglyceride levels remained as those of CAF group. Besides, esterified cholesterol levels were increased in the liver of CAF+CLA rats.**

Conclusion: **CLA supplementation is able to modify significantly the liver metabolic profile of rats with MS. CLA could improve the insulin resistance associated with MS by decreasing diglyceride levels in the liver because diglycerides repress insulin receptor activity through protein kinase C_ε (PKC_ε).**

Keywords: *metabolic syndrome, conjugated linoleic acid, metabolomics, liver*

Acknowledgements: *This research was supported by the grant AGL2013-40707-R (AEI/FEDER, UE) from the Spanish Government*

URINE METABOLOMICS PROFILING REVEALS SPECIFIC METABOLIC ALTERATIONS IN RATS FED DIFFERENT OBESOGENIC DIETS

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Metabolomics has been extensively used for metabolic phenotyping while its application in nutritional research has been rising in the last years. The aim of our study was to characterize the urinary metabolomics profiles induced by several obesogenic diets in rodents in order to define the impact of diet-induced obesity on the metabolome.

For this purpose, 7-week-old male Wistar rats were fed four different diets for 10 weeks: a non-purified standard chow as a control diet (ST); a cafeteria diet containing highly palatable and energy-dense foods (CAF); a semi-purified high-fat diet (HF) and a semi-purified high-fat diet enriched with sweetened milk (HFS). During urine collection, rats were housed individually in metabolic cages for up to 24h. All urine samples were analyzed by 1H nuclear magnetic resonance (NMR) spectroscopy.

All the obesogenic dietary treatments increased caloric intake and body fat mass, whereas only CAF diet induced a significant increase in body weight gain when compared with ST group. Principal component analysis (PCA) showed a marked separation of all three hypercaloric diet-fed groups when compared to ST. However, CAF animals presented the most distinctive urinary metabolomics pattern, mainly characterized by the greatest presence of uremic toxins. Overall, hypercaloric diet feeding reduced the excretion of microbial-host co-metabolites and perturbed metabolites involved in the TCA cycle. Conversely, there was also found an increased secretion of metabolites associated with anaerobic glycolysis, creatine metabolism, oxidative stress and metabolic inflammation.

In conclusion, cafeteria diet feeding induced the most noticeable metabolic alterations revealed at urine level, suggesting that the urinary metabolome could be differentially affected by common dietary models used for the study of diet-induced obesity in rodents.

Keywords: *metabolomics, diet, obesity*

URINARY AND SERUM BIOMARKERS OF MILK AND CHEESE INTAKE ASSESSED BY METABOLOMICS APPROACH

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Biomarkers of food intake are currently intensively investigated in the frame of the European Project "Food Biomarker Alliance" (FoodBAll) for a wide range of foods. In the future, validated biomarkers will serve as a tool for dietary assessment enabling the objective link between diet and disease outcomes in epidemiological studies.

The randomized, controlled crossover study presented here aimed to identify urinary and serum biomarkers for the intake of two products of public health relevance, milk and cheese, in addition to a soy-based control. Eleven healthy volunteers (five women, six men) participated in the acute intervention study. Urine and serum samples were collected in the fasted state and up to 24 hours postprandially and were further analysed using a multiplatform approach combining untargeted gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR).

Using multivariate analysis, several urinary and serum biomarkers for milk, cheese as well as for soy drink after their acute intake have been identified. The majority of metabolites that specifically reflected the intake of milk were derived from lactose/galactose metabolism whereas an amino acid derivative was indicative of cheese intake. The kinetics of the biomarkers from the two dairy products was different when compared to biomarkers of the plant-based control (soy drink). Comparing urine and serum, the majority of identified biomarkers were discriminative in both biofluids but urine seemed to contain a higher number of biomarkers for the intake of these three food items. Overall, the outcomes of this study will serve as a basis for future validation studies under free-living conditions.

Keywords: *biomarkers of food intake, metabolomics, cheese, milk*

Acknowledgements: *Swiss National Science Foundation*

INTEGRATING THE INFLUENCE OF HOST, DIET AND MICROBIOME VIA FECAL METABOLOMICS AND LIPIDOMICS TO REVEAL METABOLIC PERTURBATIONS IN TYPE 2 DIABETES

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Current treatment strategies for type 2 diabetes are still characterized by a number of shortcomings including side effects and lack of long-term effectiveness when using pharmaceuticals, absence of personalized guidelines for lifestyle interventions, and insufficient knowledge on the role of gut microbiota to justify fecal transplants. Moreover, current diagnostic tests have limited sensitivity and specificity, with no opportunities for large-scale screening. A better understanding of the underlying mechanisms of type 2 diabetes is thus desired to make progress in disease management and diagnosis.This study implemented a strategy of fecal metabolomics to assess the pathology of type 2 diabetes. Hereby, feces was targeted as this specimen can be obtained non-invasively and captures the interactions between the host, gut microbiota, diet, and other exposomal factors. Methodologies were established for both the polar and non-polar (lipidome) metabolome fraction. The lipidomics methodology covers all eight lipid classes (defined by LIPID MAPS) whereas the method for polar metabolomics targets ten different polar to medium-polar classes. Both methods apply generic extraction and mass detection through UHPLC-Q-ExactiveTM Orbitrap MS and were used to map the fecal metabolome, thereby considering individuals without (n=22) and with hyperglycemia (n=17) (UZ Ghent EC 2016/0673). Fecal fingerprints enclosed 7061 lipophilic and 10214 polar species, whereby associated intensity data matrices were subjected to multivariate statistics. Valid OPLS-DA models (p-values ≤ 1.4e-7) were generated by which the fingerprints of healthy individuals and diabetes patients could be compared. Hereby, significant discrepancies were noted within both metabolome fractions, leading to the selection of 96 lipids and 105 polar metabolites with discriminating power. The use of any resulting biomarkers may lead to better and personalized disease management in terms of prediction, diagnosis, treatment, and follow-up. Moreover, based on revealed metabolic networks, alternative treatment targets may arise. Eventually, coupling these data to metagenome data may support fecal transplants.

Keywords: *feces, polar metabolomics, lipidomics, type 2 diabetes, multivariate data analysis*

Acknowledgements: *VLAIO*

ASSOCIATION BETWEEN OXIDATIVE STRESS-RELATED BIOMARKERS AND RECOMMENDED FOOD SCORE IN HEALTHY SUBJECTS

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It is well known that carotenoids, as antioxidants, are efficient scavengers of radicals, while malonyldialdehyde (MDA), a marker of lipid peroxidation, is caused by excessive oxidative stress. The recommended food score (RFS) is the simple questionnaire assessing overall diet quality, consisting of food items bearing high amounts of antioxidant nutrients. Therefore, the present study aimed to assess the associations of an individual oxidative stress status and overall diet quality in apparently healthy subjects. The data were collected from Korean adults aged above 18 years who underwent an annual health examination at the Seoul National University Boramae Medical Center. A total of 916 subjects (men, n=368; women, n=407) were classified into four groups according to the number of presence of metabolic risk factors (high waist circumference, high triglyceride level, high blood glucose, low HDL cholesterol, and high blood pressure). The four groups were distributed as follows: healthy subjects (40%), subjects with one metabolic risk factor (32%), subjects with two factors (18%), and subjects with more than three factors (10%). A high-performance liquid chromatography was used for the determination of carotenoid concentrations in plasma and MDA levels in biofluids (plasma, red blood cells (RBC), and urine). In the healthy group without any risk factors, RFS showed significant positive association with total carotenoids, xanthophylls, carotenes, and all individual carotenoids. Moreover, plasma carotenoids described negative correlation to plasma MDA. No consistent trends between RFS and plasma carotenoids are seen for subjects who have metabolic risk factors. On the other hand, the association between plasma carotenoids and RBC MDA was consistent in all groups, except subjects who have two risk factors. Therefore, metabolic risk factors may contribute to individual oxidative stress status.

Keywords: *carotenoids, MDA, RFS, antioxidants, oxidative stress*

CAN DIETARY PLANT BIOACTIVES ENHANCE RESILIENCE TO OXIDATIVE STRESS?

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Platelets have been shown to beneficially respond to plant bioactives, and may undergo oxidative modifications induced by external reactive oxygen species (ROS). Using a human platelet model approach would therefore allow us to assess the efficacy by which plant bioactives affect production of resilience products, and link this to a physiologically relevant outcome, i.e. platelet activation.

Human platelets were pre-treated with cinnamaldehyde, curcumin, epigallocatechin gallate, phenethyl isothiocyanate and sulforaphane, all possessing an α, β-unsaturated carbonyl moiety, and challenged with 5 mM H2O2. Platelets were analysed for expression of platelet activation markers, antioxidant enzyme activity, and oxidative stress.

Pre-treatment with physiological concentrations of all plant bioactives significantly inhibited ADP-induced P-selectin expression, and thus platelet activation, in a dose response manner, with the concentration of 1 µM causing an average inhibition of 7%, and 100 µM an average inhibition of 65%. A 5 mM H2O2 oxidative challenge increased platelet P-selectin expression by 13%, but pre-treatment with >25uM of dietary bioactives prevented an increase in platelet activation. In unchallenged platelets, sulforaphane showed pro-oxidant (increased thiobarbituric acid reactive substances (TBARS) by 65%) and phenethyl isothiocyanate (PEITC) anti-oxidant effects (decreased TBARS by 43%). No consistent effects on antioxidant enzyme activities were observed.

