Lifestyle Genomics

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Abstracts

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1

Unravelling the Effects of Mitochondrial DNA Methylation on Hepatic Energy Metabolism

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Introduction: Mitochondria play a key role in providing energy and promoting cell survival by mediating adaptive responses to cellular stress. Indeed, mitochondrial dysfunction results in many diseases, such as non-alcoholic steatohepatitis (NASH) and type 2 diabetes. Intriguingly, differential methylation of the mitochondrial DNA (mtDNA), observed for various clinical phenotypes, has been associated with altered gene expression and mitochondrial dysfunction. Recently, we have shown that GpC, and not CpG, methylation of mtDNA downregulates mitochondrial gene expression [1]. The low levels of mtDNA methylation (<10%), however, fuel the debate on its true existence and/or its physiological relevance. In this study, we artificially-induced mtDNA methylation to study the effects on mitochondrial activity and liver cell functioning.

Methods: HepG2 cells were lentivirally transduced to stably express recombinant mitochondrion-targeted prokaryotic cytosine DNA methyltransferases (M.CviPI = GpC; MSssI = CpG) or a catalytically-inactive variant thereof (M.CviPI†). MtDNA methylation was analyzed by pyrosequencing, gene expression by qRT-PCR and cell proliferation by real-time xCELLigence. Oxidative respiration (mitochondrial function) was analyzed by Seahorse technology.

Results: Levels of mtGpC methylation were enhanced up to 39% in HepG2-mtM.CviPI cells, compared to background levels in HepG2-mtM.CviPI† and parent HepG2 cells. Only HepG2-mtM.CviPI and not HepG2-mtMSssI cells showed reduced cell proliferation. HepG2-mtM.CviPI cells also showed a strongly impaired oxygen consumption and ATP production compared to HepG2-mtM.CviPI† cells.

Conclusions: Our data suggest that artificially-induced GpC mtDNA methylation impairs mitochondrial activity and cellular fitness, which may be relevant for mitochondrial functioning in NASH. This warrants a detailed analysis of the downstream effects of mtDNA methylation and its (patho)physiological relevance in metabolic diseases, including NASH.

This study was financed by the UMCG and networking activities were supported by EU COST EpiChemBio (CM1406) and MitoEagle.

Reference

1 Van der Wijst et al., Sci Rep 2017.

2

Quantitative Profiling of One-Carbon Metabolism Donors in the Blood – Can Dietary Methyl Donors Serve as Proxies of Cellular DNA Methylation?

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Objectives: Diet and lifestyle have been extensively shown to impact DNA methylation changes. DNA methylation, the most studied epigenetic mechanism, requires methyl groups to be readily available for their addition to genomic regions. The one-carbon metabolism generates methyl groups via dynamic molecular conversions within the cycle. For instance, the SAM/SAH ratio has been shown to be an important proxy of cellular DNA methylation. Associations between the diet and DNA methylation status have been mostly evaluated using either subjective dietary reporting, or assays looking at single dietary biomarkers within the blood. The purpose of the present study is to develop an analytical method to objectively quantify the entire one-carbon metabolism cycle, and a number of selected amino acids in the blood using ultra high pressure liquid chromatography coupled with tandem mass spectrometry.

Methods: Ultra high pressure liquid chromatography/ tandem mass spectrometry is conducted on a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific). First, cold-labelled and unlabelled standards of each compound are prepared by diluting each powdered standard into 0.1% HCl or into 10 mmol/L ammonium acetate with 10% ascorbic acid and 2% tris (2-carboxyethyl) phosphine solution (TCEP) (compound-dependent dilution factors). Second, the prepared standard is mixed with TCEP, a mixed internal standard solution, and a protein precipitation reagent containing MeOH and 0.1% formic acid. Third, the individual standard solutions are injected into the mass spectrometer to establish how each compound is being fragmented, and determine the optimal conditions for the most abundant product ion of the precursor molecule of interest. Fourth, once all standards have been characterised at MS/MS level, each one is run individually to assess chromatographic separations and retention times on a Kinetex® 2.6 µm F5 100 Å 150x2.1 mm column (Phenomenex). Fifth, a mixture of all standards is injected into the LC/MS-MS to evaluate and optimise separation and detection across the standard set.

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Finally, blood-based extractions and dilutions are optimised for these different molecules from test samples, as these have different starting plasma concentrations.

Results: A panel quantifying one-carbon metabolism compounds along with a selection of amino acids is being developed and will be implemented to holistically assess methyl donor status in blood, as a dynamic interface between the diet and cellular DNA methylation changes.

Acknowledgements: Stephanie Andraos is supported by the Liggins Institute research scholarship, and the New Zealand International Doctoral Research Scholarship.

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Evaluation of the Antioxidant Properties of Table Olive from "Piantone Di Mogliano" Cultivar

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Objectives: "Piantone di Mogliano" is an olive cultivar from Marche region, mainly located in Mogliano (Macerata). Olive drupe, in general, is characterized by the presence of important metabolites which can influence the quality and aroma of the fruit. The fruit quality is influenced also by the environment in which the olive tree is located since it may influence the expression of some enzymes involved in several metabolic processes and in the development of phenolic and aromatic compounds [1]. In addition, the table olives quality and taste are influenced by the processing methods used to debittered the raw olives that could give rise to a final product not completely satisfactory from a nutritional point of view. The aim of the present work was the production of table olives from "Piantone di Mogliano" through innovative techniques able to enhance both taste and nutritional properties of the olives. For this purpose, two techniques were used: the Sevillian style and the Natural style. On each final product, the total polyphenol content and the antioxidant activity was determined and the obtained values were compared to that of the Raw Olives (RO).

Methods: The olives were subjected to Sevillian style (based on NaOH and partial fermentation) and Natural style (based only on fermentation) processes, modified in order to obtain a final product with enhanced organoleptic characteristics. On the samples RO, Sevillian and Natural Style Olives (SSO and NSO respectively) the polyphenol total content was determined according to the Folin-Ciocalteu method, the total antioxidant activity by the method of Pellegrini and coworkers [2], and the chemiluminescence assay by Gabbianelli and coworkers [3].

Results: The Polyphenol content of RO, SSO and NSO samples, revealed that RO has the highest polyphenol content (6.45 mgGAE/g), NSO keeps its phenolic composition almost unaltered (5.69 mgGAE/g), whereas the SSO have the lowest content (2.93 mgGAE/g). Chemiluminescence assay was performed using two specific probes, lucigenin and luminol, that are sensitive to superoxide anion and hydrogen peroxide, respectively. In all cases, the results indicated that the natural style olive extracts have an antioxidant activity similar to that of fresh olive extracts. As regard to the total antioxidant activity evaluation, NSO has an antioxidant activity similar to RO and proved to possess a greater antioxidant activity of SSO.

Conclusions: The results of this preliminary study proved that the Natural style olives possess enhanced organoleptic characteristics and maintains its antioxidant power and total polyphenol content with respect to the table olives produced according to the Sevillian style.

Acknowledgements: The authors would like to thank Mr. Massimiliano Andreozzi owner of the "Antica Gastronomia" Company.

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4

Epigenetic Markers of Diet Response for Personalized Weight Loss Strategies

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Objectives: 1) Determine the effect of a healthy low-fat (HLF) diet vs. a healthy lowcarbohydrate (HLC) diet on DNA methylation (DNAm); 2) Assess whether baseline DNAm may predict individual weight loss response to a diet intervention.

Methods: DNAm was analyzed in peripheral blood lymphocytes (PBL) samples collected at baseline and 12 months of the DIETFITS randomized clinical trial with 609 obese non-diabetic subjects randomly assigned to a HLF or a HLC diet (Gardner CD *et al*. 2018, JAMA). Whole genome bisulfite sequencing (WGBS) was carried out in a discovery cohort consisting of the eight "biggest losers" defined as those who lost the most weight at six months, and who also sustained their weight-loss up to the 12-month visit.

Results: Weight loss on a HLF diet or a HLC diet is associated with significant, dietspecific DNAm changes at several genomic loci including obesity- and diabetes-related genes.

Conclusions: A HLF diet and a HLC diet are associated with distinct changes in DNAm across the genome. Follow-up analyses will assess whether baseline methylation of some of these genomic loci may be used as a biomarker to predict weight loss response for personalized weight-loss strategies.

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Glycoxidative Stress and Paraoxonase-2 in Intestinal Cells: Effect of Apple Polyphenols

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Objectives: Oxidative stress is one of the primary processes underlying the initiation and progression of inflammation and tissue injury in inflammatory bowel disease [1]. Under physiological conditions, the balance between reactive oxygen species (ROS) generation and ROS scavenging is tightly controlled in intestinal cells. Elevated plasma glucose levels and advanced glycation end products (AGEs), formed during hyperglycemia, generate free radicals and can cause inflammation and cell damage. Apple fruit has been reported to have high antioxidant effectiveness that is potentially linked to its richness in polyphenolic molecules. The aim of the study was to investigate the role of apple extracts (*Calvilla apple*) on glycoxidation in intestinal Caco-2 cells. In particular, we studied the expression and activity of the antioxidant enzyme paraoxonase-2 (PON2) in cells exposed to high-glucose (HG) stress in the absence and in the presence of apple extract. PON2, a member of the multigene family of paraoxonases, is expressed in all human tissues. The enzyme localizes in mitochondria and cell membrane and exerts a protective role against ROS within cells [2] and therefore, PON2 may be involved in the antioxidative and anti-inflammatory response in intestinal cells [3].

