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Guest Editor

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Windows of Opportunity in the First 1000 days for Nutritional Programming of Metabolic Health

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The first 1000 days represents a period of focused growth and development that shapes the metabolic health of an infant during childhood and adulthood. Apart from genetics, the maternal and infant environment, represents one of the driving forces in this development. A mother's metabolic health influences the growth of the fetus, determining the birth weight of the infant, which, in turn, sets the stage for its growth trajectory during infancy and associated later life risk of non-communicable diseases. In addition, nutrition provides not only the building blocks for physical growth but influences and programs metabolic homeostasis and responsivity. The timing and duration of any period of under- or over-nutrition from conception to toddlerhood is critical since any imbalances in growth velocity may lead to suboptimal organ development, disproportionate weight gain, unfavorable fat distribution and predisposition to adverse metabolic profiles. These trajectories can be influenced by nutritional interventions during certain windows of opportunity during the first 1000 days: pre-conception, pregnancy, lactation, weaning and toddlerhood. The quantity and quality of both fat and protein can have a significant impact on growth and metabolic health, driving changes in adiposity and metabolic signaling. Human milk contains lipid globules with a unique structure; the structure as well as the fatty acid composition likely drive some of the metabolic health benefits associated with breastfeeding. In comparison, suboptimal fat intake during toddlerhood is associated with increased adiposity in early adulthood. Informed weaning practices, nutrient-dense complementary foods and the development of healthy eating habits can help mitigate any deficiencies. Understanding these windows of opportunity allows us to improve the composition of age-specific nutritional solutions and target periods of vulnerability in development. Early detection of growth challenges provides an opportunity for intervention and the possibility of reducing the incidence of metabolic diseases in later life.

2

Lasting Effects of Early Feeding and Angles of Breastfeeding Support

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Breastfeeding is the optimal feeding mode and own mother's milk the ideal food matrix for all infants. Mother's milk is a complex secretion with immense inter- and intrapersonal variations that are further affected by climate and environment. Especially preterm infants benefit from trophic and immune stimulating or infection protective effects that own mother's milk provides. Subsequently, donor human milk bank initiatives in many (European) countries are established that make an effort to secure access to human milk for preterm or (critically) ill infants when own production is too low or lacking. Often mothers of preterm infants experience difficulties to establish milk flow. Coaching and feeding technique can play a role to resolve these issues.

In severe cases of hypogalactia (lactation insufficiency), prescription or recommendation of milk inducers – so called galactogogues – can be considered. Certain medication have shown in off-label use to stimulate milk flow as have herbal or plant extracts used since ancient times in traditional medicine.

But mother's milk does not only provide immediate benefits and increases infant survival rates. Breastfeeding also affects maternal health – often long-lasting: The mother-infant dyad bonding shortly after birth is enhanced through breastfeeding and risk for some maternal birth complications are reduced through the release of oxytocin. Evidence accumulates that women benefit from breastfeeding with reduced cancer risk for several cancer types in reproduction-related tissues and organs. These benefits are related to intensity and accumulated duration of breastfeeding in the women's reproductive years.

In addition, medium- and long-term benefits – likely to be mediated through early nutrition programming – have also been observed in children or (young) adults that had been breastfed in infancy. Higher intelligence scores have been well demonstrated. Several key nutrients historically absent in alternative feeding options have been debated to contribute to these observations. Key nutrients include long-chain polyunsaturated fatty acids, particularly docosahexaenoic- and arachidonic acids, or human milk oligosaccharides. Presence, degree or composition of both nutrient groups are genetically regulated in breast milk. In contrast to higher intelligence scores, the impact of breastfeeding on later overweight and obesity, diabetes mellitus type 2 or cancer risk, particularly childhood leukemia, remain controversial due to confounders or data paucity.

In short, breastfeeding and human milk impact health of mother and child immediately yet also affect life-long health beneficially. This feeding mode and food matrix fall within the principle of Developmental Origins of Health and Disease (DOHaD) and deserve more attention.

3

Optimising Preclinical Models of Nutritional Programming for Markers of Metabolic Health

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Preclinical research is crucial in understanding the potential lasting influence of early life nutrition and other environmental conditions on metabolic health and associated pathophysiology. When designing preclinical experiments with metabolic outcomes, there are several significant factors that must be considered to ensure clarity and accurate interpretation of results. Though often overlooked in analyses, the choice of rodent (background) diet as well as (social) housing conditions, cage mate/litter mate interactions and the influence of maternal conditions and care are essential factors that can affect metabolic outcomes. A better understanding of the impact of these factors can help to optimize preclinical model design and data interpretation. We have conducted systematic literature reviews and meta-analyses and *in vivo* studies assessing the effects of social versus individual housing on metabolic health in rats and mice, as well as the effects of grain based versus semi-synthetic diets on hepatic health in rats and mice. Whilst housing did not affect body weight, both food intake and visceral adipose tissue mass were significantly higher in individually compared with socially housed animals. In addition, our findings strongly suggest that semi-synthetic diets induce insulin resistance and hyperinsulinemia, resulting in higher accumulation of triglycerides and, eventually, development of steatosis. Based on our experience with preclinical programming models, we recommend a renewed focus on accounting for variation caused by maternal unit, litter variables, and cage mates in the experimental design as well as in the statistical analysis, ensuring that the impact of these variables is accounted for during the period of early life programming. Increased awareness of the consequences of diet choice, housing conditions, and consideration of the maternal and litter units in statistical analyses are necessary to optimize preclinical programming models and other models that report metabolic health outcomes.

4

White, Brown and Pink Adipocytes: The Rainbow of the Nutritional System

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The white and brown adipose tissues are organized to form a true organ (1). They have a different anatomy and perform different functions, but they collaborate thanks to their ability to convert mutually and reversibly following physiological stimuli (2). This implies a new fundamental property for mature cells, which would be able to reversibly reprogram their genome under physiological conditions. The subcutaneous mammary gland provides another example of their plasticity (3). Here fat cells are reversibly transformed into glands composed by lipid rich epithelial cells (pink adipocytes) during pregnancy and breastfeeding. The obese adipose organ is inflamed because hypertrophic fat cells, typical of this condition, die and their cellular residues must be reabsorbed by macrophages (4). The molecules produced by these cells during their reabsorption work interfere with the insulin receptor and this induces insulin resistance, which ultimately causes type 2 diabetes. The adipose organ collaborates with those of digestion. Both produce hormones that can influence the nutritional behavior of individuals. They produce molecules that mutually influence functional activities including thermogenesis, which contributes to the interruption of the meal. The nutrients are absorbed by the intestine, stored in the adipose organ and distributed by them to the whole body between meals. Distribution includes offspring during breastfeeding. The system as a whole is therefore called the nutritional system (5).