In conclusion, this study showed that dietary bioactives can maintain platelet function upon exposure to oxidative stress, but it is unclear whether the mechanisms include resilience products - the 5 mM H2O2 challenge may not have been strong enough to enable the detection of the prevention of a rise in levels of SOD and GPx activities. We are currently assessing the effects of an acute combined supplement containing cinnamaldehyde, epigallocatechin gallate and sulforaphane on regulation of baseline antioxidant enzyme activity levels (resilience response) and whether this will prevent an oxidative stress response upon an exhaustive exercise challenge test in healthy subjects.

Keywords: *BBSRC, Science in Sport (SiS)*

UNDERSTANDING THE ANTI-OBESITY EFFECTS OF RESVERATROL: PHASE-II CONJUGATES VS GUT MICROBIOTA METABOLITES

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Introduction: **Resveratrol (RSV) has been recently reported as preventing obesity. However, as RSV is quickly metabolized by both phase-II enzymes and gut microbiota, it is necessary to simultaneously analyze its metabolites in plasma in order to understand the mechanism of action of this compound.**

Aim: **The aim of this study was to identify which blood RSV metabolites could be potentially involved in the anti-obesity effects of RSV.**

Materials and Methods: **Five groups (n=6) of Wistar rats were fed a standard (STD) or cafeteria (CAF) diet for 12 weeks and were orally treated with 0, 50, 100 and 200 mg/kg/day of RSV during the last three weeks of the study period. At the end of the treatment, RSV metabolites were quantified in plasma by liquid chromatography-tandem mass spectrometry and body fat content was assessed by RMN. MetaboAnalyst was used to perform multivariant statistical analyses.**

Results: **The consumption of RSV at doses of 50 and 100 mg/kg/day did not prevent body weight gain in our experimental conditions, and only the dose of 200 mg/kg/day reduced obesity as indicated by significant decreases in body fat mass and circulating leptin levels. According to different metabolic variables, the PCA and PLS-DA analyses showed that animals treated at dose of 200 mg/kg/day were the closest to the group of lean animals. Notably, the observed separations were significantly related to the presence of higher concentrations of microbiota metabolites at dose of 200 mg/kg/day. In fact, only microbiota metabolites (dihydroresveratrol forms), but not phase-II conjugates, showed a significant dose-response pattern. Finally, the animal's weight was significantly correlated to the plasmatic concentrations of dihydroresveratrol metabolites.**

Conclusion: **The RSV metabolites derived from gut microbiota could explain, at least partially, the contribution of high doses of RSV to reduce the metabolic disorders associated with obesity.**

Keywords: *Resveratrol, cafeteria diet, obesity, metabolites, multivariant*

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INVESTIGATING THE EFFECT OF FISH INTAKE ON METABOLIC HEALTH USING DRIED BLOOD SPOT METHODOLOGY

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Systematic review of data has shown that the relationship between fish consumption and a decreased risk of type 2 diabetes mellitus (T2DM) is mostly evident in the Asian population. Remarkably, tissue levels of omega-3 fatty acids are lower among Asian populations with T2DM. This finding is important especially for South Asian people as they are known to have a greater risk of diabetes compared to other ethnic groups. We hypothesise that fish consumption beneficially modulates omega-3 status and glycaemic control in South Asian people with T2DM living in the UK, and this response is modulated by a single nucleotide polymorphism (TCF7L2 rs7903146). The biomarkers will be assessed using dried blood spot (DBS) technology in replace of venepuncture, allowing the subjects to complete the intervention study in a home setting. DBS sampling requires minimal training, reduces transport burden, and keeps the analytes stable. Although micro-sampling reduced the sensitivity and specificity of assays and limited the use of DBS for many years, advanced analytical techniques have quickly overcome many problems. With this study we are hoping to show that combined with remote sampling, DBS technology offers a feasible alternative in epidemiological studies. We optimised and validated the measurement of the HbA1c, the omega-3 index and triglycerides in DBS, yielding recoveries of >98%, 71-90% and >90%, respectively, compared to whole blood. Furthermore, the role of platelets in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-mediated cardio-protection will be investigated by assessing the effects of EPA and DHA on platelet activation in healthy versus diabetic European and Asian subjects (2x2 design), using an ex vivo human platelet model. We hypothesise that the effective concentrations of EPA and DHA to inhibit platelet activation may be higher for diabetic compared with non-diabetic subjects as their platelets are in a more pro-coagulant state.

Keywords: *omega 3, PUFA, fish, T2DM, dried blood spot*

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FUZZIFIED DUMMY VARIABLES HAVE A BETTER FIT THAN REGULAR DUMMY VARIABLES IN STATISTICAL MODELS – AN EXEMPLARY APPROACH BASED ON DATA FROM A GERMAN NUTRITION SURVEY

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Introduction: **In statistical analyses, the classification of quantitative dependent variables grants substantial flexibility when modelling their effect on an outcome variable. An observation's membership to a specific class (e.g. a subject's age group) is traditionally coded by dummy variables taking only values of either zero or one. This, however, leads to undesired breaks at the intersection of two classes (staircase function).**

Materials and Methods: **With regular dummy variables (rDV), an observation can only be a member of a single class (e.g. a subject can only be assigned to one age group). With fuzzified dummy variables (fDV), however, an observation is simultaneously assigned to two neighboring classes. The closer the value of the quantitative variable to the center of a class, the higher the respective degree of membership (with a maximum value of one). At the intersection of two classes, the degree of membership is 0.5 to both of these classes.**

As with rDV, fDVs sum up to exactly one. Since fuzzification does not affect the number of dummy variables required for classifying a quantitative variable, the degrees of freedom of the respective statistical analysis remain unchanged.

The effect of fuzzifying dummy variables is exemplarily analyzed for the association of age and body mass index (BMI) in a subsample of a German nutrition survey (n=1500).

Results: **Models with fDV show consistently better fits (i.e. higher R^2 values) than those with rDV. The difference is the more pronounced, the lower the number of age groups.**

In contrast to rDV, fDVs do not show staircase functions in scatterplots of mean values of age and predicted BMI, but rather smooth transitions across age groups.

Conclusion: **It seems that statistical modelling with fDVs leads to a better fit than rDV. They might contribute to avoiding artifacts in data analyses possibly evoked by inappropriate staircase functions.**

Keywords: *statistical modelling, dummy variables, fuzzy logic*

LONG-TERM ADMINISTRATION OF GALLIC ACID DECREASES BLOOD PRESSURE IN METABOLIC SYNDROME ANIMAL MODEL VIA AN IMPROVEMENT OF ENDOTHELIAL FUNCTION

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Gallic acid (GA, 3,4,5-trihidroxibenzoic) is a phenolic compound, widely represented in the plant kingdom, which has been demonstrated to exert beneficial health effects, including antihypertensive effect . The aim of this study was to evaluate the chronic antihypertensive effect of GA in hypertensive cafeteria diet-fed rats and the mechanisms involved in this effect. After 8 weeks of cafeteria diet feeding, male Wistar rats (n=6 per group) were daily orally supplemented with GA (6.65 mg/kg), Captopril (50 mg/kg) as a positive control or vehicle as a negative control for 3 weeks. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by the tail cuff method and thoracic aorta was extracted to evaluate endothelium-dependent genes expression. After 3 weeks of GA administration, there was a significant reduction in blood pressure (BP) (-20 mmHg decrement of SBP and -15 mmHg decrement on DBP) when compared to animals from the vehicle group. Regarding gene expression, GA produced the upregulation of Sirt-1 and eNOS gene expression resulting in an increase of NO production and promoting a vasodilatation effect. In addition, GA downregulated the gene expression of the vasoconstrictor agent, ET-1, which could contribute to BP reduction observed after GA administration. Moreover, the antihypertensive effect of GA could be explained through the overexpression of NOX4 which has been reported to exert vasoprotective effects. Considering these results, we can conclude that GA had chronic antihypertensive effect in cafeteria diet-fed rats through an improvement in the endothelial function.

Keywords: *gallic acid, phenolic compounds, hypertension, metabolic syndrome, endothelial function*

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POSTPRANDIAL SERUM LACTOSE AFTER ACUTE INTAKE OF MILK AND YOGHURT

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The use of dietary lactose as a source of energy requires its prior hydrolysis at the intestinal brush border into glucose and galactose. Indeed, as a disaccharide, intact lactose cannot be actively absorbed. Therefore, the presence of unhydrolysed lactose in plasma or urine has mostly been associated with specific cases such as lactating women or altered gastrointestinal permeability. Nevertheless, lactose has also been detected in the blood and urine of healthy men, indicating that the presence of lactose in the circulation of healthy subjects is not incompatible with normal physiology. The present crossover study monitored, in 14 healthy men, the postprandial appearance of lactose in serum after a single intake of 800 g of milk or yoghurt. Genetic testing for lactase persistence and microbiota profiling of the subjects were also performed in order to investigate their potential contribution to postprandial lactose serum levels. Data revealed that lactose does appear in serum after dairy intake, and with delayed kinetics when compared to the actively absorbed galactose. A notable inter-individual variability was observed and, although lactose levels were inversely correlated with galactose levels, the presence of the lactase persistence allele could not explain this variability. Finally, lactose levels have been associated with the abundance of the Veillonella genus in faecal microbiota. The measurement of systemic lactose following dietary intake could provide information about lactose metabolism and nutrient transport processes under normal or pathological conditions.