Results: Our results showed a lower cell viability and a higher intracellular ROS and AGEs formation in HG-treated cells. The cell glycoxidation was associated with a significant decrease in PON2 protein levels and activity. Treatment with apple extract reduces the glycoxidative stress and induces a significant increase of PON2 levels and activity.

Conclusions: The intestine is highly vulnerable to glycoxidative damage due to its constant exposure to aerobic metabolism, high glucose concentration or oxidants and AGE from ingested nutrients. A diet rich in apple antioxidants might prevent or delay cell oxidative stress and inflammation, by increasing PON2 activity and reinforce cell antioxidant defence.

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The Influence of Phenolic Acids on Antioxidant Defence System of Cells

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Objectives: The enhancement of endogenous antioxidant defence system through dietary supplementation with antioxidants appears to be a reasonable approach to reduce oxidative stress. Unfortunately, despite the large number of publications about antioxidants, there is still no consensus which parameters should be used as the guidance in rational design of e.g. functional foods based on antioxidants. The aim of the study was to clarify the relationship between chemical and electrochemical properties of 9 phenolic acids, their biological behaviour as well as molecular implications for oxidative stress response and antioxidant defence system in colon adenocarcinoma HT29 cell line.

Methods: Reduction potentials of phenolic compounds were determined by potentiometric titration. The biological tests embraced determinations of cytotoxicity, antioxidant activity in cell culture assessed by CAA assay, as well as protection of cells against genotoxic insult using comet assay. Genomic studies concerning stimulation of gene expression involved in oxidative stress response employed RT-PCR array-based technologies.

Results: The comparative approach to establish the correlation between the chemical properties and biological potential of phenolic acid suggests the role of these phytochemicals in oxidative stress state. Studies document relationships between electrochemical properties of antioxidants and their biological effects.

Acknowledgement: This work is supported by the project "Antioxidant Power Series as a tool rational design and assessment of health promoting properties of functional food based on antioxidant phytochemicals" (number of the application 2014/14/A/ST4/00640) financed by National Science Centre, Poland in a programme "MAESTRO 6".

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Can *Nigella sativa* Oil Control Inflammation in Human Pre-Adipocytes?

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Objectives: The fixed oil obtained from the seeds of *Nigella sativa* L. (NS), also known as black cumin, is frequently used in the Mediterranean area for its therapeutic properties. Pertaining literature suggests that its anti-inflammatory, anti-oxidant and anti-cancer activities is due to thymoquinone (TQ), a main component of this oil, the bioactivity of which has been investigated mainly in cancer models. Besides TQ, NS oil is rich of components (including vitamins, amino acids, fatty acids, sterols, micro elements, etc.) that can contribute to its biological activity.

We therefore aimed at evaluating TQ concentration in a *Nigella sativa* oil extracted from seeds of cultivar produced in the Marche region of Italy, and at determining if its content, antioxidant properties and biological activity decay during storage. Cytotoxicity and anti-inflammatory properties of NS oil were tested in an *in vitro* model of low-grade inflammation of Simpson-Golabi-Behmel syndrome (SGBS) human pre-adipocytes to assess if this oil, or equal concentration of synthetic thymoquinone, could affect the production of pro-inflammatory cytokines.

Methods: Fresh extracted oil (FEO) and old extracted oil (OEO) were evaluated for their content in TQ by gas chromatography coupled to flame ionization detection (GC-FID). Total Antioxidant Activity (TAA), Oxygen Radical Absorbance Capacity (ORAC), Luminol-amplified- and lucigenin-amplified-chemiluminescence of FEO, OEO, TQ and medium from SGBS incubation (MI) were analyzed for their scavenger capacity by spectrophotometric and chemiluminescent assays. SGBS and monocytic leukemia (THP1) cell lines where cultured as previously described by Wabitsch and collaborators (Keuper et al., 2011). Cytotoxicity of NS extracts (FEO, OEO) and solutions equivalent in terms synthetic tymoquinone concentration (syntFEO) were tested by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for 24 h. Inflammation was induced in SGBS cells by conditioning their culture medium with 15% of supernatant obtained after THP1 differentiation with phorbol 12-myristate 13-acetate (PMA) for 48 h (Keuper et al., 2011). SGBS inflamed cells were treated with FEO, OEO, syntFEO, solved in DMSO for 24 h. Cytokine production was assessed using Multi-Analyte ELISArray Kits (Quiagen).

Results: the content of TQ in the NS oil from the Marche region cultivar was higher compared with other NS oils produced in the middle East and in other Mediterranean regions. The FEO contains 33% more TQ than OEO, showing that storage affects its overall quality. FEO and OEO showed similar cytotoxicity, but in both cases lower than that of pure TQ. Pro-inflammatory cytokines (e.g. IL-1alfa, IL-1beta, IL-6, IL-8 and GM-CSF) were differently modulated by NS oils.

Conclusions: NS oil produced in the Italian Marche region has a good content in TQ and a lower cytotoxicity than pure TQ. Its capacity to counterbalance proinflammatory cytokine production can be ascribed to the antioxidant properties of the other oil components.

Acknowledgements: This project was supported by *Società Agricola Vaccarini S.S.*

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Effect of Weight Loss on Mitochondrial Defects in the Ageing Human Colon

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Objectives: The objectives are to investigate the effect of surgically induced weight loss on mitochondrial DNA mutations and function in the colorectal mucosa of obese and normal weight participants.

Methods: Obese patients listed for bariatric surgery and age-matched healthy non-obese individuals (Controls) were recruited at North Tyneside General Hospital. Rectal mucosal biopsies were collected at baseline and six months post-surgery from obese participants and at baseline only from Controls. Mitochondrial oxidative phosphorylation proteins complex 1 and 4 and mitochondrial mass were quantified by immunofluorescence after staining with validated antibodies.

Results: Data were available for 16 Controls and for 31 obese participants pre- and post-bariatric surgery, all aged 21–65 years. Mean BMI of the pre-surgery obese group was significantly higher than for Controls (means 41.9 and 25.9, respectively) ($p = 0.001$) and bariatric surgery resulted in mean 26 kg weight loss. The prevalence of complex 4 ($p = 0.039$) and mitochondrial mass ($p = 0.010$) deficiency was significantly greater in older (>48 y) compared with younger (≤48 y) individuals. Obese individuals had significantly more complex 1 depleted crypts (9.2%) compared with Controls (0%) ($p = 0.046$). In addition, the obese had significantly fewer crypts with normal complex 4 (96.9%) and mitochondrial mass (93.8%), and more crypts with complex 4 (3.1%) and mitochondrial mass (6.2%) deficiency compared with Controls (100%, 99.9%, 0% and 0.04% respectively) ($p = 0.03$). However, weight loss did not change significantly the concentrations of oxidative phosphorylation proteins.

Conclusion: Advancing age and greater adiposity lead to significantly more complex 1 and 4 deficiencies in colonocytes but, at

least after 6 months, weight loss by bariatric surgery had no significant effect.

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Dietary Intervention Modifies DNA Methylation Age Assessed by the Epigenetic Clock

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Objectives: Alterations in DNA methylation patterns are correlated with ageing, environmental exposures and disease pathophysiology; the possibility of reverting or preventing these processes through dietary intervention is gaining momentum. In particular, methyl donors that provide *S*-adenosyl-methionine for one-carbon metabolism and polyphenols such as flavanols that inhibit the activity of DNA methyltransferases (DNMTs) could be key modifiers of epigenetic patterns. To elucidate the effect of supplementation with methyl donors (folic acid+vitamin B_{12}) and DNMT inhibitors (flavanols) on global DNA methylation profiles, including epigenetic models of ageing, in humans.

Methods: We assessed DNA methylation patterns in publicly available Illumina Infinium 450K methylation datasets from intervention studies with either folic acid+vitamin B_{12} (GSE74548) or monomeric and oligomeric flavanols (MOF) (GSE54690) in 44 and 13 participants respectively.

Results: Global DNA methylation levels increased in unmethylated regions such as CpG islands and shores following folic acid+vitamin B_{12} supplementation and decreased in highly methylated regions, including shelves and open-seas following intervention with MOF. However, demethylation was observed at CpG islands and shores following MOF, similar to the effects of the chemotherapeutic DNMT inhibitor decitabine with reduced magnitude. After supplementation with folic acid+vitamin $B_{12,}$ epigenetic age, estimated by the Horvath 'epigenetic clock' model, was reduced in women with the *MTHFR* T677T genotype.

Conclusions: The effects of supplementation with folic acid+vitamin B_{12} and MOF on DNA methylation age are dependent upon gender and *MTHFR* genotype. Additionally, our findings demonstrate the potential for these dietary factors to modulate global DNA methylation profiles. Further work is required to establish the potential of these dietary factors for targeted epigenetic alterations and reduction of disease risk.

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Effect of a Prebiotic Cranberry Extract on Weight Loss and Glucose Tolerance in Obese Mice Exposed to Persistent Organic Pollutants

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Objective: Persistent organic pollutants (POPs) accumulate over years, mostly in adipose tissue, and can have negative effects on metabolic health. They also cause epigenetic changes that can be transmitted through several generations. However, few treatments are known to counteract these effects. In this study, we evaluated the impact of a prebiotic on the effects of POPs release in the circulation during weight loss, and on glucose tolerance, inflammation and gut microbiota.