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The Ups, Downs, and Ups Again of Omega-3 Fatty Acids as Cardioprotective Agents

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After the glory of earlier trials with omega-3 fatty acids in cardiovascular disease, culminating in the GISSI-Prevenzione trial in 1999, documenting important effects on cardiovascular mortality and sudden cardiac death in the thrombolytic era of myocardial infarction treatment, skepticism has risen on the reproducibility of those results in current times, with several meta-analyses not being able to re-document significant efficacy. This contrasts however with the consistency of epidemiologic data relating consumption of omega-3 fatty acids with lower cardiovascular risk. Such discrepancy can be largely explained by consideration of the fundamental difference between continuous dietary intake extended for an entire life, as in epidemiological studies, as opposed to relatively short-term intakes occurring in intervention trials. Indeed the inverse relationship of omega-3 fatty acid intake with cardiovascular events, including myocardial infarction, has been confirmed even recently by studies documenting that incorporation of omega-3 fatty acids in the adipose tissue, a stable and reliable marker of omega-3 fatty acid intake, is inversely related to cardiovascular events even at extended follow-up. Recent data from the REDUCE-IT trial have again boosted the enthusiasm for these agents also in the course of an intervention trial in primary and secondary cardiovascular prevention, probably as the result of doses used, higher than before, and of an accurate selection of the target population. Such data will be discussed in the broad perspective of the history of attempts at using such agents to improve cardiovascular health.

Insights into the Infant Gut Microbiota: Bifidobacteria and the Human Gut as an Intriguing Example of Strict Microbe-Host Co-Evolution

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The formation of the human gut microbiota is mostly assumed to arise immediately after birth, including key gut microbial commensals like bifidobacteria, which are maternally inherited. The following expansion of this initial gut microbiota is driven and

modulated by certain dietary compounds occurring in the human milk, supporting selective colonization. This constitutes a very fascinating model of host-microbe co-evolution, where both partners are thought to benefit. Recently, several reports have focused on analyzing microbial infant gut communities and their cross-talks with the human host, representing a determining issue in host physiology and metabolic activities. These analyses have underlined a reduction of microbial diversity and/or an aberrant microbiota composition, described as dysbiosis, which may display itself during the early stage of life, i.e., in infants, or later stages of life. There are increasing experimental insights explaining how the early human gut microbiota influences risk factors associated to adult health conditions. This notion has stimulated the promotion of several nutritional intervention strategies, many of which are based on probiotics and/or prebiotics, aimed to manipulate the composition of the infant gut microbiota. Here, we will present the current state of the art concerning the infant gut microbiota and the role of key commensal microbes such as bifidobacteria in the establishing of the first microbial communities in the human gut.

Novel Cutting-Edge Metagenomics Approaches for the Analysis of the Microbiota

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The last decade witnessed incredible technological advances in microbial DNA sequencing that gradually led to per-base sequencing cost reduction. Thus, allowing progressively more research teams to include metagenomics approaches in their research projects. Moreover, the body of sequence data available in publicly available databases such as SRA along with datasets released by new studies represent a valuable resource for data mining and meta-analysis purposes. For these reasons, *in silico* analyses of big data now exerts a central role in supporting classical *in vitro* and *in vivo* research applications. Amongst the body of next-generation sequencing-based analyses, metagenomics arises as the golden standard for the study of complex microbial populations by allowing the dissection of the taxonomic profile of microbiota and prediction of the functional commitment of their corresponding microbiomes. Here, we explore novel cutting-edge metagenomics tools for the high-throughput analysis of shotgun metagenomics datasets.

GWAS versus EWAS: Integration of (epi)Genetic Approaches Identified *Paraoxonase-1* as a Critical Determinant of Obesity-Associated Fatty Liver Disease

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Background: The multifactorial nature of non-alcoholic fatty liver disease cannot be explained solely by genetic factors. Recent evidence revealed that DNA methylation changes take place at proximal promoters within susceptibility genes. This emphasizes the need of combining genetic and epigenetic data to provide a better understanding of the disease's pathogenesis. One such candidate gene is *paraoxonase-1* (*PON1*). Substantial interindividual differences in *PON1* activity are apparent and may influence disease risk later in life. The aim of this study was therefore to determine the different regulatory aspects of *PON1* variability and examine them in relation to the predisposition to obesity-associated fatty liver disease.

Results: A targeted multi-omics approach was applied to investigate the interplay between *PON1* genetic variants, promoter methylation, expression profile and enzymatic activity in an adult patient cohort with extensive metabolic and hepatic characterisation including liver biopsy. Alterations in *PON1* status were shown to correlate with waist-to-hip ratio and relevant features of liver pathology. Particularly, regulatory polymorphism rs705379:C>T was strongly associated with more severe liver disease. Multivariable data analysis furthermore indicated a significant association of combined genetic and epigenetic *PON1* regulation. This identified relationship postulates a role for DNA methylation as a mediator between *PON1* genetics and expression, which is believed to further influence liver disease progression via modifications in *PON1* catalytic efficiency.

Conclusions: Our findings demonstrate that vertical data-integration of genetic and epigenetic regulatory mechanisms generated a more in-depth understanding of the molecular basis

underlying the development of obesity-associated fatty liver disease. We were able to gain novel insights into how NAFLD classification and outcome are orchestrated, which could not be obtained by exclusively considering genetic variation.

A Short Lifestyle Intervention Study (4-mo RCT) is Already Sufficient to Improve Seminal Plasma Biomarkers in Healthy Young Males Living in Highly Polluted Italian Areas?

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Background: Human semen quality is declining affected by lifestyle and exposure to several environmental factors.

Objective: To evaluate the short-term effects of a lifestyle, based on diet and physical activity, intervention on semen quality of healthy young men living in three highly Italian polluted areas.

Study Design: A Randomized Controlled Trial (<https://clinicaltrials.gov/>, Protocol Registration and Results System; receipt release date: February 15, 2019; n. J59D1600132001) has been conducted recruiting 18-22 yrs old healthy young men randomly assigned to a control (CTRL) and an intervention (INT) group. The lifestyle intervention has been performed following a 4-mo Mediterranean diet and a moderate physical activity program.

Outcome Measurements: Andrological outcomes have been assessed following a WHO-based spermogram, e.g., measuring sperm concentration, motility and morphology, concentration of round cells. In addition, semen total antioxidant capacity (TAC) has been measured. Lifestyle outcomes included adherence to Mediterranean diet and physical activity.

All outcomes were measured at the enrollment (t0), at the end of the intervention (t4), upon a 4-mo follow up (t8). Data from the latter time point are not yet available.

Results: The study results have taken into account only the 263 healthy young men attending all visits, examinations and laboratory analysis: n = 126 in the CTRL group and n = 137 in the INT group.

From the time points t0 to t4, the adherence to Mediterranean diet and physical activity level increased more in the INT group than in the CTRL group. In the same period, sperm concentration, total and progressive motility, and the proportion of cells with normal morphology increased in the INT group but decreased in the CTRL group: data have been statistically significant considering the two groups at t4. From t0 to t4, TAC increased in the INT group but decreased in the CTRL group,

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Role of Histone Deacetylase 3 (HDAC3) and Downstream Players in Physiopathology of Adipose Tissue

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Objectives: Obesity is considered a global epidemic which increases risk to develop cardiovascular disease, type 2 diabetes, and also infectious diseases such as Covid-19. New therapies are needed to counteract the associated metabolic changes and comorbidities. Metabolic dysfunctions are related to epigenome modifications that increase susceptibility to obesity. Histone deacetylase 3 (HDAC3) is relevant in physiopathology of white adipose tissue (WAT) and its genetic inactivation leads to a metabolic rewiring of white adipocytes towards browning. The aims of this study are to unravel early changes caused by obesogenic diet coupled with the role of HDAC3 and to capture insights about molecular determinants of this phenotype.