Keywords: *lactosemia, milk, intestinal permeability, yoghurt, Veillonella*

POSTPRANDIAL HUMAN STUDIES - A KEY ANALYTICAL STRATEGY TO LINK FOOD AND NUTRITIONAL SCIENCES

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The dynamic, postprandial response of the human organism to acute ingestion of food is a key element of nutritional sciences as it directly associates food with its impact on human metabolism. The study of this response can thus facilitate sensitive characterization of the metabolic impact of foods that are similar but which differ in compositional or structural features. The relevance of the postprandial analysis strategy to nutritional sciences can be potentiated by (i) complementing classical biochemical analyses with 'omic' methods, such as metabolomics and transcriptomics, using blood as a reporter of the metabolic activity of the organism; (ii) conducting metabolomic analyses on foods and linking the results of these analyses to the postprandial response of subjects who ingest the food under investigation.

One important determinant of the variability in the metabolic response of the human organism to the ingestion of food is the gut microbiota, a significant fraction of the human metabolome being derived from gut microbial activity. In this context, the technological process of food fermentation by microorganisms provides an ex vivo method to modulate the postprandial response of the organism by predigesting food ("preprandial digestion").

The concepts exposed above are illustrated by presenting data from two human intervention studies that evaluated the postprandial responses to different dairy products (milk, yoghurt and cheese) in which the properties of the foods were modified by bacterial fermentation. The molecular and functional blood markers that were associated with the different foods evaluated in these studies are presented, including metabolites derived from bacterial metabolism and genes involved in metabolic pathways such as oxidative phosphorylation and inflammation.

The integrated use of these markers may help to strengthen the evidence for an association between metabolic health and food qualities, consequently narrowing the gap between food and nutritional sciences.

WHAT FACTORS DETERMINE THE ANTI-DIABETIC RESPONSE OF BILBERRY AND GRAPE SEED EXTRACTS?

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Type 2 diabetes prevalence has increased dramatically in the last decades. Diet is the first and main route for reducing diabetes risk. However, specific diets may benefit some more than others. A literature review assessing the effects of grape seed extracts and bilberry extracts on glucose and lipid metabolism in humans revealed: 320mg/d bilberry extract could lower low-density lipoproteins (LDL) by 10%, total cholesterol (TC) by 3.5%, and increase high-density lipoproteins (HDL) by 14% on average in 3 compared intervention studies. A dose of 100mg/d to 600mg/d grape seed extract in six intervention studies lowered HbA1c by 0.5%, TC by 2.8%, LDL by 1% and HDL by 6% on average. We are currently preparing a human intervention study to assess whether a commercial blend of grape seed and bilberry extract beneficially affects glucose and lipid metabolism in human subjects from various ethnic origins and at risk for developing metabolic disease, and determine which factors (bioavailability of anthocyanins and procyanidins, age, sex, baseline metabolic health including BMI, ethnicity) contribute to a beneficial response. For this, we are currently characterising the composition and metabolism of extracts of anthocyanin and procyanidin using a simulated digestion model. We are also fermenting pre-digested samples with faecal inocula to investigate the influence of gut microbiota on the digestion. We have now optimised and validated methodology to measure HbA1c and total/HDL cholesterol, yielding a recovery of >95% and ~80%, respectively. HbA1c in dried blood spots (DBS) provided stable results over a period of 124 days. We are currently optimising and validating the measurement of catechin and epicatechin, which are major metabolites of procyanidins, in DBS for use in the human study. The collection of DBS will allow subjects from all over the UK to participate in the study from a home setting.

Keywords: *T2D, bilberry, grape, DBS*

Acknowledgements: *By-Health*

EFFECT OF PERSONALISED LIFESTYLE ADVICE ON MUSCLE AND METABOLIC HEALTH AMONG ACTIVE SENIORS – A PILOT STUDY

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Worldwide, seniors make up the fastest-growing age group which is associated with a gradual loss of muscle health as well as metabolic health. Previous studies have shown that personalised feedback and advice is more effective than general information in improving health behaviour. The primary focus of the current pilot-study was to demonstrate that personalisation of lifestyle advice improves muscle and metabolic health in a population of active seniors.

Therefore, a total of 59 seniors aged 60 years or older with a sedentary lifestyle were recruited. The study was designed as a 9-week single-blind randomised controlled trial. At baseline and end, biological, genetic and socio-psychological measurements were performed. Food intake and compliance were monitored at three moments during the study. The intervention group (n=30) received personalised dietary and lifestyle advice (PA), based on their dietary intake, phenotype and genotype, whereas the control group (n=29) received generic advice (GA) based on the Dutch dietary guidelines.

Both groups showed a significantly improved muscle as well as metabolic health. Interestingly, the PA group showed a significantly greater reduction in body fat percentage and hip circumference as compared to the GA group. In terms of dietary intake both groups showed a significant improvement, but only the PA group showed increased plasma vitamin D levels as well as higher EPA and DHA levels. Although protein intake decreased in both groups, plasma levels of several amino acids increased in the PA group, including essential and aromatic amino acids.

In conclusion, this study indicates that personalised lifestyle advice results in additional health benefits as well as improved nutritional status in a population of active seniors as compared to general lifestyle advice.

Keywords: *personalised advice, elderly, lifestyle, nutrition, muscle health*

Acknowledgements: *TNO, Wageningen University*

INTEGRATING TRANSCRIPTOME AND METABOLOME SIGNATURES TO PROVIDE NEW INSIGHTS AS TO HOW DIET CAN PREVENT PROSTATE CANCER PROGRESSION

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Although there is an increase in the diagnosis of prostate cancer, most tumours remain organ-confined with little consequence to health. However, if highly aggressive clones emerge, this is a poor health prognosis. Genomic studies have demonstrated that many mutations occur in what appears to be non-cancerous tissues, and metastatic cancer may arise from relatively small tumours, with larger tumours remaining confined to the prostate. We are seeking to understand why the prostate gland becomes susceptible to multifocal carcinogenesis, and how diet may affect the tumour microenvironment to reduce the risk of emergence of aggressive clones. We report results from an intervention study designed to investigate dietary factors that reduce the risk of cancer progression. Men who had a diagnosis of organ-confined prostate cancer underwent a transperineally template biopsy before and after a 12-month dietary intervention with a soup developed from glucosinolate-enriched broccoli, along with appropriate control diets. Habitual diet was rigorously assessed through repeated seven-day diet diaries. Biopsy tissue was assessed for cancer progression, metabolome and transcriptome profiles, and quantification of targeted metabolites. The results provide insight into specific components of cruciferous vegetables that may influence the risk of cancer progression and the underlying mechanisms.

ANTI-INFLAMMATORY, PRORESOLVING AND CANCER CHEMOPREVENTIVE EFFECTS OF DOCOSAHEXAENOIC ACID AND ITS METABOLITES

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Docosahexaenoic acid (DHA), a representative ω-3 polyunsaturated fatty acid derived from fish oil, has been reported to possess a wide spectrum of health beneficial effects. We have previously reported that topical application of DHA prior to UVB irradiation attenuated the expression of cyclooxygenase-2 and NAD(P)H:oxidase-4 in hairless mouse skin. DHA pretreatment also attenuated UVB-induced DNA binding of nuclear factor-kappaB (NF-κB) through inhibition of phosphorylation of IκB kinase-α/β, phosphorylation and degradation of IκBα and phosphorylation and nuclear translocation of p65, a functionally active subunit of NF-κB. When topically applied to mouse skin or treating mouse epidermal cells with it, DHA induced the activation of Nrf2 that plays a pivotal role in cellular defence against oxidative stress and inflammatory damage. DHA also inhibited experimentally induced colitis in mice. Timely resolution of inflammation at the early stage is important in preventing further progression to chronic inflammation and related disorders including cancer. Resolution of inflammation is an active coordinated process regulated by distinct endogenous lipid mediators, such as resolvins and lipoxins that have anti-inflammatory and pro-resolving effects. Our recent studies have revealed that DHA enhances the efferocytic activity of macrophages, an essential event in the resolution of inflammation. Moreover, DHA induced M2 macrophage polarization through peroxisome proliferator-activated receptor γ activation. DHA undergoes oxidation to produce 17-hydroxy-DHA that is converted to an electrophilic 17-oxo metabolite. The 17-oxo-DHA induced activation of Nrf2 and upregulation of its target proteins, such as heme oxygenase-1 with anti-inflammatory activity. Further, transgenic mice harbouring fat-1 gene capable of generating spontaneously n-3 polyunsaturated fatty acids including DHA were found to be less susceptible to experimentally induced colitis and skin carcinogenesis.

VITAMIN D BEYOND THE BONE

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The discovery of vitamin D receptor (VDR) in many tissues opens a new era in vitamin D (VD) research, far beyond the mineral homeostasis and bone metabolism. The enormous data on the epidemic VD deficiency focused the attention on its role for prevention of numerous diseases, such as cancer, diabetes etc. The calcitriol molecular mode of action ranges from immediate nongenomic responses to long-term genomic effects, linked to cell proliferation, differentiation and survival. In vitro and in vivo studies demonstrate the antiproliferative role of VD. In accordance with this data we have studied prostate cancer patients and determined that VD deficiency was negatively correlated with tumor aggressiveness. The immunomodulatory role of VD has been recognized for many years. Recently, multiple studies have been focused on the role of the VD status on immune response to infection and on the novel effects of VD related to gut microbiota. Studying the effect of VD status in hepatitis C viral infection patients we found VD deficiency correlating with the viral load, liver fibrosis and the effect of antiviral therapy. In an effort to examine the role of VD on gut microbiota, we studied the relation between VD status and the frequency and severity of diarrhea syndrome in toddlers. In all studied children VD deficiency was related to a more severe diarrhea syndrome. By its noncalcitropic effects VD regulates the function of numerous organs and systems including endocrine pancreas, liver, and fat tissue. It is supposed that subclinical VD deficiency may result in insulin resistance, beta-cell dysfunction, diabetes and metabolic syndrome. Our study on normal weight and obese prepubertal children revealed VD deficiency in more than 50% of them, correlating with poor metabolic status. In relation to the noncalcemic effects of VD it might be supposed that defining and correcting the VD status of risky groups may reduce the risk of development of socially significant diseases, such as cancer, metabolic and immunodeficient diseases .