Methods: The animals were exposed to a high-fat high sucrose diet containing POPs for twelve weeks, after which they were fed a low-fat low sucrose diet for four weeks to induce weight loss. During the last six weeks of the protocol, they received a polyphenol-rich cranberry extract (200 mg/kg) or vehicle (water) treatment. Oral glucose tolerance tests were performed at week 9 and 16, while feces were collected throughout the protocol for metagenomic analyses. At the end of the protocol, tissues were collected, weighted and conserved for subsequent analyses (i.e. epigenetic modifications, measurement of inflammatory markers, POPs quantification in adipose tissue).

Results: Our initial results show a significant greater weight loss in the obese mice treated with the cranberry extract compared to the vehicle. Furthermore, the cranberry extract significantly lowered fasting glycemia and tended to improve glucose tolerance.

Conclusions: The administration of a cranberry extract accentuated weight loss and improved glucose tolerance in mice switched from a high-fat high sucrose to a low-fat low sucrose diet. Future analyses are underway to determine whether this extract facilitates the elimination of circulating POPs in association with changes in gut microbiota composition.

Acknowledgements: We thank V. Dumais, C. Dion, C. Dallaire and J. Morissette for their expert help with animal experimentation.

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Elucidating the Multifactorial Causation of Metabolic Disease: The Interplay between Environmental Epigenetics and Obesity-Associated Genetic *Pon1* Variants

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Objectives: Obesity is a highly heritable complex disorder that imposes an enormous burden on human health. Although studies have shown that 40–70% of the interindividual variability in BMI is attributed to genetic factors, only 2.7% has currently been explained. This leaves a substantial component still missing for which other forms of variation need to be considered. An emerging hypothesis proposes that genetic factors within susceptibility genes interact with environmental factors through epigenetic regulation. A potential candidate for this is the paraoxonase 1 (PON1) gene. Research has identified a protective role of PON1 against adverse environmental exposure, obesity, and its comorbidities non-alcoholic fatty liver disease and atherosclerosis.

Methods: Associations between the clinical metabolic phenotype and *PON1* genetic variants, epigenetic DNA methylation variation and enzyme activity were examined in an obese patient cohort (N = 700). Genetic variation was determined by SNP genotyping for *PON1* polymorphisms –108C/T (rs705379), L55M (rs854560), and Q192R (rs662). A pyrosequencing assay was designed to define DNA methylation values at the promoter region in relation to the found genetic variants. Differences in PON1 activity were measured by spectrophotometry. In addition, we considered the potential interaction of PON1 with polychlorinated biphenyl exposure as well as with diet, bariatric surgery and drug induced weight loss.

Results: Preliminary data associated the promoter polymorphism rs705379 with alkaline phosphatase, liver cell injury and non-alcoholic fatty liver disease (respectively $p = 0.011$; $p = 0.019$; $p = 0.044$). The potential link with non-alcoholic fatty liver disease was also perceived for rs854560 ($p = 0.005$) while a correlation was observed between adiponectin levels and rs662 ($p = 0.033$). Further multivariate analysis will be performed to integrate the effect of rare variants, DNA methylation status, enzyme activity and exogenous factors.

Conclusions: Genetic analysis proposes a role for *PON1* in obesity and its comorbidities. Incorporation of supplementary information will enable us to explore the onset and development of metabolic alterations in obesity.

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The Impact on the Human Gut Microbiome's Functional Profile Caused by the Antidiabetic Drug Metformin

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Objectives: Metformin is an antidiabetic drug used to reduce blood glucose levels without causing hypoglycemia, however, ~25% of patients under its treatment develop gastrointestinal side effects. Studies have been carried out about metformin treatment and its effects on type 2 diabetes patients, though there is no full clarity about metformin and gut microbiome interaction.

The aim of this work was to find out how metformin affects gut microbiome taxonomic and functional profiles of healthy individuals.

Methods: Study was conducted in a time-series design. Fecal samples were collected at three time points – before first dose of metformin (M0), 24 hours after metformin introduction (M24h) and seven days after starting metformin therapy (M7d). All samples were collected in two aliquots. Microbial DNA was extracted from fecal samples with *FastDNA® Spin Kit for Soil* (MP BIOMEDICALS). Analysis for metagenomic libraries were performed employing massive parallel sequencing on *Ion Proton™* sequencer. Clinical trial registration number: 2016-001092-74 (www.clinicaltrialsregister.eu).

Results: Results display several trends across all analyzed variables – taxonomic groups, pathways and gene families – beginning already at 24 hours after first metformin dose, and these trends are also observed after seven days of metformin use. Metformin treatment significantly modified *Barnesiella* genus, but tendencies of change in abundance can be seen at *Bifidobacterium* and *Escherichia* and other genera as well. Further analysis revealed no significant changes in pathway abundance but observed several tendencies in abundance of gene families.

Conclusions: The results partly complement already existing knowledge about metformin and its interaction with gut microbiome. It can be added that the observed changes in abundance of *Barnesiella* genus, represent one of possible mechanisms how metformin ensures its antidiabetic properties.

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Centaurium erythraea Methanol Extract Attenuates SNP-Induced Oxidative Stress in Pancreatic B-Cells

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Objectives: *Centaurium erythraea* (CE) is traditionally used in Serbia for diabetes management. Since oxidative stress represents one of the major pathogenic factors that lead to diabetes and its complications, this study investigated protective effect of CE methanol extract against oxidative stress-induced pancreatic β-cell death.

Methods: Rin-5F, rat insulinoma pancreatic β-cells, were incubated for 24 h with 1.25 mM sodium nitroprusside (SNP) with/without CE extract (0.2 mg/mL) and processed immediately. Rin-5F cell viability was estimated using the MTT viability assay. Lipid peroxidation was assessed using the thiobarbituric acid-reactive substance (TBARS) assay, while the DNA damage was estimated by alkaline comet assay. Catalase (CAT) activity was determined by the rate of H_2O_2 decomposition, whereas superoxide dismutase (SOD) activity was estimated by the epinephrine method. The activities of glutathione peroxidase (GPx) and glutathione reductase (GR) were determined by monitoring NADPH oxidation. Relative gene expression of CAT, MnSOD, CuZnSOD, GPx, GR and insulin was determined by RT-qPCR. Relative protein level of antioxidant enzymes was estimated using immunoblot analysis.

Results: CE methanol extract enhanced β-cell viability and insulin gene expression by reducing oxidative stress in SNP-treated cells. CE extract lowered DNA damage and lipid peroxidation provoked by SNP treatment and adjusted antioxidant enzyme activities. CE treatment increased SNP-mediated attenuation of CAT, GPx and GR activities and reduced CuZnSOD and MnSOD activities that were stimulated in SNP treated cells. SNP-induced increase in gene expression of CAT, GPx, GR, MnSOD and CuZnSOD was accompanied by decrease of CAT, GPx and CuZnSOD mRNA level after CE treatment. In addition, SNP treatment increased protein levels of CAT, GR, GPx and MnSOD and decreased CuZnSOD protein level. CE extract reduced CAT and MnSOD and partially restored CuZnSOD protein level.

Conclusions: The CE methanol extract protects pancreatic β-cells from oxidative damage by improving antioxidant defence system. Detected attenuation of oxidative stress in β-cells *in vitro* provide a useful platform for *in vivo* investigation of antioxidant and antidiabetic effect of CE extract and its potential usage as an effective supplement for diabetes treatment.

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Milk Alpha-S2 Casein Protein Provided Different Physiological Function on Signal Transduction Gene Cascade and Tissue Microstructure

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Objectives: Goat milk is rich in whey and casein proteins contain mainly in αS1-, αS2-, β- and κ-casein, of which those types of casein have different properties and bioactive functions that exhibit important physiological and biochemical functions which have crucial impacts on human metabolism and health. Recently our studies have been reported on functional properties of bioactive αS2-casein protein in caprine milk of the Ethawah breed as local goat at Java, Indonesia. This study focuses on elucidating the biological function of caprine milk alpha-S2 casein protein provides by using the rat rheumatoid arthritis model, and in silico analyses.

Methods: Twenty-four rats were randomly divided into six groups: control (untreated) group (C), CM group was treated with CSN1S2 protein of goat milk, CY group was treated with CSN1S2 protein of goat yoghurt, RA group, RA group treated with CSN1S2 protein of goat milk (RAM), and RA group treated with goat yoghurt (RAY). Mineral elements and mesostructure of bone and ileum were analyzed using X-Ray Fluorescence and Scanning Electron Microscopy. To identify the cytokine related with anti-inflammation was analyzed using western blotting and in silico analysis.

Results: The caprine CSN1S2 protein in RA disease is also provided for repairing the crystallinity, collagen structure, and trabecular mesostructure as anti-osteoporosis through RAGE-AGE signaling. CSN1S2 protein of caprine milk has reduced the erythema, swelling, and inflammation in lower extremities and decreased the TNF-α and RAGE expression of ankle joint synovial membranes. While this protein also could repair the ileum microstructure and suppress inflammatory processes through an IL-10 elevation in RA disease which is may act as an anti-inflammatory agent via JAK-STAT3 signal transduction cascade. This milk casein can also act as chelating agent that is capable of binding to the metal ions and may induce the replacement of sulfur with selenium in methionine residue in the ileum.

Conclusions: These results indicate the caprine CSN1S2 has a broadly properties function as anti-osteoporosis, anti-inflammation, and immunomodulatory.

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Nucleic Acids as Food Components – May They Have an Impact on Human Epigenome?