Methods: To pursue our objectives we performed RNAseq and ChIPseq (H3K27ac) analyses of subcutaneous WAT of 12 weeks old Hdac3 knockout (H3fatKO) and floxed control (FL) mice, fed high- (HFD) or low-fat diet (LFD) from 8 weeks of age.

Results: Integrated bioinformatic analysis confirmed the existence of the futile cycle of β -oxidation and lipogenesis which is the hallmark of H3fatKO mice. In addition, in H3fatKO mice we found enriched pathways related to amino acid metabolism and ferroptosis, whether pathways associated with cytoskeleton and extracellular matrix were downregulated. By further examination of transcriptomic data, we discovered that two genes inversely correlated with browning and linked to triglyceride accumulation

were in the top 10 downregulated genes (-94- and -17-fold change) in KO LFD vs. FL LFD. Interestingly, the epigenomic analysis uncovered a hypoacetylated region 39 Kb upstream the promoter of the most downregulated gene. This is consistent with RNAseq data and suggests us a non-canonical role of HDAC3 as a possible coactivator in the regulation of these genes.

Conclusions: HDAC3 is a key factor in determining WAT phenotype and its inactivation triggers a cascade of events which leads to browning. This study identified novel processes involved in establishing the phenotype observed in HDAC3 ko mice. The discovery of novel players in browning of WAT may reveals new therapeutic targets for the treatment of obesity and comorbidities.

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Ideabril Packaging Capability in the Preservation of Raw and Cooked Ham. A Comparative Study

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Food packaging contributes to the preservation and shelf-life of food.

In literature, several studies demonstrate that active packaging, enriched of bioactive compounds like the essential oil of *Rosmarinus Officinalis* [1], and modified atmosphere packaging can positively influence the preservation of food. The study of new types of packaging is continuously increasing, mostly in terms of environmental impact and food preservation.

In this study three types of packaging provided by Essequattro company were tested in order to assess their capability in the preservation of food. *Ideabril*® packaging, a three layers pack of polyethylene high density layer, metallic layer and cellulose with long fiber layer, combined with *Ideabril*® sacchetto salvafreschezza was compared to paper coupled with wings alone and combined with *Ideabril*® sacchetto salvafreschezza. The study was conducted on raw and cooked ham preserved in the packaging described above through the quantification of biogenic amines (BAs) at day 0, 3, 5 and 7. BAs can be considered markers to evaluate the freshness and the quality of food. In particular, a higher concentration of BAs is related to a higher deterioration degree of food. BAs were extracted, derivatized with dansyl chloride, purified with a SPE C-18 and then analyzed with an HPLC-DAD method. This study, in combination with microbiological study, shows that *Ideabril*® packaging combined with *Ideabril*® sacchetto salvafreschezza showed the best conservation capability for raw and cooked ham when compared with others. Moreover from an eco-friendly point of view, *Ideabril*® packaging layers can be easily separated in order to encourage recycling.

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Effects of Early Life Exposure to Famine on Adulthood Metabolic and Cognitive Outcomes: A Historical Cohort Study from 1983 - 1985 Ethiopian Great Famine

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Background: The Ethiopian Great Famine was one of a severe form of global famines ever documented in Africa as well as in the recent history of the world. Nutritional insult in early life brings adaptive changes in body structure and functioning, which could remain throughout the affected individual's life course. Previous famine studies, as natural experiments, had tested the association of early life famine exposure with metabolic syndrome and cognitive function in adults, and reported heterogeneous findings. Hence, this study aimed at investigating the effects of early life exposure to the 1983-1985 Ethiopian great famine on metabolic syndrome and cognitive function in adults.

Methods: A historical cohort study was conducted among adult men and women. Exposure status for the famine was categorized into prenatal-exposed (age = 34-36), postnatal-exposed (age = 37-38), non-exposed groups (age = 30-32). A total of 447 and 1047 participants were recruited to assess metabolic syndrome and cognitive function, respectively. The International Diabetes Federation (IDF) criterion and Montreal Cognitive Assessment (MoCA)-score was used to assess metabolic syndrome and cognitive function, respectively.

Results: The findings showed that, adjusted for covariates, adults who had prenatal exposure to famine were 2.94 times more likely to develop metabolic syndrome compared to non-exposed groups (AOR = 2.94; 95% CI: 1.66, 5.27). Famine exposure during prenatal life was associated with increased waist circumference (mean difference (MD) = 2.27cm; 95% CI: 0.28, 4.26), diastolic blood pressure (MD = 2.47 mmHg; 95% CI: 0.84, 4.11), triglyceride (MD = 14.52 mg/dl; 95% CI: 4.56, 25.47) and fasting blood glucose (MD = 4.28mg/dl; 95% CI: 0.80, 7.75) compared with the control groups. Likewise, postnatal (birth to 2 years) exposure to famine resulted in 2.26 (β = -2.26; 95% CI -3.12, -1.36) points lower cognitive function score compared to non-exposed groups. Prenatal famine exposure had 1.26 (β = -1.26; 95% CI -2.35, 0.94) points lower cognitive function score although not statistically significant.

Conclusion: Early life exposure to famine was associated with metabolic syndrome, risky anthropometric and dyslipidemic parameters and cognitive decline in adults. The results substantiated the theory of Developmental Origins of Adult Diseases (DOHAD) implying the need for promoting optimal nutrition during pregnancy and early postnatal life to prevent emerging epidemics of chronic non-communicable disease.

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Organic and Antibiotic-Free Chicken Meat: A Proteomic Approach

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Antibiotics have been extensively used in animal husbandry, mostly as growth promoting agents or for prophylactic purposes to reduce bacterial pathogens. Their continuous use in animal production raised concern associated with emergence of resistance in meat consumers [1]. In 2018, "antibiotic-free" meat attracted the attention of more than 43% of consumers, an always growing percentage [2]. Consequently, farmers adopted several strategies such as antibiotic-free and organic methods. Antibiotic-free animals are not treated with any kind of antibiotic, while organically raised animals cannot be fed with non-natural or synthetic substances, and cannot be treated with synthetic chemotherapeutic drugs, only phytotherapy and vaccinations are allowed. A deepening research of biomarkers to differentiate antibiotic-free, organic, and conventional meat is increasingly necessary [3].

Proteomic is a critical platform in nutrigenomics and can be used to identify, quantify, and characterize new dietary biomarkers and bioactive peptides [4].