Keywords: *vitamin D, non-calcitropic effects, cancer, immune deficiency, obesity*

CHEMOPREVENTIVE EFFECTS OF DIALLYL TRISULFIDE DERIVED FROM GARLIC

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Garlic has been widely used as a therapeutic agent as well as a spice for more than 3000 years. Besides its well-known cardioprotective effects, garlic is also considered to possess potential cancer preventive properties. The chemopreventive effects of garlic have been attributed to its oil-soluble sulfur ingredients, such as diallyl sulfide, diallyl disulfide, and diallyl trisulfide (DATS), but their underlying molecular mechanisms remain largely unresolved. In the present study, we have compared the effects of aforementioned allyl sulfides on the growth of cultured human breast carcinoma (MCF-7) cells. DATS inhibited the growth of MCF-7 cells to a greater extent than did the other allyl sulfides as determined by the MTT assay. DATS also induced apoptosis in MCF-7 cells, which was mediated through accumulation of reactive oxygen species with subsequent activation of JNK that catalyzes phosphorylation of Bcl-2 at Ser70. In another experiment, DATS prevented tumor formation in a mouse xenograft model. Aberrant upregulation of cycloocygenase-2 (COX-2) has been frequently observed in several types of cancer cells and is considered as a molecular target for cancer chemoprevention. Topically applied DATS inhibited the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced COX-2 expression in the dorsal skin of female ICR mice. DATS inhibited the DNA-binding activity of AP-1, which is one of key transcription factors regulating the inflammation and expression of COX-2. DATS inhibited TPA-induced phosphorylation of Akt and JNK, which are major MAPKs regulating AP-1. A pharmacologic Akt inhibitor LY294002 and a JNK inhibitor SP600125 abrogated the TPA-induced COX-2 expression, suggesting that suppression of COX-2 expression by DATS in TPA-stimulated mouse skin is mediated by blocking the PI3K-Akt and JNK signaling. Topical application of DATS protected against mouse skin carcinogenesis induced by 7, 12-dimethylbenz[a]anthracene plus TPA. In addition, DATS strongly inhibited the DNA-binding activity of NF-κB compared with other allyl sulfides in human mammary epithelial (MCF10A) cells treated with TPA. DATS inhibited the transcriptional activity of NF-κB, phosphorylation of IκBα, and activity of IKKβ. Inhibition of NF-κB DNA-binding activity and IKKβ activity by DATS were blunted by addition of the antioxidant N-acetyl-L-cysteine (NAC) and the reducing agent dithiothreitol. Therefore, the anti-inflammatory activity of DATS may be associated with oxidation or covalent modification of thiol groups contained in IKKβ.

Keywords: *garlic, diallyl trisulfide, chemoprevention*

DNA ADDUCT MARKERS ASSOCIATED WITH THE GASTROINTESTINAL DIGESTION OF RED MEAT

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Epidemiological research has demonstrated that the consumption of red meat contributes to colorectal cancer (CRC) risk. At the time, the main hypothesis on the red meat-CRC relationship is based on the fact that red, but not white meat consumption, has been linked to CRC, and that red meat contains more heme iron than white meat. More specifically, the heme hypothesis states that the ingestion of heme iron stimulates the formation of N-nitroso compounds (NOCs) and lipid peroxidation products (LPOs). Both NOCs and LPOs can exert geno- as well as cytotoxic effects, and as such contribute to carcinogenesis. In this study, beef (model for red meat) and chicken (model for white meat) were digested in vitro (static model) as well as in vivo (Sprague-Dawley rats) to investigate the gastrointestinal formation of DNA adducts upon red vs. white meat digestion. DNA adduct formation was assessed by means of a state-of-the-art UHPLC-HRMS DNA adductomics platform (Q-Exactive™) and in-house DNA adduct database, after which univariate (e.g. t-test) as well as multivariate (e.g. OPLS-DA) statistics were employed for red meat associated DNA adduct marker discovery. Combining the results from 3 independent in vitro and 1 in vivo digestion experiment(s), 7 DNA adduct types, including O6-carboxymethylguanine, dimethyl- or ethylthymine, methylguanine, heptanalguanine, a malondialdehyde-guanine adduct, and a malondialdehyde-cytosine adduct could be singled out as potential red meat digestion markers. This is highly relevant to the red meat-CRC hypothesis because the formation of the retrieved DNA adduct types may be linked to DNA alkylation and/or oxidation by e.g. NOCs and/or LPOs. Therefore, follow-up research should focus on the role of DNA adduct formation in the red meat-CRC pathway, as well as the mutagenic potential and human in vivo relevance of the proposed DNA adduct markers.

Keywords: *colorectal cancer, DNA adductome mapping, gastrointestinal digestion, red meat*

HESPERIDIN MODULATES HYPERTENSION AND THE URINARY METABOLIC PROFILE IN CAFETERIA DIET-FED RATS

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Introduction: **Diet seems to play a crucial role in the development and prevention of cardiovascular disease (CVD). In particular, fruit-rich diets can be an important modifiable factor for reducing CVD-related comorbidities. Hence, orange juice (OJ) consumption has been reported to exert beneficial effects on some CVD risk factors such as blood pressure an endothelial function. Hesperidin represents more than 90% of the flavanones found in OJ but its role on OJ-mediated beneficial effects is far from fully understood.**

Aim: **The principal aim was to evaluate hesperidin's effect and underline the mechanisms after a chronic consumption in rats fed cafeteria (CAF) diet.**

Materials and Methods: **Eight-week-old male Sprague-Dawley rats (n=40) were fed CAF or standard (STD) diet for 9 weeks. After this, each diet group was supplemented either with vehicle or hesperidin (100 mg/kg) for 8 weeks. Systolic blood pressure (SBP) and body weight were measured every week during treatment. At the end, urine samples were collected and the urinary metabolic profiles were analysed by 1H-NMR spectroscopy.**

Results: **CAF diet significantly increased body weight, fat content, and SBP compared to STD diet. Hesperidin supplementation had no effect on body weight or fat content in either diet group. However, hesperidin treatment reduced SBP in rats fed CAF diet, but did not change it in STD diet-fed rats. In addition, hesperidin supplementation had a significant effect on the urinary metabolic profiles of both diet groups. Thus, it resulted in changes in several gut microbially derived uremic toxins (DMA, PAG, 4-C), and a reduction in the excretion of metabolites related to inflammation and oxidative stress (2-deoxyciditine, allantoin, pseudouridine, NAG, and fucose).**

Conclusion: **Our findings are consistent with the beneficial effect of hesperidin on blood pressure and the urinary metabolic profile, which could be in part related to a change in the microbiota composition and/or functionality after hesperidin supplementation.**

Keywords: *hesperidin, hypertension, metabolism, CVD*

DIFFERENTIAL EFFECTS OF ANIMAL VERSUS PLANT PROTEIN DIET ON CIRCULATING CONCENTRATIONS OF TRANSFORMING GROWTH FACTOR B IN DIABETIC PATIENTS

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Introduction: **Transforming growth factor β (TGFβ) is a pleiotropic cytokine known to exert both pro- and anti-inflammatory effects. Elevated concentrations of TGFβ are reported in patients with type 2 diabetes (T2D). However, little is known about the effect of diet on circulating TGFβ concentrations. We recently demonstrated beneficial metabolic effects of a high-protein diet in diabetic subjects.**

Aim: **We aimed to determine changes of TGFβ concentrations after a 6-week high-protein diet intervention in T2D patients and to evaluate potential differences according to dietary source of protein (animal versus plant).**

Materials and Methods: **37 individuals with T2D (age 64±6 years, HbA1c 7.0±0.6%) were randomly assigned to either high-animal protein or high-plant protein diet (30% protein, 40% carbohydrates, 30% fat) for 6 weeks. Clinical examinations were performed at the beginning and the end of the study. Levels of TGFβ and other cytokines were determined in blood serum using ELISA and Luminex technology.**

Results: **Levels of TGFβ were elevated in all participants [mean(±SD)=7594.1±2021.4 pg/mL]. Baseline TGFβ correlated inversely with body mass index (Rho=-0.10) and insulin levels (Rho=-0.32). After intervention, a non-significant decrease in TGFβ concentrations by 5% (7986.4±2226.6 pg/mL to 7594.4±2934.7 pg/mL, P=0.604) was observed in the high-plant protein group. In contrast, an increase of TGFβ by 13% (7155.7±1724.4 pg/mL to 8105.0±1903.2 pg/mL, P=0.004) was detected in the high animal protein group. Parallel evaluation of additional biomarkers revealed that the negative change in high-plant protein diet correlated negatively with changes of adiponectin, while the positive change in high-animal protein diet correlated negatively with changes of C-reactive protein and interleukin-8.**

Conclusion: **These data provide first line of evidence on the differential effects of animal versus plant protein diet on circulating concentrations of TGFβ in subjects with T2D. Further research is warranted to investigate the mechanism explaining potential role of protein diet in modulating TGFβ inflammatory cascade.**

Acknowledgements: *Federal Ministry of Food and Agriculture, German Center for Diabetes Research*