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Objectives: Nucleic acids are present in food in a form of genomic DNA and functional RNA structures. All of them are present in raw food products of animal and plant origin. Nucleic acids play an important role as a source of nucleotide-related molecules and participate in many biosynthesis pathways. The major role of dietary nucleic acids is to provide building blocks for the synthesis of cellular DNA and RNA by the salvage pathway, as well as to facilitate the *de novo* nucleic acid synthesis. Recent studies suggest that dietary nucleic acids can survive the digestion process in alimentary tract, pass through the intestinal barrier and circulate in the blood system.

The small regulatory nucleic acids – RNAs – are presumed to regulate gene expression in the consumer's body, to influence epigenome and, in consequence, to affect metabolism. In the contexts of epigenetics the role of ingested DNA fragments seems to be an important subject. It is the part of the sentence "We are what we eat".

In our study, the presence of nucleic acids in high and low processed food products after thermal treatment was investigated.

Methods: The raw and processed meat was thermally treated and the presence of nucleic acids was investigated. The addition of RNase enzymes during isolation procedure was evaluated. The isolates were obtained with conventional phenol/chloroform isolation procedure and analyzed with capillary electrophoresis.

Results: The presence of high molecular mass nucleic acids was observed in thermally and non-thermally treated meat samples, as well as in high processed meat products. The addition of RNase enzymes during the isolation procedure did not significantly influenced nucleic acid fragmentation profile.

Conclusions: The ~20 k bp nucleic acid fragments are present in processed meat after culinary treatment. In the context of nucleic acid migration from the intestine to the blood stream, their presence is an important point for further investigations on their possible impact on human epigenome.

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Catechins as Potential Epigenetic Modulators

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Objectives: Year by year, a growing public interest in relationship between diet and ageing can be observed. In particular, this refers to antioxidants, a group of compounds present in foods and beverages that are expected to have beneficial health effects. This group includes catechins (favan-3-ols), which are known to play a role in modulation of cellular redox homeostasis and various signalling pathways.

Our previous results have indicated that in HT29 cell line, changes in expression of some genes associated with oxidative stress response are dependent on concentration of catechins. In the subsequent experiments, we aim at examining the methylation profiles in the promoter areas of up-regulated and down-regulated genes. The objective of the research is to find out whether the observed dose dependence is caused by epigenetic modulation of gene expression.

Methods: The investigation of the impact of different catechins on the expression of genes involved in redox homeostasis was conducted using Oxidative Stress and Antioxidant Defense PCR Array from Qiagen, while DNA methylation is examined using Methylation-Specific PCR (MS-PCR) and Methylation-Sensitive High Resolution Melting (MS-HRM) methods.

Results: A significant up-regulation of 3 genes (ALB, CCL5, HSPA1A) out of 84 included into antioxidant response gene array was observed after treatment of HT29 cell line with investigated catechins at 1 μM concentration. No such up-regulation was seen at 10 μM catechins. Moreover, at this higher dose, (-)-epigallocatechin caused down-regulation of SRXN1 gene within the examined gene panel.

Conclusions: Our methylation analysis included two antioxidant response genes, SRXN1 and HSPA1A, whose expression is affected by catechins. HSPA1A gene suppression due to its promoter methylation is already documented. Our analysis of the SRXN1 gene regulatory region revealed presence of 706 bp long CpG island that encompasses promotor region and transcription start site, hence conducive to epigenetic silencing via DNA methylation. The impact of DNA methylation on gene expression in control and catechin-treated HT29 cells is in progress.

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Integrated Analysis of Expression Data Using Directed Signalling Network Identifies Regulatory Pathways in Human Obesity

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Objectives: Obesity is an important health problem increasing the risk of many health issues like diabetes, hypertension and coronary heart disease. Obesity also is a complex, multi-factorial disease that develops from the interaction between genotype and the environment. Genetic influences are difficult to elucidate and identification of the genes is not easily achieved in familial or pedigree studies. In other words, obesity may be caused by perturbation of regulatory pathways rather than the dysfunction of a single gene. That is why, the contribution of genetic and epigenetic factors should not be ignored.

MicroRNAs, small endogenous non-coding RNAs, play a critical role in many cellular processes and have been linked to the control of signal transduction pathways. Omics studies demonstrated that changes in miRNA profiles of various tissues (e.g., pancreas, adipose tissue, and liver) correlate with obesity and several metabolic diseases.

The aim of our study was to identify the potential active TF-miRNA-gene regulatory pathways involved in obesity via integrating miRNA and gene expression profiles and SIGNOR directed signalling network.

Methods: We downloaded the miRNA and gene expression profiles from gene expression omnibus (GEO) database. We derived the differentially expressed genes (DEGs) and differentially expressed miRNAs (DEmiRs) in adipose tissues from obese and non-obese subjects. SIGNOR database of causal relationships between signalling entitites is used as a signed directed network and TF-miRNA-gene bidirectional regulatory network is constructed. Then, DEGs and DEmiRs are mapped to the curated TF-miRNA-gene regulatory network as active nodes. In addition, obesity related known genes and miRNAs are marked on the regulatory network. We connected the active nodes with their first degree neighbors and obtained the potential regulatory TF-miRNA-gene subnetwork. By using BFS algorithm, we identified the potential active TF-miRNA-gene regulatory pathways. We used hypergeometric test to detect TF-miRNA-gene regulatory pathways related to obesity.

Results: In this study, we systematically analyzed obesity-related mRNA and miRNA expression profiles as well as curated transcription factor (TF) and miRNA regulation to identify active TF and miRNA regulatory pathways in obesity.

We identified DEmiRs, DEGs and active TF-miRNA-gene regulatory pathways and our final results will be discussed.

Conclusions: This study provides bioinformatic support for further research on the molecular mechanism of obesity.

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Intake of SFA Compared to PUFA Induce Lower Postprandial LDI Receptor Gene Expression in PBMC in Subjects with and without FH

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Objectives: The long-term cholesterol lowering effect of replacing intake of saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA) is well established, however not fully explained mechanistically. We examined the postprandial response of meals with different fat quality on expression of lipid genes in peripheral blood mononuclear cells (PBMC) in subjects with and without familial hypercholesterolemia (FH).

Methods: Thirteen subjects with FH (without current lipid-lowering treatment) and 14 normolipidemic controls were included in a randomized controlled double-blind crossover study with two meals, each with 60 grams (~70 E%) of fat, either mainly SFA (~40 E%) or PUFA (~40 E%). Expression of 34 lipid genes in PBMCs were analysed by qPCR from fasting and 6 h postprandial blood samples. A linear mixed model for repeated measures was used.

Results: 1. Intake of SFA compared to PUFA induced lower gene expression of LDL receptor ($p = 0.01$) and higher of ABCG1 ($p = 0.002$), with no interaction with group.

2. FH compared to control subjects had significantly lower gene expression of FDPS, SREBP2, ACC, CPT1A/2, MSR1, NR1H3 and higher of MYLIP ($0.007 \leq p \leq 0.05$), with no interaction with meal (except for NR1H3) or time point (except for SREBP2, ACC and CPT1A).

3. In controls only, the postprandial gene expression was significantly reduced for DHCR24, LSS, SREBP1/2, FADS1/2, HMGCS1 and ACC ($0.001 \leq p \leq 0.03$), with no interaction with meal.

4. In FH subjects only, the postprandial gene expression was increased for SORL1 ($p = 0.03$), with no interaction with meal.

Conclusions: Intake of SFA compared to PUFA induced lower postprandial gene expression of LDL receptor and higher of ABCG1 in PBMCs. This may potentially result in increased plasma cholesterol level.

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Methylation of *SSRP1* Gene is Correlated with Aluminum and Arsenic Serum Concentration in Obese Women

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Objectives: Obesity has been linked to lifestyle and recently have been associated to environmental toxicants, it is widely accepted that these xenobiotics have a negative impact on human health and may contribute to the development of diseases and its comorbidities. Metals exposure is a very concerning event, and in this context, arsenic (As) is a metal that is commonly used in industry in combination with sulfur and other metals. The contamination occurs mainly by the respiratory (particulate matter) and oral (grains, cereals, seafood, drinking water) routes. Aluminum (Al) is also a metal that may reach people through diet. This type of contamination usually occurs due to food packaging and utensils composition, which may contaminate food during preparation and storage processes. The mechanisms by which these compounds cause disease have not been fully elucidated. DNA methylation changes have been associated with many diseases including obesity and its comorbidities, maybe alterations in the adipogenesis and lipid storage processes may contribute to the pathological state. Therefore this study aimed to 1. evaluate arsenic and aluminum serum concentration and DNA methylation profile in obese and normal weight women, and 2. Evaluate possible associations between metal concentrations and DNA methylation patterns.

Materials and Methods: This transversal study enrolled Obese (Ob) and Eutrophic (Eut) women. Anthropometric data such as weight (kg) and height (m) were collected. As well as peripheral blood samples after 12 hours fasting for biochemical and genetic analysis. DNA extraction and bisulfite conversion were performed in order to hybridize the samples on the 450 k Infinium Methylation Beadchip. Serum concentration of metals was determined by inductively coupled plasma mass spectrometry (ICP-MS). Statistical analysis included the Shapiro-Wilk, independent t test, Pearson's correlation and Benjamini-Hochberg analysis ($p < 0.05$).