The aim of this work is to compare the proteome of organically and antibiotic-free produced poultry meat. Proteomic analysis was performed on sarcoplasmic proteins combining two-dimensional electrophoresis for separation and high-resolution mass spectrometry (UHPLC-QTOF MS) for characterization. Preliminary results showed that most of identified proteins are glycolytic enzymes. PDQuest quantitative analysis underlines statistically significant differences in expression of specific proteins, such as pyruvate kinase, β -enolase, and creatine kinase. These results were compared with several recent studies which use the same proteomic approach. They underline how over or under expressions of specific proteins affect meat quality in terms of tenderness and color. [5-7] Further studies need to be performed to confirm these preliminary results, furthermore, the next step would be the study of membrane proteins using the same proteomic method.

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Role of *Saccharomyces Cerevisiae* Supplemented to Glial and Neural Cells

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Objectives: Baker's and brewer's yeasts (*Saccharomyces cerevisiae*) have been used as dietary supplements due to their high contents of vitamin, amino-acids and trace minerals. We tested whether or not the activation of different yeast metabolic pathways may be involved in the induction of growth and phenotypic as well as a functional switch in glial and neural cell lines following yeast addition.

Methods: We evaluated in specially-treated *Saccharomyces cerevisiae*, a probiotic, yeast-added cell cultures: i) yeast-related toxicity by Trypan Blue assay, ii) cytoskeletal reorganization by immunofluorescence, iii) anti-inflammatory activity counteracting LPS inflammatory stimulation by Real-Time PCR.

Results: No toxicity was observed up to the concentration of 10⁵ yeast added to 5x10³ cells. The shape of microglial cells remained unchanged after yeast addition; on the contrary, they became enlarged and round following liposaccharide (LPS) stimulation; but if the LPS treatment was performed on cells preincubated with specially-treated *Saccharomyces cerevisiae* the cells largely retained the morphology of untreated cells. To confirm the immune-system affecting activity of specially-treated

Saccharomyces cerevisiae, we evaluated the expression of iNOS and Arg-1, pro- and anti-inflammatory markers, respectively. We used yeast grown in different metabolic conditions to analyze a possible effect of differential metabolic yields: YP rich medium or SD synthetic defined w/o amino acids, supplemented with 2% glucose, which allows only fermentation or 3% glycerol, a respiratory carbon source. Microglia cells incubated with yeast grown in YP Gly 3% or SD Glu 2% were positive for Arginase in immunofluorescence experiments. iNOS staining was detected in microglia challenged with LPS, but that positivity decreased in cells treated in the presence of specially-treated *S. cerevisiae* grown in YP 3% GLY.

Conclusions: Much interest is focused on the relation between diet and epigenetic modifications. Our findings outline the immunomodulating activity exerted by specially-treated *S.s cerevisiae* added to microglia cultures, promoting the polarization towards a new phenotype, probably dependent on epigenetic mechanisms. Previous studies showed that SIR2 purified from *S. cerevisiae* deacetylates histones and several transcription factors, silences some genes located near telomeres and behaves as a pro-longevity factor, outlining the involvement of yeast derived factors in several epigenetic mechanisms.

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The Regulation of Central Metabolism by the Food Derived Bioactive Sulforaphane

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Objectives: Substantial epidemiological evidence has shown that diets rich in cruciferous vegetables are linked with a reduction of a wide range of diseases such as prostate and bladder cancer along with cardiovascular diseases. One promising food bioactive is Sulforaphane (SF), derived from sulphur-rich phytochemicals present in broccoli, which has been linked to many health benefits in model systems. Recently, evidence from human intervention studies, suggests that metabolic regulation by SF may play a big role in the observed protective effects, but the molecular pathways involved are not yet understood.

The aim of the study was to determine the molecular mechanisms by which SF regulates energy metabolism in the liver upon exposure to varying concentrations of glucose that represent different cellular metabolic states.

Methods: Established liver cells (HepG2) were treated with physiological concentrations of SF (10 µM) under varying glucose concentrations; No (0mM), Basal (5mM) and High Glucose (25mM). Real time energy production was assessed using the Seahorse Extracellular Flux Analyser. Whole transcriptome was obtained through RNAsequencing and Differentially Expressed Genes were obtained through EdgeR and limma, followed by Gene Set Enrichment Analysis. Quantification of metabolites involved

in Glutathione biosynthesis were determined through Liquid Chromatography and Mass Spectrometry (LC-MS).

Results: Data from the Seahorse Extracellular Flux Analyser have shown that SF changes metabolic fluxes to allow liver cells to adapt to varying levels of glucose. Transcriptome data analysed through the Gene Set Enrichment Analysis using the KEGG database reveal that most of metabolic changes are observed in the absence of glucose and at high glucose availability. SF promoted the antioxidant response by inducing Phase II detoxification genes (NQO1, GCLC, GCLM etc), in both the Basal and the high glucose environments. This effect was not observed in a no glucose environment. In a high glucose environment data, SF increased glutathione biosynthesis and upregulated genes involved in One Carbon Metabolism.

Conclusions: The results shed light to the mechanistic understanding of the dietary bioactive SF as a promising regulator of metabolic homeostasis.

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The Association of Dietary Calcium Intake and Serum Vitamin D with Leptin and Apelin Gene Expression from Visceral and Subcutaneous Adipose Tissue among Adults

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Objectives: The purpose of the study was to investigate the association of dietary calcium and vitamin D levels with leptin and apelin gene expression in visceral and subcutaneous adipose tissues in adults.

Methods: This cross-sectional study was conducted on 91 participants (n=40 non-obese and n=51 obese), aged ≥ 20 years, who underwent an abdominal open surgery with minimal impact on dietary intake. Visceral and subcutaneous adipose tissues were obtained during the surgery. Before the surgery, dietary calcium intakes were collected using a valid and reliable food-frequency questionnaire and fasting blood sample were gathered and then the 25(OH)vitamin D concentration was measured by means of the electrochemiluminescence assay. The leptin and apelin gene expression in visceral and subcutaneous adipose tissues was measured by Real-Time PCR.

Results: The mean age was 39.6 years and body mass index for obese and non-obese subjects were 35.3 and 25.6kg/m², respectively. Apelin gene expression was more increased in obese than non-obese participants in both subcutaneous (3.42 vs 0.49, $P<0.05$) and visceral adipose (3.65 vs 0.19, $P<0.05$) tissues; however, the leptin mRNA levels was equal. After adjustment for body mass index, total energy intake, and age, visceral adipose tissue apelin gene expression was associated with calcium intake ($\beta=-0.571$, $P=0.014$) and vitamin D concentrations ($\beta=-0.314$, $P=0.034$) in the total population. Among non-obese participants, calcium intake was associated with visceral adipose tissue apelin ($\beta=-0.617$, $p=0.008$) and leptin ($\beta=0.417$, $P=0.018$) gene expression. Leptin gene expression in subcutaneous adipose tissue was associated with serum vitamin D ($\beta=-0.481$, $P=0.016$) among obese participants. Moreover, among obese participants, we found a significant association between visceral adipose tissue leptin mRNA expression and serum vitamin D ($\beta=-0.353$, $P=0.40$).