ASSOCIATION BETWEEN PHYSICAL ACTIVITY AND COLON CANCER IS MEDIATED BY BIOMARKERS OF IMMUNITY AND VITAMIN D METABOLISM: A NESTED CASE-CONTROL STUDY

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High physical activity is associated with a lower risk of colon cancer; however, the underlying biological mechanisms for this association remain largely unclear. We aimed to determine the extent to which body fat and biomarkers of various biologically plausible pathways account for the association between physical activity and colon cancer. We conducted a nested case-control study in a cohort of 519,978 men and women aged 25 to 70 years participating in the European Prospective Investigation into Cancer and Nutrition Cohort (EPIC) followed from 1992 to 2003. A total of 713 incident colon cancer cases were matched using risk-set sampling to 713 controls on age, sex, study center, fasting status, and hormonal therapy use. The amount of total physical activity during the past year was expressed in metabolic equivalent of task [MET]-hours/week. Anthropometric measurements and blood samples were collected at study baseline. Biomarkers in the following pathways were evaluated: metabolic dysfunction, insulin growth factors, adipokine secretion [adiponectin, leptin, soluble leptin receptor (sOB-R)], lipid metabolism, oxidative stress, vitamin D metabolism [25[OH]D], inflammation and immune response. High physical activity was associated with a lower risk of colon cancer (Relative Risk ≥91 MET-hours/week versus < 91 MET-hours/ week = 0.75 [95% confidence interval (CI): 0.57 to 0.96]. In mediation analyses, this association was accounted for by waist circumference [proportion explained effect (PEE) = 17%; CI: 4% to 52%] and the biomarkers: soluble leptin receptor (sOB-R) [PEE = 15%; 95% CI: 1% to 50%] and 5-hydroxyvitamin D (25[OH]D) [PEE = 30%; 95% CI: 12% to 88%]. In combination, these factors explained 45% (95% CI: 20% to 125%) of the association. Beyond waist circumference, sOB-R and 25[OH]D additionally explained 10% and 23% of the association, respectively. Promoting physical activity and maintaining metabolic health and adequate vitamin D levels could represent a promising strategy for colon cancer prevention.

Keywords: *physical activity, colon cancer, mediating biomarkers, vitamin D metabolism, immunity*

A NOVEL PLATFORM FOR TAXONOMIC AND FUNCTIONAL PROFILING OF THE GUT MICROBIOME

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The advent of metagenomic approaches revolutionized our understanding of the role of gut microbial communities and their interactions with the host. However, due to the dominant perception that the majority of bacteria are unculturable, their functional and phenotypic characteristics remain unknown. To address this problem, we developed a novel platform for high-throughput specific phenotypic selection of environmental microbial strains and communities. We collected samples from gastrointestinal tract of the herbivorous fish Kyphosus sydneyanus, which is a hindgut fermenter. Applying the two main approaches, 16S ribosomal RNA gene amplicons and shotgun metagenomics, we profiled the taxonomic composition and functional diversity of the microbial species and compared the ones presented in the original samples to those that grew in the lab. A total of 96 strains were isolated, representing 52 novel species from 10 families belonging to the phyla Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria In addition, of the 52 species cultured, 47 were strict anaerobes. The metabolic analysis also revealed that the variance in dietary algae, may influence the gut microbiota, due to the difference in nutritional composition between algal groups. In conclusion, our approach enables not only the large-scale culturing and phenotyping of novel microbial species but also unlocks the fish intestinal microbiota for metabolic capabilities. The potential application of seaweed degrading microbes for fishmeal protein production will be discussed.

Keywords: *microbiome fish nutrition*

Acknowledgements: *New Zealand Government Funding*

UNTARGETED METABOLIC PROFILING BY MEANS OF UHPLC-QTOF/MS FOR THE IDENTIFICATION OF MEAT AND DAIRY PRODUCTS INTAKE BIOMARKERS

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Meat intake has been associated with positive as well as negative health outcomes. Meat is essential from a nutritional point of view but, on the other hand, has been associated with increased risk of several diseases. However, a causal relation between meat consumption and negative health outcomes is still controversial. In order to shed light on this issue, a reliable method for assessment of meat intake is needed. Meat intake has often been assessed using very imprecise methods. Dietary biomarkers measured in biological fluids by means of untargeted metabolomics have been proposed as a possible way to find objective biomarkers for measurement of the actual intake of specific foods.

A meat intake marker should be able to differentiate meat consumption from that of other protein-rich foods, such as dairy products. For this purpose, a randomized, controlled, cross-over single meal study was conducted on 17 subjects to collect urine samples at several time points after the ingestion of red meat or dairy product based meals.

An ultra-high-performance-liquid chromatograph coupled via an electrospray source to a Q-TOF mass spectrometer was used for the separation and metabolic profiling of the urine samples. Data were analyzed and group differences compared by both univariate and multivariate data analysis and results combined. As a result, 37 compounds were found to be highly important for discriminating the two diets; in particular 20 markers were higher after the dairy-based meal, while 17 markers were higher after the meat-based meal. These markers were fragmented and tentatively identified. Some of them were synthesized to allow confirmation of their identity. Combinations of these markers should be further validated as meat or dairy product intake biomarkers.

Keywords: *metabolomics, meat, untargeted, biomarkers, high resolution mass spectrometry*

QUALITY OF NUTRITIONAL LIPIDS OF MYTILUS GALLOPROVINCIALIS FROM THE BLACK SEA

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Over the past few decades the interest in marine mussels as popular seafood has increased rapidly. Marine mollusks are valuable healthy food, low in calories and fats and high in proteins. Among the three major groups of mussel primary metabolites, lipids have shown the highest potential for developing functional foods or dietary supplements with beneficial effects on health. Mussel lipids are a good source of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) and have shown to contain other minor lipid components with their own beneficial effects on human health. Black mussel (*Mytilus galloprovincialis*) is one of the most commercially important species from the Bulgarian Black Sea waters. Farmed *Mytilus galloprovincialis* are considered to be a sustainable source of LC-PUFA as they require no dietary inputs, gaining nutritional lipids by filter-feeding sea water.

The present study was conducted to investigate biologically active substances in lipid extracts of farmed *Mytilus galloprovincialis* from the Black Sea. Total lipids, phospholipids, fatty acids, fat-soluble vitamins and carotenoids were analyzed.

Mussel lipid extracts and fractions are obtained by solvent extraction and are purified by chromatographic separation. The saponifiable lipid fraction was derivatized into fatty acid methyl esters (FAMEs) and analyzed by gas chromatography–mass spectrometry (GC-MS) to identify and quantify the fatty acids. The fat soluble non-saponifiable lipids were identified by high pressure liquid chromatography coupled with UV/Vis and fluorescence detectors (HPLC-UV-FL).

The study revealed that farmed *Mytilus galloprovincialis* from the Black Sea is a good natural source of high quality nutritional lipids to be part of a proper or preventive diet. Moreover, black mussels show high potential for developing functional foods and/or dietary supplements with beneficial effects.

Keywords: *Mytilus galloprovincialis, lipids, fatty acids, vitamins, carotenoids*

Acknowledgements: *Bulgarian National Research Fund support, Project DM 09/2 from 15/12/2016*

EARLY NUTRITION, METABOLIC EFFECTS AND LONG-TERM HEALTH

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GENOTYPE FREQUENCY IN THE B-CAROTENE CONVERSION TO VITAMIN A: IMPLICATIONS ON REDUCING VITAMIN A DEFICIENCY IN THE PHILIPPINES

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Vitamin A is required for normal organogenesis, immune function and visual cycle regeneration. Worldwide, vitamin A deficiency(VAD) is estimated to be 190 million individuals in preschool-age children. VAD prevalence among children in the Philippines is 11.1%, which is considered a public health problem of moderate severity. The requirements for vitamin A can be satisfied either from animal foods or from plant foods containing provitamin A, such as carotenoids. The key enzyme responsible for carotenoid conversion into the active form of vitamin A is β-carotene 15,15'-monooxygenase(BCMO1). However, conversion efficiency of carotenoid into vitamin A is highly variable in different individuals or population groups due to genetic variants in BCMO1 gene. The study determined the BCMO1 single nucleotide polymorphism (SNP) frequency among 6-18-year-old children of the 2013 Philippine National Nutrition Survey (NNS), particularly rs7501331 and rs12934922. A total of 555 individual survey participants with BCMO1 SNPs rs7501331 and 723 respondents with rs12934922 were analyzed. Results showed that 56.0%, 37.7% and 6.3% of CC, CT and TT for rs7501331 genotype were found among Filipino children, while 80.4%, 19.5% and 0.14% genotype frequencies of AA, AT and TT were obtained for rs12934922. Genotype frequencies of both SNP targets follow the Hardy-Weinberg equilibrium indicating good data quality. The results suggest that a portion of the Filipino children (43.96% for rs7501331 and 19.6% for rs12934922) carries the risk allele that may decrease β-carotene to vitamin A conversion efficiency. Pre-determination of the proportion of the population who are at risk for VAD by genetic profiling is an important step in prioritizing those that need intervention either by increasing intake of β-carotene or preformed vitamin A supplements. We can envisage adapting recommended dietary intake of carotenoids, or carotenoids incorporated in functional foods/supplements, to population groups with genetic variants that affect carotenoid status.