Results: A total of 32 subjects participated in the study: 16 obese (IMC = 45.1 ± 5.4 kg/m^2; Age = 39.3 ± 11.5 years old) and 16 normal weight (IMC = 22.5 ± 1.7 kg/m^2; Age = 39.1 ± 13.4 years old) women. Serum concentration of Al and As were different between groups (Ob-As:13.48 ± 1.10 µg/L, Eut-As:12.65 ± 0.46 ug/L, $p = 0.01$; Ob-Al:83.69 ± 65.60 ug/L, Eut-Al; 43.30 ± 20.05 µg/L, $p = 0.02$). We found that obese group showed a lower methylation level of adipogenesis-related gene *SSRP1* than eutrophic women ($\Delta\beta = 0.31$, FDR < 0.05, $p = 0.01$). Also, we observed significant correlation between gene methylation levels and metals serum concentration (As-*SSRP1*: r = –0.62, $p = 0.01$; Al-*SSRP1*: r = –0.51, $p = 0.01$).

Conclusion: Serum concentration of toxic metals are different between obese and normal weight women and correlates with *SSRP1* methylation level.

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Oral L-Carnitine Supplementation Increases Trimethylamine-N-Oxide But Not Markers of Atherosclerosis in Healthy Aged Women

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Objectives: Human microbiota are responsible for converting L-carnitine and other dietary quaternary amines (e.g., choline, glycine betaine, and phosphatidylcholine) to trimethylamine (TMA), which are subsequently oxidized by host hepatic flavin monooxygenases to trimethylamine N-oxide (TMAO), a molecule that promotes atherogenesis through its interaction with macrophages and lipid metabolism.

The objective of the present study was to assess whether L-carnitine supplementation may promote TMAO-induced atherosclerotic risk.

Methods: The study participants were originally recruited to another study aimed at evaluating the impact of carnitine supplementation on skeletal muscle function. The research protocol was approved by the Local Ethics Committee. Before the start and after completing the 24-weeks supplementation protocol, fasting blood samples were taken from the antecubital vein. Plasma free L-carnitine, γ-butyrobetaine (GBB) and TMAO were determined by the UPLC/MS/MS method as described previously [1]. Serum proteins were determined by the enzyme immunoassay method using commercially available kits. Total cholesterol, HDL-cholesterol, LDL-cholesterol, triglicerides, urea, creatinine and uric acid have been determined using standard automatic analyzer Cobas6000 (Roche Diagnostics, Mannheim, Germany).

Results: L-carnitine supplementation elevated fasting plasma carnitine in the mid-point of our study and it remained increased until the end of supplementation period. Moreover, it induced tenfold increase in plasma TMAO concentration, but did not affect lipid profile, white blood cells and platelets counts or serum C-reactive protein, interleukin-6, tumor necrosis factor-α, L-selectin, P-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1 or lipid profiles markers.

Conclusion: We demonstrated that although oral L-carnitine supplementation significantly increased plasma TMAO concentration, no lipid profile changes or other markers of adverse cardiovascular events were detected in healthy aged subjects.

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Epigenetic Regulation of Hibernation: An Endogenous Switch for Safe Metabolic Suppression?

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Objectives: In this study, we investigated the expression of candidate genes and whole genome epigenetic modifications in hibernation. We hypothesize that epigenetic modifications such as histone acetylation and DNA (hydroxy)methylation are underlying metabolic regulation during torpor (cold phase) and arousal (rewarming phase).

Methods: Syrian hamsters were housed at a photoperiod of 14 h light and 10 h darkness at an ambient temperature of 21°C. To induce hibernation, hamsters were kept in constant darkness at an ambient temperature of 5°C. Animals were sacrificed in different phases of hibernation. Livers were collected from all animals and snap frozen. Expression of candidate genes (e.g. RBM3, NRF2, PGC1α, and ACADVL) was analyzed using qRT-PCR. Histone acetylation was measured using antibodies specific to H3ac, H3K9ac, H3K27ac, and H3K27me3 using immunoblot. DNA (hydroxy)methylation was measured using antibodies specific to 5(h)mc using dot-blot.

Results: Cold-shock protein RBM3 expression has a trend of increase in arousal compared to control animals. Regulator of mitochondrial biogenesis, PGC1α, also showed a trend towards increased expression in all hibernation phases compared to control animals. Although candidate genes did not show significant differences, global epigenetic modifications changed during hibernation phases. Pan-acetylation of histone 3 (H3ac) is significantly increased in arousal vs torpor and were correlated to rectal temperature. More specifically, acetylation of H3K27 has a trend of increase from torpor to arousal. H3K9 is not significantly differently acetylated in liver. In addition to differences in histone acetylation, DNA methylation was also increased significantly between control animals and arousal.

Conclusion: Epigenetic modifications change during different phases of hibernation, potentially leading to gene expression changes. Histone 3 acetylation is altered in hibernation, possibly due to differential production of acetyl-donor Acetyl-CoA by mitochondria. In addition, DNA methylation is altered, suggesting that gene expression is regulated by (promoter)methylation during hibernation. Epigenetic modifications in hibernation could function as drug targets for safe metabolic suppression using e.g. Epigenetic Editing.

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Dietary Oxidized Phospholipids: Digestion, Absorption and the Potential Dietary Risk Factor

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Objectives: The results of numerous epidemiological studies indicate that the type, quality and intake of food-delivered lipids contribute to the prevention or promotion of diet related and metabolic diseases such as: type 2 diabetes, obesity or atherosclerosis. Among the food-delivered lipid compounds, phospholipids (PLs) attract increasing attention due to their high nutritional value and functional properties. PLs, especially those containing essential unsaturated fatty acids (FAs) exhibit a number of key biological activities. At the same time, polyunsaturated FAs incorporated in the structure of natural occurring PLs are particularly susceptible to oxidation. Oxidized phospholipids (OxPLs) delivered with food and products of their digestion can be potentially toxic molecules for to the epithelial cells of digestive tract. Accumulation of OxPLs can lead to gut pathologies as a result of cell membrane modifications as well as DNA and protein damage. In addition, previous research suggested that lipid hydroperoxides, which were not hydrolyzed during intestinal digestion, may be released to the blood stream after absorption by enterocytes and thereby contribute to the pathogenesis of atherosclerosis. Additionally, OxPLs may lead to pathological conditions in cells by aberrant regulation of numerous genes implicated in cell proliferation, differentiation, cellular stress, inflammation and lipid metabolism. Among transcription factors activated by OxPLs are such important gene expression regulators as PPARs, Nrf-2 or ATF4.

Methods: Long-chain oxidized PLs generated by LOX-1 catalyzed oxidation were characterized using *offline* two-dimensional liquid chromatography coupled with DAD, CAD and MS detection. Kinetics of non-oxidized PLs (n-OxPLs) and OxPLs digestion by porcine pancreatic phospholipase A_2 were studied using pH static method (pH = 8). Effect of n-OxPLs and OxPLs on human colon adenocarcinoma cell line (HT-29) growth was determined using MTT test.

Results and conclusion: Our research suggests that OxPLs are digested slower by *porcine pancreatic phospholipase* A_2 in comparison with their native counterparts. This may result from a decreased phospholipase A_2 activity towards its substrates when chemically modified, e.g. by oxidation as is the case with OxPLs. In addition, OxPLs exhibited higher toxicity against model cells of the gastrointestinal tract.

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Ischemic Preconditioning Causes Overexpression of Genes Involved in Iron Metabolism in Young Men

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Objectives: This study was performed to evaluate the effect of ischemic preconditioning on the expression of ferritin H (*FTH*) and ferritin L (*FTL*) in peripheral blood mononuclear cells (PBMC).

Methods: 34 healthy, physically active men (aged 20.7 ± 1.22 years) volunteered to take part in this study. The experiment was twofold. First part was based on the crossover study procedure and was performed to evaluate the effect of one-time IPC on *FTH* and *FTL* gene expression. The second part was a parallel study aiming to evaluate the effect of 10 days of IPC sessions on *FTH* and *FTL* gene expression. Parallel study consisted of two groups: experimental (E-IPC) (n = 17, age, 19.7 ± 0.64 y, body mass, 73.6 ± 18.9 kg, height, 177 ± 6.18 cm, BMI, 23.8 ± 2.38 kg/m^2) which was assigned for 10 days IPC sessions, and control (C-IPC) (n = 17, age, 20.1 ± 1.88 y, body mass, 74.9 ± 15.4 kg, height, 177 ± 5.78 cm, BMI, 23.4 ± 2.24 kg/m^2). During both experiments blood samples were collected before and after the experiment to assess gene expression via quantitative-PCR. Statistical analysis was performed afterwards.

Results: After 10 days of IPC the over-expression of *FTH* and *FTL* was observed in IPC group while changes in control group were small and not statistically significant. In experimental group mean value significantly increased from 2^254.2 to 2^1678.6 (p = 0.01) for *FTH* and 2^81.5 to 2^923 (p = 0.01) for *FTL*. Differences in expression between groups after 10 days of ischemic sessions were also significant (2^143.5 in control group and 2^1678.6 in experimental group, p = 0.009 for *FTH* and 2^106.8 in control group and 2^903, p = 0.01 for *FTL*). No significant differences in *FTH* and *FTL* mRNA were observed after one time upper limb IPC. Mean value for *FTH* increased from 2^34.22 to 2^57.81 and for *FTL* from 2^8.88 to 2^9.42.

Conclusions: Our findings suggest that 10 days of IPC sessions cause significant increase in expression of *FTH* and *FTL* in PMBC. This phenomenon might have a protective role in stress exposition to any tissue.