Conclusions: Dietary intake of calcium and serum vitamin D were inversely associated with leptin and apelin gene expression in visceral and subcutaneous adipose tissue independent of body mass index.

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Characterization of Gut Microbiome in Type-1 Diabetic Children in Qatar Population

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The prevalence of type 1 diabetes mellitus (T1DM) and obesity in Qatar is one of the highest rates among few countries worldwide. Microbiome is a potential factor in NCD's pathogenesis; however, no study has examined its pattern in T1DM children in Qatar. Interestingly, some of the T1DM children are obese, unlike the majority. Hence, the aim of this study is to characterize peculiar gut microbiome profiles in T1DM and T1DM-obese children in Qatar. This study involves 120 paediatric subjects recruited from Sidra Medicine. Inclusion criteria are: 6-12 years old, no recent antibiotic treatment, no chronic diseases other than T1DM and obesity, and not newly diagnosed for T1DM. The participants are divided into 4-categories: healthy control (HC), T1DM, T1DM-Obese and Obese. Anthropometric parameters, clinical biomarkers, treatments, and 24-hrs dietary recalls are collected. The microbiome characterization is based on 16S rDNA sequencing on Illumina Miseq. QIIME 1.9.0 pipeline, R package, LefSe and Picrust are used for microbial analysis. Dietary analysis is performed using ePhood software. Statistical analysis is performed using R-package with two-sided p -value <0.05 .

We present here preliminary results of microbiome compositions in the four groups. The α -diversity is significantly lower in the 3 groups compared to HC ($p < 0.001$). LefSe showed peculiar microbiota profile in each group (particularly, T1DM – Lachnospiraceae, Collinsella, Blautia, Clostridium; T1DM-Obese – Klebsiella). Nutrients strongly correlated with specific bacteria in T1DM (Phosphorus – Bifidobacterium, Enterococcus, bifidobacteriaceae, Megaspheara; VitB1, VitB2, VitD – Clostridiaceae and Escherichia; soluble glucids – unclassified Peptococcaceae).

The relative abundance of Bacteroidetes in T1DM is significantly higher (63.4%) while Firmicutes is significantly lower (29.2%) compared to the T1DM-Obese (29% and 55.2%).

We propose that dietary pattern is the culprit behind the taxonomy microbial differences between T1DM and T1DM-obese subjects.

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***In vitro* Fecal Fermentation of Broccolo di Torbole Ecotype (*Brassica Oleracea* var. *Botrytis*): Analysis of gut Microbiota Composition**

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Introduction and Objectives: Our diet can strongly influence gut microbiota (GM) composition, which is also connected with our metabolism and health. Particularly, gut bacteria are able to transform dietary compounds, especially the ones that escape human digestion becoming available for colonic bacterial fermentation, such as fiber and plant secondary metabolites. Several studies confirm health beneficial effects of phytochemical, a class of dietary molecules derived by plant: these compounds can be transformed by GM into the derivative metabolites with *in vivo* activity. Moreover, GM breaks down complex dietary fibers with production of short chain fatty acids and gas as main fermentation end products. The aim of this study is to analyze the effect of *in vitro* faecal fermentation of Broccolo di Torbole ecotype, rich in polyphenols, glucosinolates and fibers, on GM populations and metabolites production.

Methods: Fecal samples were collected from 5 donors (age between 20 and 50 years, no antibiotic treatment in the 3 months preceding the experiment), diluted 1/10 (wt/vol) in PBS and used as fermentation inoculum at 1% (wt/vol). *In vitro* anaerobic batch cultures fermentations were carried out at 37°C for 24 hours and at pH between 5.5 and 5.9, to simulate the proximal colon conditions. Samples were collected from each vessel at hour 0, 5, 10 and 24 for microbial 16SrRNA sequencing analysis and MS-based metabolite profiling. As fermentation substrates, inulin (positive control), cellulose (negative control) and Broccolo di Torbole (steamed-cooked leaves and fruit in equal proportion), were employed at 1% of the total fermentation volume after *in vitro* upper digestion.

Results: Fecal microbial α and β -diversity were significantly different ($p < 0.05$). Inulin gives the strongest bifidogenic effect,

with an increase in Actinobacteria. Broccolo di Torbole promotes the growth of Actinobacteria, giving also a strong increase of Bacteroidetes, whose growth is sustained throughout 24 hours of fermentations. The ratio Bacteroides/Firmicutes is significantly higher during all fermentation timepoints of broccoli compared to inulin.

Conclusions: Faecal fermentation of a local Trentino ecotype of *Brassica oleracea* showed to modulate gut microbial composition over time. Broccolo di Torbole promotes Bacteroidetes growth, probably thanks to the availability of substrate and complex carbohydrates. All these changes will be related to production of *Brassicaceae*-derived microbial metabolites in fermentation supernatants and to systemic metabolites produced after *in vivo* long-term clinical nutrition study in obese subjects.

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Organic Meat in Compostable Packaging Solution: A Preliminary Study

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Nowadays, intensification of farming, use of fertilizers and pesticides, and industrialization of food production scare many consumers. They tend to be more conservative in food than in any other field, so chemical safety of food products is probably the major concern of this period. Organic food was born on this line.

Organic farmers must respect strict rules for feeding, as they want to obtain meat and derivatives of the best quality, safeguarding animal welfare. Biologically raised animals cannot be fed with GMOs, non-natural or synthetic substances, and cannot be treated with synthetic chemotherapy drugs, while phytotherapy, vaccinations and homeopathy are allowed. The principle aim of this work is to combine organic meat from an eco-sustainable farming with an eco-friendly compostable packaging solutions. As preliminary study, we research in literature differences between organic and conventional meat. Although the current interest topic, out of pesticides area, there are not many studies about that. Some of them compare organic meat to the corresponding conventional products, but, they consider the overall production system; thus, we cannot separate effects of single factors, such as rearing system, diet or genetic type. Consumers' safety perception of organic products support the growth of the organic food market. However, organic livestock production is not designed to reduce pathogen loads in food animals. [1] Residues of veterinary drugs represent the main difference between organic and conventional meat. [2]

A deepening of research of chemical tracer markers that differentiate organic and conventional meat is increasingly necessary. To achieve this, we are working on developing an UHPLC-QTOFMS analytical method to carry out an untargeted screening to identify unknown compounds that can differentiate organic meat from conventional one, supported by metabolomics and proteomics insights. Acquisition of these informations allows designing some functional biopackaging prototype, then tested to choose the best for organic meat, using completely reusable and recyclable materials respecting the environment.

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A longitudinal Study to Determine the Effect of Gene-Based Personalised Diet and Physical Activity Advice on Healthy Eating Motivation in University Students

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The prevalence of obesity continues to rise and is associated with an increased risk of chronic disease. The transition to higher education and subsequent years at university is a period of risk for weight gain. Weight loss is difficult to maintain therefore prevention rather than treatment of obesity is a more favourable approach. A gene-based personalised approach to dietary recommendations may motivate individuals to maintain or adopt positive dietary behaviours.