Keywords: *BCMO1, genotyping, nutrigenetics, vitamin A deficiency*

GREEN TEA EXTRACT INCREASES THE TELOMERE LENGTH OF GRADE III OBESITY WOMEN

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Introduction: **Some factors related to obesity, such as inflammation and oxidative stress may play a substantial role in telomere attrition and, consequently, obesity is associated with telomere shortening. Emerging evidences have demonstrated that green tea and its most abundant catechin (polyphenol epigallocatechin-3-gallate, EGCG) had anti-inflammatory and antioxidant effects. In line of this, green tea may be positively related to telomere length (TL).**

Aim: **In this context, the present study aimed to evaluate the effect of decaffeinated green tea supplementation in obese women on TL.**

Materials and Methods: **We conduced a cross-sectional interventional study with 10 obese (BMI>40 kg/m²) and 8 normal weight (BMI >18.5 and <24.9 kg/m²) women (age between 27 and 48 years). We did not include patients with alterations in liver biomarkers. The supplementation was carried out with capsules (one capsule daily, each containing 450.7 mg of epigallocatechin-3-gallate) for 8 weeks. Anthropometric (weight, height and body mass index – BMI) and dietary intake assessment and blood collection [for biochemical (glycemia and lipidic profile) and TL analysis by quantitative PCR] were performed before (day 0) and after supplementation (day 56). Normal weight patients were evaluated at a single moment. Shapiro-Wilk test, paired t-test, independent t-test, Pearson correlation and multiple regression analysis were performed as statistical analyses ($p<0.05$).**

Results: **There were not any significant weight loss (119.7±17.8 to 120±18 kg, $p=0.772$) or BMI (45.3±4.7 to 45.4±5gm², $p=0.695$) changes linked with the intervention. However, we observed a significant increase on TL after supplementation (1.57±1.1 to 3.2±2.1 T/S ratio; $p<0.05$). Moreover, we found shorter TL in obese patients (day 0) when compared to normal weight individuals (3.2±1.9 T/S ratio; $p<0.05$) and an inverse association between TL and BMI, even after age adjustment (beta=-0.527; $r^2=0.286$; IC= -0.129, -0.009). There was no correlation between the biochemical profile and TL.**

Conclusion: **Obesity is related to shorter telomeres. Green tea supplementation for 8 weeks promotes telomere elongation in obese women.**

Keywords: *obesity, epigallocatechin-3-gallate, green tea, telomere length, supplementation*

Acknowledgements: *This work was supported by grants #2013/08916-4 and #2014/00669-0 from the São Paulo Research Foundation (FAPESP) and grant #166191/2015-9 from the National Council of Scientific and Technological Development (CNPq)*

PHYSICAL TRAINING PROMOTES TELOMERE ELONGATION IN OBESE WOMEN

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Introduction: **While sedentary and obese individuals have shorter telomere lengths (TL) due to their inflammatory status, physical activity may be associated with longer TL. In line of this, physical activity may protect against the deleterious effects of obesity on shortening TL. This study aimed to evaluate the changes of TL of obese women submitted to a concurrent physical training intervention.**

Materials and Methods: **This is a cross-sectional interventional study conducted with 7 obese (BMI>30 kg/m²) women (35.1±7 years). The concurrent exercise intervention (strength and aerobic) was performed for 8 weeks (3 times a week with high intensity). Anthropometric (weight, height and body mass index – BMI), body composition assessment (fat mass, by Deuterium oxide), blood collection for TL (by quantitative PCR) and inflammatory cytokines analysis (by Multiplex assays using Luminex) were performed before and after intervention. Shapiro-Wilk test, Wilcoxon test, and Spearman correlation were performed as statistical analyses ($p<0.05$).**

Results: **There was a significant increase on TL after the intervention (0.007±0.008 to 0.084±0.1 T/S ratio; p=0.043) and a decrease of fat mass (40±4.9 to 38.2±5.8 kg, p=0.018). However, there were no significant weight loss (83.2±7.7 to 84.2±7.7 kg, p=0.204) or changes of cytokines (IL-6: 1.51±0.42 to 1.33±0.41; p=0.091; IL-10: 1.37±0.25 to 1.33±0.19; p=0.336; IL-4: 26.2±28.1 to 23.6±24.4; p=0.176; IL-1beta: 7.6±9.7 to 6.5±7.2; p=0.172; IL-17a: 1.15±0.15 to 1.1±0.1; p=0.317). Indeed, we observed a tendency for positive correlation between baseline TL and weight changes (r= 0.750; p=0.052). We did not find a correlation between TL and inflammatory biomarkers.**

Conclusion: **Concurrent physical training intervention promotes telomere elongation in obese women, fat mass decrease, but the obesity inflammatory status is not associated with TL.**

Keywords: *obesity, exercise, telomere length*

Acknowledgements: *National Council of Scientific and Technological Development (CNPq)*

AGING AT THE MOLECULAR LEVEL: CAN BACTERIA SHED LIGHT ON IT?

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Recent studies have challenged the paradigm that bacteria do not age and are immortal. Stewart et al. provided intriguing evidence that during division Escherichia coli K-12 exhibits functional asymmetry, leaving behind a mother cell with delayed growth and survival rate and a juvenile daughter cell, empowered to successfully perpetuate the species over time. In view of this new finding, bacteria, and in particular E. coli, emerged as a promising model for exploration of basic mechanisms of aging. It is not yet clear to what extent pro- and eukaryotic cells age similarly but at least some episodes of aging, especially those playing at the molecular level, should be common. Spontaneous chemical reactions including hydrolysis, oxidation and glycation (the Maillard reaction) are well known to deteriorate macromolecular structure and function. The Maillard reaction, yielding the so-called advanced glycation end products (AGEs) on proteins, DNA and amino-lipids, has long been associated with diseases (diabetes, Alzheimer's and Parkinson's diseases) and aging in humans. We have demonstrated that despite the short life span of E. coli of tens of minutes to hours, its chromosomal DNA accumulates AGEs under normal growth conditions. Such progressive modification of a key biological molecule provides an independent line of evidence for E. coli aging. Subsequent studies led to the identification of several E. coli genes (hchA, yajL, yhbO, elbB, gcp), which are implicated in anti-glycation defense and are highly conserved. Thus, it appears that common molecular mechanisms underlie aging in all three domains of life (Bacteria, Archaea and Eukaryota), which gives us hope that E. coli may provide an excellent platform for deciphering the intimate mechanisms of aging.

Keywords: *aging, Escherichia coli, Maillard reaction, glycation, AGEs*

Acknowledgements: *Contract No. DH1/5-16.12.2016 from the Bulgarian National Science Fund and Contract No. DFNP-79 A-1/28.04.2016 from the Bulgarian Academy of Sciences*

ASSESSING OBESITY AND INSULIN MOLECULAR PATHWAYS INVOLVED IN WEIGHT LOSS INDUCED BY ROUX-EN-Y GASTRIC BYPASS

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Introduction: **Given the role genetics plays in weight regulation, the magnitude of weight loss after Roux-en-Y gastric bypass (RYGB) can also be genetically determined. The present study aimed to evaluate how the expression of genes involved in insulin metabolism and obesity pathways changed after RYGB.**

Materials and Methods: **Blood samples were obtained from 13 women (35.9±9.2 years) with severe obesity before and six months after RYGB. Whole blood transcriptome was assayed by microarray.**

Results: **A total of 1966 up- or downregulated genes were identified in pre- and postoperative periods of bariatric surgery. From these analyses, we observed that genes involved in obesity and in insulin pathways were upregulated after RYGB, especially the genes IKBKB, PIK3R1, and PLA2G4A ($p<0.05$).**

Conclusion: **Microarray is a powerful tool for identifying biomarkers in obese patients in the pre- and postoperative periods of RYGB by analyzing differential gene expression profiles. Our findings in blood support previous studies documented in adipose tissue. RYGB promotes upregulation of genes involved in obesity and insulin pathways, which is associated with improved glucose metabolism. Inflammation due to acute weight loss and surgical procedure per se may explain the upregulation of IKKB. Based on our findings in blood, it may be possible to promote the development of therapeutic targets for specific altered genes.**

Keywords: *obesity, microarray, gene expression, bariatric surgery, weight loss, insulin*

Acknowledgements: *São Paulo Research Foundation (FAPESP) (#grant No. 2013/12819-4) and National Council for Scientific and Technological Development (CNPq) (#grant No. 480763/2013-5)*

METHYLATION ANALYSIS OF INFLAMMATORY GENES OF WOMEN WITH GRADE III OBESITY BEFORE AND AFTER HYPOCALORIC DIETARY INTERVENTION

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Introduction: **Obesity is a chronic disease that affects millions of individuals and is related to several metabolic alterations. The interactions between genes and environment have been studied to identify possible factors that influence the responses of individuals to different metabolic conditions. In line with this DNA methylation is an important epigenetic marker in the determination of various biological processes and diseases in humans, including obesity.**

Aim: **The aim of this study was to identify the modifications in DNA methylation pattern involved with inflammation pathway genes before and after hypocaloric dietary intervention in women with grade III obesity.**

Materials and Methods: **This was a cross-sectional study with a sample of women admitted to a university hospital, classified into two different groups: Intervention group (G1) – grade III obesity women (body mass index (BMI) ≥40 kg/m2] pre and post hypocaloric dietary intervention (1200 kcal/day) for 6 weeks; Control Group (G2) - healthy eutrophic women (BMI between 18.5 and 24.9 kg/m2). Anthropometric data [weight, height, BMI and abdominal circumference (AC)], body composition [fat-free mass (FFM) and fat mass (FM)] and peripheral blood for methylation analysis were collected. Normality test (Shapiro-Wilk), descriptive statistics (mean and standard deviation), t-tests (paired and independent) were used for the statistical analysis. Significance level was accepted for $p<0.05$.**