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Evaluation of the DNA Methylation Status of Procalcitonin Gene as a Biomarker of Local and Systemic Inflammation

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Objectives: Procalcitonin (PCT) has recently emerged as an important biomarker for an early and accurate diagnosis of bacterial infection, hence suggested as biomarker of periodontal disease caused by oral pathogenic microorganisms. Altered DNA methylation of PCT coding gene – calcitonin-related polypeptide α (*CALCA*) has been shown during systemic inflammation. The aim of this study was to evaluate the influence of local and/or systemic inflammation present during periodontitis and diabetes on DNA methylation status of *CALCA* and its potential use as epigenetic-based biomarker of these chronic inflammatory conditions.

Methods: The study included 65 individuals divided in three groups: healthy control (n = 17), periodontitis (n = 27) and diabetes/periodontitis group (n = 21). Periodontitis was diagnosed using International Workshop for a Classification of Periodontal Diseases and Conditions criteria (1999) while type 2 diabetes assessment was performed according to WHO criteria (2013). DNA methylation profile of *CALCA* promoter in buccal epithelial cells was analyzed by methylation specific polymerase chain reaction (MSP).

Results: Decrease in DNA methylation of *CALCA* promoter was observed in periodontitis and even more pronounced in diabetes/periodontitis compared to control group, although without statistical significance. Correlation analysis revealed statistically significant relationship between the extent of DNA methylation of the *CALCA* promoter and glycosylated hemoglobin. Even though it is known that life style affects DNA methylation patterns, there was no difference in DNA methylation of *CALCA* promoter between smokers/non-smokers and normal/overweight individuals.

Conclusions: Presented results suggest that local periodontal inflammation contributes to the change, but that only systemic inflammation significantly alters the DNA methylation profile of *CALCA* in buccal cells. Those results imply that DNA methylation status of *CALCA* reflects systemic inflammation, but additional studies are needed to estimate usefulness of this epigenetic-based biomarker for periodontal disease.

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Finding Intermediate DNA Methylation Biomarkers of Early Life Exposures and Later Life Obesity

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Objectives: Many early life exposures have been associated with childhood adiposity, however the mechanisms remain largely unresolved. DNA methylation is hypothesised to be a potential mechanism underlying this phenomenon, with differences in DNA methylation having been associated with adiposity. However evidence linking exposures with both methylation and adiposity is limited. Thus our aim was to investigate the associations between the early life factors, blood DNA methylation, and subsequent adiposity outcomes.

Methods: Using data from the Avon Longitudinal study of parents and children (ALSPAC), associations between early life exposures and methylation at individual CpG sites (at ages 7.5 and 17) were investigated. Exposures investigated included older maternal age at birth, rapid weight gain (RWG), adversity and antibiotic exposure in the first year. DNA methylation for 1,000 study members was measured using the *Infinium* HumanMethylation450 BeadChip (at ages 7.5 and 17). Epigenome-wide association studies were carried out for each exposure with independent surrogate variable analysis. Associations between overweight/obese (OW/OB) and the CpG loci with significant methylation differences were investigated using logistic regression adjusted for confounders.

Results: RWG was associated with differential methylation in childhood for one CpG (5% false discovery rate correction). The significant loci was annotated to upstream of the gene encoding a 5' nucleotidase that localizes to the mitochondrial matrix (NT5M). The mean difference between those who were exposed compared to unexposed was 1% increase in methylation. Methylation at this loci was not associated with OW/OB in childhood in adjusted regression analyses, however there was an association between methylation at this loci at age 17 and OWOB at age 17. Validation of this target in other populations is ongoing. No other early life exposures demonstrated significant associations with DNA methylation in childhood or adolescence.

Conclusion: Overall there were few early life exposures associated with changes in methylation in childhood in this cohort. This study identified a small but significant increase in methylation at one CpG site in blood in childhood in association with early life rapid weight gain. The significant CpG loci could be a potential biomarker of exposure predictive of later life adiposity pending further study.

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Understanding the Bioactivity of Pomegranate Ellagitannins in Humans: Results of a Literature Review

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Objectives: There is strong evidence in animal models suggesting that pomegranate fruit exerts health benefits relating to their antioxidant and anti-inflammatory properties [1]. Beneficial effects are certainly the consequence of the presence of the pomegranate polyphenols, mostly consisting of ellagitannins (ETs). However, studies in humans often failed to show clear associations between pomegranate intake and health outcomes, possibly due to inter-individual variation in absorption, distribution, metabolism, and excretion (ADME) of ETs.

Methods: A literature review was conducted using the PubMed and Scopus databases including all original research articles on the relationship between inter-individual variability and ADME of ETs in humans. Data were summarized in a tabulated summary containing: study design, population, description of the intervention, duration, outcomes relevant to inter-individual variability in bioavailability and metabolism of ETs.

Results: From 2004 to date, most of the research studies are mainly related to cardiometabolic risk biomarkers. Intervention studies are carried out using pomegranate juice or phenolic extracts at different doses of ETs. Additionally, the study designs used differ for each trial. Data on the four criteria ADME were not available in all publications. Results showed that urolithins are the predominant metabolites following pomegranate consumption. Anyhow, few works are still focused on the bioconversion of pomegranate ETs to their active metabolites.

Conclusions: Urolithins are colonic microbiota metabolites of ETs and are considered responsible for *in vivo* health effects. The recently discovered existence of human metabolic phenotypes or metabotypes [2] could explain the variability seen in diet intervention studies. An understanding of the ADME of ETs in relation to the inter-individual variability is crucial for the elucidation of the mechanisms responsible for the health benefits of pomegranate and other ET-rich foods.

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20-Hydroxyecdysone Protects Pancreatic Islets and Liver in Streptozotocin-Induced Diabetic Rats

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Objective: 20-hydroxyecdysone (20HE), a steroid hormone that modulates molting response in insects exerts many pharmacological effects in mammals, most of which appear beneficial. The aim of this study was to investigate whether 20HE is able to reduce the destruction of beta-cells of the islets of Langerhans and ameliorate hyperglycemia induced changes in liver tissue in streptozotocin (STZ)-induced rat model of diabetes.

Methods: An experimental model of diabetes was induced in rats by the administration of 35 mg/kg STZ intraperitoneally for 4 consecutive days. 20HE was administered orally at a dose of 20 mg/kg body weight for four weeks, starting from the last day of STZ administration. Pancreas tissue sections were analyzed by hematoxylin and eosin staining and immunohistochemical staining with insulin. Estimation of oxidative damage of DNA and lipids in the liver were detected by comet assay and thiobarbituric acid-reactive substance assay, respectively. Liver sections were analyzed by hematoxylin/eosin and Masson's trichrome staining.

Results: Diabetic rats treated with the 20HE displayed several improved biochemical parameters in the circulation: reduced hyperglycemia, lower triglyceride concentration and reduced glycated hemoglobin. The administration of 20HE to diabetic rats also led to positive histological changes of pancreatic islets and increase in the number of insulin-positive cells in the islets which was accompanied by increased serum insulin level. These results show that 20HE administration to diabetic rats restrained islet destruction and partially restored the number of insulin-positive cells. In addition, treatment of 20HE attenuated diabetes-induced liver damage in rats according to lower level of DNA damage and reduction of oxidative damage of lipids. This result is in accordance with observed improvement of liver architecture in 20HE treated diabetic rats. Staining of collagen and increase in E-cadherin and decreases in α-smooth muscle actin revealed that administration of 20HE to diabetic rats attenuated fibrotic process in the liver.

Conclusions: 20HE administration seems to be beneficial for improving the hyperglycemia by increasing beta-cell mass and preventing diabetic complications in liver by attenuating fibrotic process.

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Effects of Sulphur-Containing Mineral Water on Classical Biochemical Markers in Human Interventional Study

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Objectives: Varna basin mineral waters are rich in hydrogen sulfide and soluble sulfides. Sulphur plays an important role in many processes, such as maintenance of redox balance, metabolism of xenobiotics, intracellular cell signaling. The purpose of the present research was to study the effects of Varna mineral waters on human metabolism with respect to their potential usage as a drinking remedy.

Methods: An 8 weeks human intervention study with mineral water intake was performed. Healthy volunteers (n = 50, M/F = 6/46, 40–65 y old) were enrolled in the study. Their usual type of drinking water was replaced by Varna mineral water (20 ml/kg). All participants signed an informed consent and were interviewed for nutritional and water-drinking habits, physical activity, and lifestyle.

Blood samples and 24-hours urine were taken before and after the intervention. Classical biochemical markers (gamma-glutamyl transferase (GGT), lipid profile, creatinine, aldosterone, high sensitivity C-reactive protein (hsCRP) and electrolytes) were analyzed in blood serum and in 24-hours urine (diuresis, urinary specific gravity, pH). The index of glomerular kidney filtration (eGFR) was calculated (Cockcroft Gault Calculator). Spectrophotometric, immunochemical and potentiometric methods have been used.

Statistical analyzes were performed by the application GraphPad Prism 5.0. Student's paired t-test was used with statistical significance $p \leq 0.05$.