Objectives: To determine the efficacy of genetics based personalised dietary and physical activity advice on healthy eating motivation in university students.

Methods: First year undergraduate students at St Mary's University ($n = 153$), aged 18–25 years, were recruited to take part in the study. Baseline measures (participant characteristics, height, weight, body mass index (BMI), waist circumference (WC), body fat percentage (BF%), healthy eating motivation and physical activity) were collected during September and October 2019. Participants were genotyped for a SNP in the *FTO* gene (rs99396090), stratified randomisation (based on genotype and BMI) was used to allocate participants to three different groups (1. *Control*: no advice, 2. *Non-genetic personalised advice*: dietary and physical activity advice based on BMI and reported physical activity 3. *Genotype-based personalised advice*: dietary and physical activity advice based on genotype, BMI and reported physical activity). Participants allocated to groups 2 and 3 were provided with appropriate dietary and physical activity advice via email and all participants were asked to complete the healthy eating motivation questionnaire for a second time.

Results: Following non-genetic personalised advice participants informed of a BMI or body fat percentage above recommendations reported an increased in the importance of the food they eat to help control their weight ($p < 0.05$). This was not observed in participants following genotype-based personalised advice or no advice.

Conclusion: Personalised advice appears to increase importance of food in the control of body weight in young adults who are overweight or obese. Provision of more targeted personalised health advice for young adults starting university may increase their motivation to adopt or maintain healthy eating behaviours

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An Emerging Vegetable, (*Crithmum maritimum* L.), as a Source of Nutraceuticals: Extraction, Purification and Chemical Characterization of Polar Extracts

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Objectives: *Crithmum maritimum* L., sea fennel or rock samphire, is an emerging and promising vegetable crop. It is consumed and appreciated, especially in the Mediterranean area, for its aromatic traits. Moreover, this plant has been used in the folk medicine as antiscorbutic, tonic, carminative, diuretic, depurative and vermifuge^[1] and recent studies have demonstrated being rich in bioactive compounds such as vitamin C, ω -3 and ω -6 fatty acids, iodine, carotenoids, minerals, organic acids and phenolics^[2]. The aim of this study was the qualitative-quantitative chemical characterization of *C. maritimum* extracts.

Methods: *C. maritimum* aerial parts were dried, ground and extracted by percolation with ethanol 70%. Then the extract has been purified using an Amberlite® XAD7HP sorbent resin. The identification and quantification of phytochemicals have been performed using HPLC-DAD-MS/MS system.

Results: The extract was mainly composed of phenolic compounds, among which the two main classes were hydroxycinnamic acids and flavonoids. Among the former chlorogenic acids were the most abundant; they were mostly represented by 5-O-caffeoylquinic acid (32.04–166.21 mg g⁻¹), 3,5-di-O-caffeoylquinic acid (8.44–38.86 mg g⁻¹) and 4,5-di-O-caffeoylquinic acid (5.70–25.85 mg g⁻¹). Among flavonoids rutin (1.60–4.33 mg g⁻¹) and kaempferol-3-O-rhamnoside (0.07–0.33 mg g⁻¹) were the main constituents.

Conclusions: This study may improve the use of this plant as a new source of antioxidant compounds^[3].

This work shed light on the potential of *C. maritimum* to be used as a functional food or in nutraceuticals as a source of antioxidant compounds^[3].

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Dietary Fiber Intake May Influence the Associations between FTO Genetic Variants and Obesity-Related Parameters

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Objectives: Genome-wide association studies (GWAS) have identified the fat mass and obesity-associated (FTO) gene as a gene with one of the strongest significant correlation with obesity. Environmental factors such as dietary fiber may influence the associations between genetic risk and obesity development. However, dietary fiber appears to be significantly associated with a lower risk of developing hypertension, diabetes and obesity, the associations between FTO single nucleotide polymorphisms and dietary patterns need further investigation. The aim of the study was to evaluate whether dietary fiber intake could modify the association between some common genetic variants of the FTO gene and obesity.

Methods: From the study conducted among 1549 Caucasian volunteers of Polish origin, genotyped for the FTO SNPs (rs3751812, rs8050136, and rs9939609), 819 subjects were selected for gene–diet interaction analysis. We performed anthropometric measurements, total body fat content and distribution, oral glucose tolerance test (OGTT), and lipid profile. Dietary fiber intake was analyzed based on the three-day food records, and daily physical activity levels were evaluated using the International Physical Activity Questionnaire Long Form (IPAQ-LF).

Results: We observed that carriers of the GG genotype of rs3751812 presented lower hip circumference (GG vs GT, $p=0.029$) and higher total cholesterol (GG vs GT, $p=0.017$) and LDL levels (GG vs GT, $p=0.012$), when subjects were stratified to the high dietary fiber intake quantiles (≥ 18 g). Similar results were observed for rs8050136 CC genotype carriers. Moreover, we noted that carriers of TT and AA of both of the mentioned above loci, respectively, presented lower visceral fat content (AA vs AC, $p=0.015$), when subjects were stratified to the high dietary fiber intake quantiles. Additionally, we observed lower corrected insulin response (CIR) at 120 minute of the OGTT test (GG vs GT, $p=0.019$ and CC vs AC, $p=0.019$), in subjects stratified to the low dietary fiber intake quantiles.

Conclusions: Findings from our study provide new insights into the role of the interactions between daily fiber intake and selected FTO SNPs. These observations are very intriguing, especially in the current interest in dietary fiber, antioxidants and other

dietary factors. The newly published advances in this field bring us closer to the development of genome-customized healthy diet recommendations for prevention and treatment of chronic diseases.

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miR-29a Affects the Progression of Alzheimer's Disease through One Carbon-Metabolism

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Objectives: The metabolic Homocysteine (Hcy) pathway, defined as "one-carbon metabolism", involves the folate cycle, the transsulfuration pathway and the methionine cycle, leading to the production of S-adenosylmethionine (SAM), the main biological methyl donor in transmethylation reactions, and is modulated by B vitamins. microRNAs (miRNAs), considered emerging epigenetic factors and linked to DNA methylation, are associated to several disease. Therefore, we decided to test whether the modulation of one-carbon metabolism, induced by B-vitamin deficiency and SAM-supplementation, could causes relevant changes in the expression of miRNAs.

Methods: miRNAs expression has been assessed by total RNA extraction, miRNAs specific retrotranscription and Real-time PCR. miR-29a was selected for its involvement in methylation processes and Alzheimer's Disease (AD) after a preliminary screening in human SK-N-BE neuroblastoma cells, cultured in control and B-deficient medium with or without SAM-supplementation. miR-29a was also analyzed in mice (under the described B-deficient and SAM-supplemented conditions) and healthy and AD patients brain samples. Then we studied in vitro the effects of miR-29a silencing/overexpression by assessing its specific targets as BACE1 (involved in AD) and TET1 (involved in DNA demethylation).