Results: **Twenty-one women were evaluated, 10 normal weight and 11 obese. The mean age was 35.5±6.8 years for G1 and 33.5±11.2 years for G2. A significant weight loss of 8.4±4.3 kg (5.2%, $p<0.001$) was observed in G1 after 6 weeks of dietary intervention, in addition to a reduction in BMI (58.5±10.5 to 55.3±9.1kg/m2; $p<0.001$), FFM (65.4±8.6 to 63.1±7.1 kg; $p=0.009$) and FM (89.5±23.0 to 83.4±21.0 kg; $p=0.001$). Indeed, BMI, AC, FFM and FM from obese individuals' values remained statistically different from the G2 group both in the pre- and post-dietary periods.**

Conclusion: **Our results show that individuals with obesity who received hypocaloric dietary intervention had significant weight, BMI, FFM and FM reduction. The next stage of the present study will be to identify the DNA methylation changes of inflammatory genes of women with grade III obesity before and after dietary intervention and to compare them with the control group.**

Keywords: *obesity, methylation, inflammatory genes, dietary intervention*

Acknowledgements: *São Paulo Research Foundation (FAPESP), Coordination of Improvement of Higher Level Personnel (CAPES)*

SALT SENSITIVITY: GENETIC AND PHYSIOLOGICAL MARKERS AND ITS EFFECTS ON SALT TASTE PERCEPTION AND INTAKE

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Salt sensitivity is an independent cardiovascular disease and mortality risk factor, present in both hypertensive and normotensive population. It is genetically determined and it may affect the relationship between salt taste perception and salt intake. The aim of this study was to explore the genetic predisposition to salt sensitivity in young and middle-aged adult population and its effects on salt taste perception and salt intake. Salt sensitivity was investigated in 20 normotensive subjects and defined as the change in blood pressure (BP) after 7 days of low-salt (51.3 mmol sodium/day) and 7 days of high-salt diet (307.8 mmol sodium/day). Salt taste perception was identified using British Standards Institution sensory analysis method (BS ISO 3972:2011). Salt intake was assessed with a validated food frequency questionnaire. DNA was genotyped for single nucleotide polymorphisms in the SLC4A5, SCNN1B and TRPV1 genes coding for sodium and ion channels and transporters. The subjects with the rs7571842 (SLC4A5) AA genotype exhibited the highest increase in BP (systolic BP (ΔSBP)=7.75 mmHg, p=0.002; diastolic BP (ΔDBP)=6.25 mmHg, p=0.044; mean arterial pressure (ΔMAP)=6.5 mmHg, p=0.014). There was an association between salt taste perception and salt sensitivity (rs=0.551, p=0.041) with salt intake being unaffected. There is a genetic predisposition to salt sensitivity and it is associated with salt taste perception. Unaffected salt intake suggests that factors other than taste sensitivity and/or other genetic variants may have more pronounced effects in healthy adults. Moreover, experiments are conducted in our laboratory to increase the understanding of the SLC4A5 effects on salt-sensitive changes in BP. Urine samples have been collected from subjects phenotyped for salt sensitivity to measure the expression of this co-transporter. If differently expressed in salt-sensitive and salt-resistant subjects, together with its genotype, it may serve as a personalised salt sensitivity biomarker.

Keywords: *blood pressure, genetics, salt intake, salt sensitivity, taste*

ASSOCIATION BETWEEN A FATTY ACID DESATURASE 1 GENE POLYMORPHISM AND BLOOD PLASMA CHOLESTEROL BIOMARKERS IN BULGARIAN ELDERLY

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Introduction: **A particular single nucleotide polymorphism (SNP), rs174547, of the fatty acids desaturase (FADS) 1 gene has been significantly associated with low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglyceride blood plasma concentrations according to published data. FADS1 and FADS2, encoded by FADS1 and FADS2 genes, are membrane-bound key enzymes, rate limiting in the synthesis of long-chain polyunsaturated fatty acids - arachidonic, eicosapentaenoic and docosahexaenoic acid from their dietary precursors linoleic (C18:2 n-6) and α-linolenic acid (C18:3n-3).**

Aim: **The study aims to detect and determine individual variants of the rs174547 SNP among Bulgarians and analyze a possible association between the allele variants and blood plasma cholesterol levels, being strong indicators for cardiovascular health and lipid metabolism.**

Materials and Methods: **Bulgarian volunteers (N=123) were randomly selected and following standard DNA extraction from buccal swabs, genetically tested for their allele variant (C/T) of the rs174547 SNP in FADS1 gene by real-time polymerase chain reaction. Laboratory assessment for cholesterol profile, triglycerides and blood sugar was performed, together with bio-impedancemetry for body composition analysis. Dietary information was obtained by a modified diet history methodology combining a Food Frequency Questionnaire, two 24-hour Food Recall Questionnaires and General Lifestyle Questionnaire.**

Results and Conclusion: **Individual genotypes were determined and results were analyzed in order to unveil the magnitude of influence of the given SNP over the studied metabolic pathways. The rs174547 SNP could provide valuable information concerning individual's fatty acid metabolism and may support nutritionists when nutritional requirements for omega-3 and omega-6 fatty acids are estimated. It can also play a predictive role for developing future chronic illnesses and conditions. If so, this molecular biomarker could be successfully integrated in panels of nutrigenetic profiles concerning lipid and cholesterol metabolism and thus enhance the development of personalized nutrition and medicine.**

Keywords: *genetic variants, FADS1 genes, plasma cholesterol levels, personalized nutrition*

Acknowledgements: *National Center of Public Health and Analyses, Sofia and Medical University – Pleven*

DOES ROUX-EN-Y GASTRIC BYPASS INFLUENCE THE TELOMERE LENGTH? PRELIMINARY RESULTS

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Introduction: **Telomere lenght is physiologically associated with ageing but it may be influenced by oxidative stress and chronic inflammation, linked to obesity. In several studies short telomere length has also been associated to obesity in adults. However, there are still few studies evaluating the influence of obesity, diet and bariatric surgery on telomere length.**

Aim: **This study aims to evaluate the telomere length in obese women before and after Roux-en-Y gastric bypass (RYGB), and associate these data with anthropometric variables and body composition.**

Materials and Methods: **A prospective and longitudinal study has been conducted with severely obese women from a mixed population, aged 25 to 50 years, undergoing RYGB. Anthropometric data (weight and height), body composition, and peripheral blood for analysis of telomere length were collected before and 6 months after the surgical procedure. The telomere length was measured by a polymerase chain reaction (PCR) method described by Cawton and obtained by the T/S ratio for each sample [ΔCt = Ct (Tel) - Ct (36B4); T/S ratio = 2 (-ΔCt)]. Preliminary results: Thriteen women with grade III obesity were selected before the bariatric surgery. The mean age was 38.64±6.2 years, the mean weight was 110.9±14 kg and the BMI was 42.8±4.1 kg/m². The body composition assessment showed that the mean fat mass was 53.3±9.3 kg and the fat-free mass was 58.95±6.8 kg. The mean telomere length of these patients was 0.16±0.13 bp.**

Conclusion: **The next step will be to relate the anthropometric, biochemical and food intake data of 50 patients before and 6 months after the bariatric surgery and to verify the influence on telomere length.**

Keywords: *bariatric surgery, obesity, telomere length*

DETERMINING THE EFFECTS OF THE STINGING NETTLE (URTICA DIOICA) EXTRACT ON BIOFILM FORMATION OF SALMONELLA ENTERICA SEROVARS

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The increasing population leads ti an increasing demand for fresh produce all around the world. Making provisions for inhibiting foodborne pathogens, especially the ones producing biofilms, such as Salmonella spp., might remain impossible during the process, due to the cross-contaminations. Thus, foodborne outbreaks can be accompanied by biofilms. Chemical disinfectants are not efficient in disintegrating the biofilms since they cannot penetrate into the biofilm matrix. At this point, plant extracts with correct exposure time on fresh produce are crucial to take timely precautions against the attachment of bacteria to fresh produce. The effects of the water-soluble extract of stinging nettle were determined for biofilm formation capabilities on food product and in vitro, and swimming motilities and curli expressions of Salmonella enterica Virchow, Newport, Typhimurium, Enteritidis, Othmarschen and Mikawasima serovars. Moreover, rpoS, mlrA, ycfR, fimA, spiA and csgA, biofilm-related genes, were also screened. Results have revealed that 2 log reduction of bacterial strains named above, forming biofilm formation on spinach, have been observed by the help of the extract of stinging nettle with 1-hour exposure time. In vitro, different inhibitory capabilities of the extracts have been observed regarding the concentrations, ranging from 2 mg/ml to 20 mg/ml. The extract has significantly decreased the swimming motilities of serovars, except Enteritidis. Weak and strong biofilm producers have been observed in curli expression. In conclusion, stinging nettle extract plays an important role in eliminating biofilm formation of Salmonella enterica serovars.

Keywords: *biofilm, Salmonella, plant extract*

COMPARISON BETWEEN INDIRECT CALORIMETRY AND PREDICTION EQUATIONS FOR WOMEN WITH GRADE III OBESITY

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Resting metabolic rate (RMR) is an important parameter to guide nutritional therapy of class III obese patients. The objective of the present study was to compare indirect calorimetry (IC) with other predictive equations. This is a cross-sectional study on women with class III obesity (body mass index > 40 kg/m²). Weight, height, fat-free mass, fat mass and resting metabolic rate of all individuals were measured. Bland–Altman plot was used to analyze the agreement between IC and predictive equations' results. A total of 101 women with obesity class III, mean age of 36.3±10 years were evaluated. Harrys-Benedict and WHO equations showed similar bias and limits, (181.6 kcal, +2SD=765.5, -2SD=-402.2), (156.4 kcal, +2SD= 799.4, -2SD= -486.6, respectively). The Mifflin-St. Jeor and Owen equations showed large clinical bias (mean of 239.2 kcal and 463.9 kcal, respectively), and a tendency to overestimate RMR. The prediction equations tested in the study had low accuracy in estimating RMR of women with class III obesity.