Results: A statistically significant decreases in creatinine (76.35 ± 9.65 μmol/L vs 73.27 ± 9.35 μmol/L), hs-CRP (2.62 ± 2.25 mg/L vs 1.88 ± 1.21 mg/L) and an increase in eGFR (87.81 ± 21.69 ml/min vs 93.15 ± 21.93 ml/min) were established as a result of the intervention. A tendency of increase of aldosterone levels after 8 weeks of water intake was observed (0.33 ± 0.20 nmol/L vs 0.39 ± 0.24 nmol/L; $p = 0.09$). All other serum and urine parameters remained unchanged. After water intervention a significant increase of diuresis was detected. It could be suggested that increased diuresis leads to loss of sodium and the adrenal gland increases the secretion of aldosterone to compensate this loss. The reason for the lower creatinine levels after the intervention could be due to the increased diuresis. The statistically significant decrease of the hs-CRP is in agreement with the earlier established anti-inflammatory, keratoplastic and anti-pruriginous potential of sulphurous mineral waters. More research is needed to study the involvement of sulphur in these effects.

Conclusions: These are first biochemical clinical data on the metabolic effects of the long-term intake of Varna basin mineral water in humans.

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High-Fiber Diet of Pumpkin and Sweet Potatoes Improve Hypertriglyceridemia by Suppressing SREBP-1c Gene Expression in the Liver and Adipose Tissues of Dyslipidemic Rats

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Objectives: High-fat and fructose diet contribute to the development of hypertriglyceridemia. Sterol regulatory binding protein-1c (SREBP-1c) is involved in the regulation of lipogenic gene expression. A high-fiber diet was reported to suppress SREBP-1c gene expression.

The objective is to evaluate the effect of pumpkin and sweet potatoes fibers on SREBP-1c gene expression and triglyceride in dyslipidemic rats.

Methods: Twenty-five male Wistar rats were divided into 5 groups: 1) normal (N); 2) high fat and fructose diet (HFFD); 3, 4, and 5) HFFD with substitution of pumpkin and sweet potato containing fiber (g) per day: 20% (2.6 g), 40% (5.2 g), and 60% (7.8 g), respectively. The triglyceride levels were measured before and after the intervention, whereas SREBP-1c expression in the liver and white adipose tissues were measured at the end of the intervention. HFFD-inducted dyslipidemia was performed 7 weeks and high fiber diet intervention for 6 weeks.

Results: Pumpkin and sweet potatoes substitution reduced triglyceride levels in 25%, 47%, and 49% of dyslipidemic rats after 6 weeks of intervention. This intervention also suppresses the expression of the SREBP-1c gene in the liver by dose-dependent but not on white adipose tissue.

Conclusion: Pumpkin and sweet potatoes decreased triglyceride of dyslipidemic rats through suppression of SREBP-1c gene expression in liver and white adipose tissues.

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Monovalerin and Trivalerin Increase Brain Acetic Acid, Decrease Liver Succinic Acid, and Alter Gut Microbiota in Rats Fed High-Fat Diets

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Objectives: Short-chain fatty acids (SCFA) are known for their anti-inflammatory properties and may also prevent against the development of metabolic diseases. This study investigates possible effects of two valeric acid esters, monovalerin (MV) and trivalerin (TV) in rats fed high-fat diets.

Methods: Four groups of rats were given a low-fat diet (LF) or a high-fat control diet (HFC) with or without supplementation of MV or TV (5 g/kg) for 3 weeks (n = 7 per group). SCFA (caecum, blood, liver and brain), succinic acid (liver), microbiota (caecum), lipid profile (liver and blood) and the inflammatory biomarker, lipopolysaccharide-binding protein (blood) were analysed at the end of the experiment.

Results: Supplementation of MV and TV to a high-fat diet increased the amounts of acetic acid in the brain and valeric acid in serum, while succinic acid in the liver was reduced. Furthermore, liver LDL/HDL ratio was lower in the MV group, while liver triglycerides levels were higher in both MV and TV groups compared with the LF group. There was a concurrent alteration in caecal microbiota composition, with 3-fold higher abundance of Bacteroidetes and higher ratio of Bacteroidetes/Firmicutes in the MV group compared with HFC and LF groups. Acetic acid in the brain was negatively correlated with TM7, family S24-7 and *rc4-4*, and positively associated to Tenericutes and *Anaeroplasma*.

Conclusions: The present study shows that MV and TV in the specified dose can affect caecal microbiota composition and therefore bacterial metabolites in the liver, serum and brain as well as the lipid profile in the liver.

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MeFISH: Fluorescent Detection of Target Methylated Cytosines within the Genome

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Objectives: This study aim to implement a novel method, methylation-specific fluorescence *in situ* hybridization (MeFISH), based on microscopic visualization of DNA methylation/hydroxymethylation status at specific DNA regions in individual nuclei after pancreatic cell treatment with different compounds that possess a pronounced DNA (de)methylation capacity.

Methods: The DNA (de)methylating properties of two selected compounds caffeine (Co) and azacitidine (A) were evaluated in a Rin-5F pancreatic beta-cell line. Rin-5F cells were spin down on microscopic slides and further processed for preparing HALOs (relaxed DNA with preserved contacts with non-soluble nuclear proteins). The fluorescent visualization was achieved using ICON probe that covers region of interest in the promoter of the CXCL12 gene and target C positioned on the +26 bp, osmium for methylated cytosine (5mC)-dependent crosslinking and Tyramide Signal Amplification Systems for enhanced fluorescent staining.

Results: In control and Rin-5F cells treated with Co we were able to detect clear, single fluorescent signal that correspond to 5mC positioned on the +26 bp within the promoter region of the CXCL12 gene using MeFISH. Confirmation for the *in situ* hybridization specificity was achieved by omitting the crosslinking reaction with osmium. We observed a clear difference between control and Co treated samples, indicating that Co acts as pronounced DNA methylating compound. Treatment of cells with A lead to the appearance of a specific signal in a limited number of HALO preparations confirming demethylating property of A.

Conclusions: The Co acts as a pronounced DNA methylating agent in contrast to A, which demethylates CXCL12 gene and subsequently promotes higher gene expression. Higher methylation of the CXCL12 gene after cell treatment with Co leads to suppression of the gene which was observed by RT-qPCR. The analysed C, positioned on the +26 bp, may represent one of the major sites whose methylation is important for the regulation of the CXCL12 gene expression *in vivo*.

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The Role of Iron on Marathon-Induced Changes on the EPO-Erythroferrone-Hepcidin Axis

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Objective: The aim of this study was to investigate whether a marathon affects changes in key hormones responsible for iron metabolism: Erythroferrone (ERFE) and Hepcidine (Hpc). Moreover, we assessed if bearing mutations of the *HFE* gene, responsible for controlling the absorption of iron, would influence iron metabolism.

Methods: Twenty-nine healthy man (mean age 38 ± 5), who took part in the Gdansk marathon were studied. HFE gene mutation using PCR technique was evaluated. Blood iron, ferritin, ERFE, and Hpc level were assessed before, immediately and 9 ± 2 days after the marathon.

Results: ERFE increased after the marathon and remained elevated one week later, but only in the runners whose hepcidin decreased ($p < 0.05$). Athletes characterized by low blood iron concentration before the marathon (<105 μg/dl) presented with a significant increase in ERFE after the marathon, while those with high blood iron (>105 μg/dl) had a stable ERFE concentration ($p < 0.05$). Blood ferritin concentration had no effect on changes in ERFE induced by the marathon. Despite high prevalence of *HFE* gene mutation (11 out of 29 runners) no differences in all study parameters (iron, ferritin, Hpc, ERFE) were observed.

Conclusion: In the presented study, we demonstrate that alterations in hormones regulating iron metabolism (ERFE, Hpc) induced by a marathon are significantly dependent on blood iron concentration at baseline. Contrary to our expectations, athletes who were HFE heterozygotes demonstrated similar changes in iron metabolism to wild type ones.

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Antioxidant Effects of Milk-Derived Bioactive Peptides on Human Colorectal Adenocarcinoma Cells

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Objectives: Milk-derived bioactive peptides display various functions resulting in antihypertensive, antimicrobic and antioxidant effects. In particular this study is focused on their antioxidant activity in a cellular model. Four synthetic peptides, N-6-R, A-7-R, K-8-K, A-11-M, whose sequences correspond to human and bovine caseins, were used to evaluate the potential antioxidant role of the bioactive peptides in signalling pathways.

Methods: Human colorectal adenocarcinoma cells (Caco-2) were cultured in the presence of the four peptides. Subsequently, cells were treated with H_2O_2 and the activities of thioredoxin reductase (TrxR) and glutathione reductase (GR) in cell lysates were determined spectrophotometrically at 412 nm and 340 nm, respectively. ROS production and lipid peroxidation were analysed in Caco-2 cells pre-treated with N-6-R, A-7-R, K-8-K, A-11-M and oxidative stress was induced by 250 μM tert-butyl hydroperoxide (TbOOH).

Results: The obtained results showed that TrxR and GR activities were preserved against oxidative stress by the four bioactive peptides, in particular in the presence of K-8-K, A-7-R and A-11-M. Similarly to what observed in enzymatic activity analysis, K-8-K and A-7-R protected cells against ROS production induced by TbOOH. At last, we observed that bioactive peptides, in particular K-8-K, decreased lipid peroxidation in Caco-2 cells.

Conclusions: In this work we show that the four bioactive peptides (N-6-R, A-7-R, K-8-K, A-11-M) exert a protective effect in Caco-2 cells against oxidative stress. The obtained results highlight a possible role of the four peptides on modulation of gene expression. We think that they can be involved in the regulation of Nrf2/Keap1 pathway and we plan to further analyse the relationship between the bioactive peptides and this transcription factor.