Results: miR-29a was up-regulated at a ratio over 2-fold in SAM-supplemented versus control cells, showing an expression pattern counterintuitive with a methylation-mediated regulation: it was down-regulated in B-deficiency (hypomethylation) and up-regulated with SAM (hypermethylation) both in cells and in mice brain. miR-29a was also repressed in post-mortem AD brains. When silenced or overexpressed, miR-29a affects some putative target genes involved in AD and methylation metabolism.

Conclusions: These observations support that some miRNAs are regulated by DNA methylation both directly and indirectly, confirming that one-carbon metabolism can interfere with the pathological processes associated to AD through mechanism not limited to gene-specific modulation. Silencing and over-expression experiments have the purpose to evaluate the effects of the miR-29a in AD model, claiming for using it as a biomarker. Moreover, we would propose miR-29a as novel therapeutic target in addressing nutritional-based interventions.

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Glucocorticoid Signaling Alterations Induced by Late-Onset Dietary Restriction Aggravate Metabolic Inflammation in the Liver of Old Wistar Rats

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Objectives: Dietary restriction (DR) is the approach often used to delay the development of age-related disorders. One of the unresolved questions is how late beginning and short duration of DR affects disturbed metabolic balance caused by ageing. Glucocorticoid hormones have significant role in the regulation of energy metabolism and inflammation, especially during ageing when their systemic concentration arise. The aim of this study was to examine the impact of glucocorticoid signaling alterations induced by the late-onset DR on metabolic inflammation in the liver of old Wistar rats.

Methods: The experiments were conducted on 6- and 24-month-old male Wistar rats on *ad libitum* diet and 24-month-old animals on restrictive diet (60% of *ad libitum* daily intake) from 21st to 24th month (late-onset DR). The gene expression of proinflammatory cytokines was measured by qPCR, while protein levels of nuclear factor κ B (NF κ B) and antioxidant enzymes were determined by Western blot. Glucocorticoid signaling was analyzed at the level of glucocorticoid prereceptor metabolism and subcellular distribution of glucocorticoid receptor (GR). Liver corticosterone concentration was measured by ELISA.

Results: Decreased levels of antioxidant enzymes observed during ageing were accompanied with augmented inflammation, characterized by increased nuclear NF κ B protein level and higher expression of Toll like receptor 4 and TNF α . Corticosterone concentration in the liver of old rats was increased despite unchanged level of proteins involved in glucocorticoid prereceptor metabolism. Late-onset DR reduced adipose tissue and liver mass of old animals, and further stimulated inflammation in the liver. Decreased level of hepatic corticosterone after DR was a consequence of increased expression of 5 α -reductase which was in agreement with the decreased GR protein level in the nuclear fraction.

Conclusion: Late-onset DR did not improve expression of antioxidant enzymes and led to progression of age-related inflammation in the liver. This was accompanied with decreased levels of corticosterone and GR in the nucleus implying that late-onset DR aggravates inflammatory response through decreased glucocorticoid signaling in the liver of old rats.

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Impact of Cocoa/Methylxanthines Supplementation on Liver Glutathione Level in Aged Mice

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Objectives: Redox imbalance is one of the main features that characterize aging process, and strongly affects human metabolism. It has been confirmed that age-initiated reactive oxygen species formation and particularly increased liver sensitivity to oxidative damage can lead to various diseases. The causes of such events are, among others, changes in enzyme activity and redox substrate concentration on the hepatocyte antioxidant protection. The goal of this study was to appraise cocoa/methylxanthines prevention of hepatocyte glutathione depletion in aged healthy C57BL/6 male mice.

Methods: Animals in intervention group were treated by six months supplementation with cocoa powder or methylxanthines at quantity equivalent to human daily cocoa powder dose of 7.3 g. The activity of liver antioxidant enzymes, glutathione peroxidase (GSH-Px) and glutathione reductase (GR), as well as the glutathione and GSH-Px protein content were measured in both the control and intervention group.

Results: Concerning GSH-Px activity, a slightly increase was observed in mice supplemented with methylxanthines compared to control and cocoa group, but statistically significant difference was absent. It is interesting that the same group had a significant increase in GSH-Px protein level. This finding indicates that hepatocytes regulate activity of this enzyme post-translationally, i.e. activity is not affected by increased protein level. Glutathione content and glutathione reductase activity were not altered due to mentioned dietary interventions.

Conclusions: The obtained results indicate that liver antioxidant enzymes are very complexly regulated on transcriptional, translational and post-translational levels, and it could be assumed that a certain post-translational modification appears reducing the synthesized GSH-Px protein activity.

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Nucleic Acids - Underrated Food Components

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One of the basic components of every cell are nucleic acids, which play key role in coding and synthesis of proteins as well as in regulation of many metabolic processes. Most research on

nucleic acids focuses on their genetic role. However, these compounds are also chemical components of food, the same as proteins, lipids or saccharides. Reports on the nutritional significance of nucleic acids are very limited and mainly concern supplementation of animals, GMO detection and the occurrence of regulatory miRNAs in milk, especially human milk. Although absorption and metabolic processes of these food components are partly known [1], there is still little information about the quantitative and qualitative composition of nucleic acids in food products and their nutritional role. The aim of this study was the preliminary characterization of nucleic acids found in food products of various origins. Animal (chicken leg, breast, liver), plant (green bean seeds, celery, kohlrabi, potato) and fungal (champignon) tissues were analyzed.

In first step, the paraffin block method, commonly used in histopathology research, was applied to prepare microscope slides from the tested food products. The prepared samples immersed in paraffin were cut into sections used to prepare microscope slides and then stained with different dyes. This approach enabled the visualization of nucleic acids in tissues *in situ*, observation of their location, size and quantity of cell nuclei in analyzed samples. In the next step, employing comet assay, a popular method of detection of genotoxicity of chemical compounds [2], was used to assess the level of DNA fragmentation in analyzed food samples. Cell nuclei extracted from the tested samples were suspended in agarose gel and then subjected to comet assay protocol. Analysis of obtained SybrGreen stained comets enabled the comparison of the levels of genetic material integrity between tissues of various origins.

The results of the study revealed significant differences in distribution, size and quantity of cell nuclei and nucleic acids integrity in tested samples. The level of degradation of nucleic acids in animal tissues was much higher than in plant tissues. Also, differences in chromatin integrity between tissues with different functions (liver compared to the leg and breast) and stage of development (bean seeds compared to potato flesh) were observed. The most extensive fragmentation of polynucleotides in meat samples may also suggest DNA damage and justifies further research on the interaction of this kind of modified molecules with gastrointestinal cells.

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Genetic Risk Score to Predict the Likelihood of Dental Caries in Finnish Adolescents

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Background: We have previously identified marginal differences in saliva microbiota between children with and without caries. Still, dental caries is a widely prevalent disease with an early onset. We hypothesize that dental caries is likely a multifactorial

disease involving both genetic and environmental factors. A recent meta-analysis of 33000 children (1), identified two genome-wide significant hits (in *ALLC* and *NEDD9*) for caries. Genetic variants may be more prominent in genetically homogenous cohorts with well-defined caries traits.

Objectives: We aim to incorporate known caries causing genetic variants into a genetic risk score (GRS) and investigate its association with caries in a homogenous cohort.