Keywords: *resting metabolic rate, obesity, equation, women*

Acknowledgements: *Grant#2015/06624-1, São Paulo Research Foundation (FAPESP)*

MLX EXPRESSION IS MODIFIED IN WOMEN SIX MONTHS AFTER BARIATRIC SURGERY

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University of Sao Paulo, Brazil

Introduction: **The mechanisms of genetic contribution to the development of obesity need to be precisely established in interventions for weight loss, such as bariatric surgery. The gene MAX-like protein X (MLX) is a key mediator in the detection of intracellular glucose, responsible for regulating genes involved in glycolysis and in lipogenesis. Therefore, the analysis of this gene expression may elucidate the understanding of the changes occurring in energy metabolism in obesity**

Aim: **The aim of the present study was to evaluate the MLX expression in women with obesity before and after bariatric surgery.**

Materials and Methods: **The sample consisted of 13 women with grade III obesity before and six months after bariatric surgery. The nutritional status classification was performed using weight and Body Mass Index (BMI). The analysis of the genetic profile was performed in peripheral blood by the microarray methodology, BeadChip - Illumina™ and with subsequent validation by the quantitative Real Time PCR method. Data were analyzed by statistical software SPSS version 20 with a significance level $p<0.05$.**

Results: **Differences were observed in weight variables (115.4±19.5 vs. 86.7±13.8 kg, $p<0.01$), body mass index (42.5±7.0 vs. 32.1±5.6 kg/m^2 $p<0.01$), in the final stage of the study. There was an increase in MLX expression six months after the surgery (fold change 2.2E+06, $p=0.01$).**

Conclusion: **There was significant weight loss and increased MLX expression 6 months after the surgical treatment. Understanding this pathway related to energy metabolism, can lead us to future treatments for obesity**

Keywords: *obesity, energy metabolism, gene expression, microarray*

Acknowledgements: *Grant#2015/06624-1, São Paulo Research Foundation (FAPESP)*

INTERLEUKIN-6 - 174G/C POLYMORPHISM IN NON-ALCOHOLIC FATTY LIVER DISEASE – PREVALENCE AND METABOLIC CHARACTERISTICS

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The C allele of rs1800795 single nucleotide polymorphism (SNP), or "-174G/C", in the promotor of Interleukin-6 (IL-6) gene is associated with an increased rate of metabolic risk, insulin resistance (IR) and diabetes (1), especially in the Caucasian populations. However, Asian populations are almost monomorphic for the G allele, which is also associated with obesity comorbidities and diabetes in this population. Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome with IR the driving factor in its development. The aim of this study was to determine prevalence of IL-6 – 174G/C polymorphism in patients with NAFLD and to investigate the differences in metabolic phenotype between genotype groups. This cross-sectional analysis included 28 participants with NAFLD. Anthropometric, body composition, biochemistry and homeostatic model of assessment of insulin resistance (HOMA-IR) were recorded. Patients underwent magnetic resonance spectroscopy to determine intrahepatic lipids (IHL). Polymorphisms were genotyped by Fitgenes Limited. Forty-three per cent of patients with NAFLD were CC homozygote or GC heterozygote and 57% GG homozygote carriers. The C+ genotype consisted of 75% Caucasian and 25% non-Caucasian, in contrast to 25% Caucasian and 75% non-Caucasian for GG. Prevalence of diabetes was higher in C+ allele carriers compared to G homozygotes (67% vs. 31%, respectively). Subjects with the C+ genotype compared to GG had significantly greater waist circumference (114.4±22.4 cm vs. 100.2±10.6 cm), hip circumference (118.4±21.4 cm vs. 102.4±7.7 cm), fat mass (44.7±8.4% vs. 35.9±5.4%) and high-sensitivity C-reactive protein (5.3±3.6 vs. 2.8±2.6 mg/L), ($p<0.05$). C+ carriers also displayed higher plasma insulin concentrations, HOMA-IR and IHL, albeit non-significantly. Conclusion: Although numbers are small, results highlight that IL-6 – 174C variant is associated with Caucasian ethnicity, prevalence of diabetes, and greater anthropometry and body composition measurements in NAFLD. We envisage that these differences will be more apparent as patient numbers increase.

Keywords: *non-alcoholic fatty liver disease, IL-6 – 174G/C, metabolic risk*

REPAIR OF GLUCOSE-6-PHOSPHATE DAMAGED DNA IN ESCHERICHIA COLI K-12

Elitsa Boteva[1], Milena Tileva[1], Elena Krachmarova[1], Monika Pischetsrieder[2], Roumyana Mironova[1]

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That DNA may react with reducing sugars in vitro has been shown long ago but it is a discovery of the last decade that reducing sugars may react with DNA in living organisms. In a chemical reaction, called the Maillard reaction (non-enzymatic glycation or glycation), the NH2 groups of primary amines react spontaneously (no catalysis) and reversibly with the carbonyl groups of glucose and other carbonyl compounds to form Schiff bases (aldimines). Then, at neutral pH, the Schiff bases undergo an intramolecular rearrangement to more stable Amadori products (APs) (ketoamines). In the late stage of the Maillard reaction, because of additional chemical transformations, APs give rise to the so-called advanced glycation end products (AGEs). In previous studies we have demonstrated that the chromosomal DNA of Escherichia coli K-12 accumulates both APs and AGEs under normal growth conditions and that these DNA adducts represent an important endogenous source of spontaneous mutations in E. coli. The repair of DNA modified with APs and AGEs is poorly studied. As far as APs are AGEs precursors, we supposed that E. coli may have mechanisms for repair of APs damaged DNA, because maturing of APs to AGEs results in DNA structural alterations and mutability. Indeed, we have observed that E. coli lyses are capable of catalyzing the removal of APs derived from the interaction of DNA with glucose 6-phospate. In this study, we report data from experiments designed to identify the carrier(s) of the observed DNA repair activity.

Keywords: *glycation, Amadori products, DNA repair*

Acknowledgements: *NSF Contract № DN 01/5/16.12.2016*

NMR METABOLOMICS AND PROTEOMICS ON CELL MONOLAYER STUDYING THE EFFECT OF SYMBIOTICS

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Introduction: **The gut microbiota has shown to be involved in changes of the body fat mass in humans. The aim of this study was to investigate if symbiotics may enhance the gut health benefit of pre- and probiotics. By growing a Caco-2 cell monolayer on permeable membranes in-vitro, the cell model can be used to mimic the small intestine epithelium. Stimulations were performed by adding selected fecal water samples from a large clinical trial on symbiotics to the apical side of the Caco-2 cell monolayer. To explore the mechanistic effects, NMR metabolomics was used to detect indirect effects of the Caco-2 cell monolayer and fecal water on the metabolic profiles of the cell media. Proteomics on the cells were carried out to determine if proteins were regulated by symbiotic treatment.**

Methods & Results: **Stimulations were performed by adding fecal samples diluted in PBS and sterilized by filtration through a 0.22-micron filter from 26 subjects. The samples belong to 5 different groups: Placebo, A_responder, A_non-responder, A+B_responder, A+B_non-responder, where A+B is the symbiotic treatment. Caco-2 cells were seeded onto filter inserts and incubated in 24-well plates for 18 days. Stimulations were performed for 24 hours with 4 replicates. A hundred µL sterile fecal water and 300 µL cell media was added to the apical side. Four replicates of control samples on each plate were obtained by the addition of 400 µL cell media to the apical side. Cell media from the apical and basolateral sides are collected at the end of the intervention. CaCo-2 cells were harvested, lysed and digested.**

^{1}H NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer equipped with a Prodigy CryoProbe using standard 1D NOESY experiments. The 1H NMR spectra were automatically aligned, phased and binned prior to being analyzed by multivariate statistics. Proteomics were performed by using a nanoflow-LC connected to an Orbitrap Fusion. Proteins were identified using SEQUES HT + Percolator and quantified label-free in GeneData Expressionist. Regulated proteins were analyzed by Ingenuity Pathway Analysis (IPA) software. Results in a total of 7057 proteins were identified using 1% false discovery rate (FDR). Statistical filtering of the preliminary quantitative results showed that 145 proteins were significantly regulated. Pathway analysis of regulated proteins in IPA, showed that several pathways were affected including inflammatory responses, immunological disease, and lipid metabolism. PCA analysis using the 145 proteins or the binned NMR spectra showed a weak tendency for subjects to separate between responders and non-responders in the group who received symbiotics.

Conclusion: **TEER measurements on the Caco-2 cell monolayer model indicate a stronger gut barrier integrity as an effect of the symbiotic group. Differences in the metabolic profiles was found on the apical side between symbiotic responder vs. non-responder groups. No clear metabolic effect of the caco-2 cell monolayer could be found. Proteomics suggests that the effect from the symbiotic treatment effects pathways involved in cell proliferation of gut epithelium cells and ion transport across the cell layer. Regulated pathways observed in this study needs to be further investigated for their relationship to a symbiotic diet.**

Keywords: *metabolomics, proteomics, symbiotics, Caco-2 cell monolayer*

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