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Every Other Day Feeding Affects Histone K14 Acetylation of the BDNF Gene in the Hippocampal Area in Low- and High Running Capacity Rats

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Objectives: Dietary Restriction (DR) has long been associated with multiform mechanisms which includes longevity, mitochondrial biogenesis, oxidative stress and disease prevention. The prevalence of diseases like diabetes, circulatory events and various cancers can be reduced in animal models using calorie restriction. It seems that the better cognitive performance linked to dietary interventions has a strong relation with synaptic plasticity governing brain-derived neurotropic factor (BDNF) and its release is not independent of intrinsic running capacity.

Methods: In our study we were interested whether running capacity as a phenotypic trait has any impact on the epigenetic modulation of the BNDF gene expression in alerted dietary conditions. An experimental model was created for the running capacity of rats (Koch and Britton, 2001). Low capacity runners (LCR) and high capacity runners (HCR) were selected by rotational breeding. In our experiment one group from each line was exposed to DR (Every Other day Feeding). Our research focused on the histone H3 K14 acetylation of the BDNF gene ~28 Kb downstream to the actual coding sequence where the IV promoter region is located. The IV promoter is reported to be activity dependent and it is shown to have instructive roles in BDNF expression.

Results: BDNF content was increased significantly in the hippocampus of HCR-DR groups compared to other experimental groups. To investigate the epigenetic environment of the BDNF gene, total hippocampal acetylation level of histone H3 were measured and elevation was detected in the LCR-DR group. The specific binding between acetylated histone H3 and BNDF promoter IV region was increased by every other day feeding in the LCR and HCR animals. Remarkably, the increases in these bindings did not resulted in elevated mRNA of BDNF in the HCR.

Conclusions: The regulation of BDNF gene expression is a highly complex, multi-layered mechanism as our result suggests with many. This complicated process seems to differ substantially in populations with low and high running capacity. In case of brain health and memory functions it is essential to understand the individual needs in terms calorie consumption. According to our finding DR not only results in better cognitive function and elevated BDNF levels in HRC rat hippocampus, but it also has the capacity to modify specific chromatin structures.

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Plasma Fatty Acid Levels and Gene Expression Related to Lipid Metabolism in Peripheral Blood Mononuclear Cells: A Cross-Sectional Study in Healthy Subjects

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Objective: Solid evidence indicates that intake of marine n-3 fatty acids lowers serum triglycerides, and that replacing saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA) reduces plasma total cholesterol and LDL-cholesterol. The molecular mechanisms underlying these health beneficial effects are however not completely elucidated.

The aim of the study was to investigate the expression of genes related to lipid metabolism in peripheral blood mononuclear cells (PBMC) depending on plasma levels of n-6 and n-3 fatty acids and SFA to PUFA ratio.

Methods: Fifty-four healthy subjects were grouped into tertiles (n = 18) based on plasma levels of n-6 and n-3 fatty acids and SFA to PUFA ratio. PBMC gene expression levels among subjects in the highest versus the lowest tertiles were compared. In total, 285 genes related to cholesterol and triglyceride metabolism were selected for this explorative study.

Results: Among the 285 selected genes, 161 were defined as expressed in the PBMCs. The plasma SFA to PUFA ratio was associated with the highest number of significantly different expressed genes (25 gene transcripts), followed by plasma n-6 fatty acid level (15 gene transcripts) and plasma n-3 fatty acid level (8 gene transcripts). In particular, genes involved in cholesterol homeostasis were significantly different expressed among subjects with high compared to low plasma SFA to PUFA ratio.

Conclusion: Genes involved in lipid metabolism were differentially expressed in PBMCs depending on plasma fatty acid levels. This finding may increase our understanding of how fat quality influence lipid metabolism at a molecular level.

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Salivary Metabolomics as a New Tool for Unravelling Stress Obesity Pathways in Adolescents from a Clinical Public Health Perspective

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Objectives: The high prevalence and treatment resistance of obesity urges further exploration in early diagnosis and prevention. This is especially important at young age, when metabolic and psychological development are ongoing. Also psychological factors like stress can favor obesity, but researchers still struggle with the complex processes towards disease susceptibility. This project aims to elucidate pathways in the bidirectional stress-obesity relation via salivary metabolomics in adolescents.

Methods: Saliva can be non-invasively collected while its metabolic composition parallels that of blood. Hence, the associations between the metabolome from different matrices (plasma, faeces, saliva) in the stress-obesity axis of 260 adolescents will be investigated using state-of-the-art ultra-high performance liquid chromatography hyphenated to high-resolution mass spectrometry. Studying metabolomics-based differences between combinations of subgroups (low to high stress and healthy weight to obese) will explain why not all high stress adolescents develop obesity and why not all obese adolescents develop stress. Herein, both acute and chronic stress are considered by including self-reports, chronic stress biomarkers (e.g. hair cortisol, inflammation) and a laboratory study with acute stress induction. The use of longitudinal data will allow more insight in the cause-effect direction. Finally, salivary metabolite markers will be compared with existing clinical biomarkers of stress physiology, appetite, energy balance and inflammation.

Results: Data collection is foreseen from March until May 2018. In a proof of principle experiment, I investigated a bulk saliva sample of 3 adolescents resulting in 5171 features, comparable with earlier studies in our lab that found 6765 features in plasma.

Conclusions: Salivary metabolomics will demonstrate the crucial working mechanism between chronic stress and obesity. Results will highlight its potential in the diagnosis and treatment for obesity. We expect the results will also inspire new prevention strategies.

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Nutritional and Molecular Monitoring of Pregnant Women and the Infants from the Birth Until Two Years of Age

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Objectives: this study aims to investigate the effect of diet on maternal and infant microbiome profile and on the epigenetic signature during pregnancy, at delivery, and during the first two years of life of the babies. This study is part of a wider project aiming to identify biomarker signatures predictive of pre-term birth using a multi omics approach among which blood transcriptomics, placenta epigenetics, vaginal and saliva microbiome, that will not be discussed in this abstract.

Methods: the study will include 400 pregnant women of Karen or Burmese ethnicity that are applicable to the inclusion criteria (18–49 years age, healthy and without medical or obstetric complications, with viable singleton first trimester [8+0 to <14+0 weeks] pregnancy, delivered at SMRU) and their babies. For microbiome profiling we will collect stool samples (6 samples from mothers: one each trimester, one at delivery and two post-partum; 18 samples from the infants: at birth, during first week of life, every month for the first year of life and every three months for the second year), placenta samples at the delivery, and breast milk in three different maturation stages: colostrum, translational, and mature milk. The microbiome will be analysed by 16S rDNA sequencing on the Illumina MiSeq platform. For epigenetic profiling, cord blood will be collected at delivery and it will be analysed using DNA methylation array on Illumina iScan platform. Dietary habits will be recorded using 24 hrs dietary recall at the same time of the stool sample collection, and the food intake will be calculated using ePhood software for nutritional analysis.

Data will be analyzed first as individual "omics" analyses and it will focus on identifying changes during the course of pregnancy and the early life. For this we will rely on linear mixed model analyses. We also will investigate any significant deviations from the "healthy" molecular trajectories that may occur or may precede complications or adverse clinical outcomes, such as pre-term birth. These results will be incorporated in a multi-omics analyses from the other omics approaches and a data integration will be run to identify possible interaction of the identified biomarkers from the single approach.

Expected Research Outcomes/Conclusions: this study will potentially identify early biomarkers among gut, placenta, breast milk microbiome and blood epigenetics profiles, influencing individual health trajectories later in life by applying high-resolution molecular monitoring based on different associated aspects: diet, microbiome pattern, and epigenetics.

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The Triple Interaction Diet-Microbiome-Epigenome: A Novel Approach to the Non-Communicable Diseases

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Objective: This study aims to identify specific nutrients that modulate gut microbiome; to define different microbiome composition and metabolites; and to define differential methylated regions (DMR) that is possibly affected by nutrients, gut microbiome and its metabolite in type 1 diabetes mellitus (T1DM) and obese pediatric patients.

Methodology: Pediatric patients will be recruited from Sidra OPC based on major inclusion criteria, (such as age should be between 6–12 yrs, no antibiotic treatment in the past 3 months, no chronic diseases except T1D and no history of cancer) and divided into four groups: (1) healthy lean children (5–84th percentile of BMI), (2) Obese (≥95th percentile of BMI;), (3) T1DM and (4) Obese T1DM. A comprehensive set of physical measurements (body weight, height and waist circumference), clinical biomarkers for diabetes and obesity (blood glucose level, lipid and liver profile, HbA1c will be done with blood sample) and family history of diabetes, treatment history will be collected and most importantly dietary habits will be collected by 24 hrs food recall. Two sets of stool samples (one for microbiome analysis by 16S rDNA-sequencing; and one for short chain fatty acids analysis by gas chromatography) and two set of blood samples (one for DNA extraction for methylation analysis by Illumina DNA-methylation Array and one for RNA extraction for gene expression analysis using Fluidigm platform) will be collected from each subject. Data analysis will investigate any association of diet to the clinical phenotypes comparing the four groups of subjects, using logistic regression; two-sided *P*-value of <0.05 will be considered statistically significant.

Possible Research Outcomes/Conclusion: At the end of pilot study, we can able to define the (1) methodology workflow, (2) nutrients list or diet patterns that increase the risk of T1DM in obese children, (3) specific microbiome pattern, in terms of composition and metabolite, in obese T1DM children, and (4) specific nutrients and microbiome metabolites that alter DNA-methylation and gene expression in obese T1DM children. This methodology workflow will be applied to other studies on nutrition-related diseases.

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