Material and Methods: We utilize the genetic and register data from the participants of the Finnish Health in Teens (Fin-HIT) cohort. Out of 11000 young participants, we have randomly chosen and genotyped approximately 1000 samples on Illumina 610Quad array. The genotyped data will be further imputed to generate up to 10 million variants. The GRS will be generated using PLINK v 1.9. Caries is defined with a sum of Decayed, Missing, and Filled Teeth (DMFT) obtained from the national register for primary healthcare visits.

Results: The mean (SD) age of children was 11.7 (0.4) years and 56% of participants were girls. The mean DMFT of the children was 0.86 (1.97) in permanent dentition, signifying relatively good dental health. We will derive and test a weighted genetic risk score for dental caries in permanent dentition in these participants.

Conclusions: GRS for caries will help identify children with an elevated risk of caries, which might have clinical relevance. Furthermore, we will verify our results with the longitudinal data and also test the association of GRS with other factors such as saliva microbiota and body mass index (BMI).

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Metabolomics - Based Machine Learning Method for the Discovery and Characterisation of Biomarkers Implicated in Non-Alcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. However, the diagnostic approaches for NAFLD detection is challenging due to the limited availability of non-invasive biomarkers. Metabolomics coupled to

machine learning can pave way to identify diagnostic biomarkers, understand disease mechanisms, and evaluate the treatment of various diseases.

Here, targeted metabolomics was performed by liquid chromatography – mass spectrometry on healthy adults and those with NAFLD. 6 machine learning approaches were applied to the metabolomics dataset – Artificial Neural Network (ANN), K-Nearest Neighbour (KNN), Logistic regression, Support Vector Machine (SVM), Decision tree and Ensemble. These were randomly split into training, validation and test sets, and included dimension reduction, feature selection, and classification model development. The accuracies of these 6 models were tested. ANN pattern recognition model has the highest area under the curve (AUC) in classifying the subjects with and without NAFLD.

The study demonstrates the potential of ANN for NAFLD metabolomics data classification in realistic situations. Further model development and independent validation testing in other cohorts are warranted.

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Effect of Extra Virgin Olive Oil Polyphenols Pattern on Anti-Inflammatory Activities: Analytical Aspects

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Objectives: The best example of a health-promoting dietary regimen is the Mediterranean Diet (MD) whose beneficial effects are especially attributed to the consume of the extra virgin olive oil (EVOO). Even if the healthful effects of EVOO are mainly ascribed to the major components (saponifiable fraction, in particular to the oleic acid), several recent papers reported an emerging role for the minor components (unsaponifiable fraction) too, especially hydrophilic phenolics. The anti-inflammatory activity of EVOO polyphenols in chronic inflammatory diseases is extensively demonstrated by several studies. However, only some of them associated their anti-inflammatory activity to the chemical composition. Therefore, the aim of this work is to study the analytical aspects of Extra virgin olive oil extracts polyphenolic pattern in order to correlate them on anti-inflammatory activities on of BMDCs (Bone Marrow-derived Dendritic Cells).

Methods: The characterization of the EVOO extracts was performed by HPLC-UV-MS/MS analyses which allowed to identify 32 phenolic compounds and the amounts of these compounds were successively obtained by MRM (Multiple Reaction Monitoring) experiments and expressed as µg/g of oil.

Results: Our data demonstrated a good correlation between the chemical characterization of EVOO extracts (Cima di Mola/Coratina and Casaliva extracts) and their biological functions in terms of anti-inflammatory activity. Based on the gathered findings, a group of polyphenols (including some secoiridoids, lignans and flavonoids) in the EVOO extracts seemed to be synergistically able to modulate the maturational process of BMDCs toward an anti-inflammatory profile after LPS stimulation.

Conclusions: In conclusion even if the quantity of polyphenols is extremely important to dictate the beneficial effects for human health, as also indicated by the European Food Safety Authority (EFSA) Health Claim for the anti-oxidative effect of EVOO polyphenols (EFSA, 2011), a specific EVOO polyphenols combination could be crucial to induce the biological effects.

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Maternal and Paternal Dietary Quality, Dietary Inflammation Status, and Offspring DNA Methylation

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Objectives: Maternal diet and chronic inflammation may influence early-life offspring health. Recent observations indicate that early nutritional exposures may contribute to epigenetic modifications in the progeny. However, few studies have evaluated the effect of parental dietary quality on offspring DNA methylation. We aim to fill this gap by elucidating the influence of whole-diet maternal and paternal dietary quality and inflammatory potential on DNA methylation in their children at nine years in the Lifeways Cross-generation cohort.

Methods: Families were recruited around 16 weeks of gestation in the Republic of Ireland between 2001 and 2003. Maternal dietary intake during the first trimester and paternal diet of the 12 previous months were assessed with a food-frequency questionnaire. We used the healthy eating index (HEI-2015), a measure of overall diet quality, by scoring adherence to dietary guidelines and the energy-adjusted dietary inflammation index (E-DII). DNA methylation in saliva samples of 264 children was assessed using the Illumina Infinium HumanMethylationEPIC (EPIC array). Using an agnostic epigenome-wide approach we examined associations of each dietary score with methylation β-values of each CpG site, including child sex, batch effect, smoking, cellular composition as covariates.

Results: After adjusting for covariates and multiple tests, maternal HEI-2015 scores were inversely associated with DNA methylation at 1 CpG site (cg21840035, p-value=5.5×10⁻⁸) located near the PLEKHM1 gene, whose functions involve regulation of bone development. An increase in paternal HEI score was related to lower methylation at one CpG site (cg22431767, p-value=4.1×10⁻⁸) located near cell signaling gene LUZP1. No significant associations between maternal or paternal E-DII and DNA methylation at individual CpG sites were observed.

Conclusions: We report, for the first time, that parental dietary quality, determined by the HEI-2015 score, in the prenatal period was associated with long-term epigenetic changes in their children. Our results encourage further research in larger populations with

different dietary intake and investigation of the functional implications of the genes involved to understand how they may affect offspring health.

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Influence of Renal Disorder and Dietary Factors on the Incidence of Caries in Children

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Objectives: The common health status of child's organism, respectively established disturbance of the functionality of some organs and systems, has to be taken into consideration with its reflections upon the multi-aspect characteristics of oral-dental state. Not to neglect the correlation between excretory system disorders and individual dynamics of oral health. For the purpose of optimization of child's oral-dental health is accentuated on the role and impact of the disease pyelonephritis and dietary regime characteristics in childhood. In the context of our investigation have taken part 28 children with diagnosed pyelonephritis and 9 healthy controls of child's age.

Methods: Sociological, statistical and clinical methods.

Results: Regarding the consumption of carbohydrates, especially the intake of sugar-containing foods and drinks, among the investigated 28 children with diagnosed pyelonephritis 15 of them are characterized with incessant sugars' intake. The other 13 children consume fermentable carbohydrates containing foods only as a desert. The group of children characterized with incessant sugars' intake have 1.74 times higher number of reversible carious lesions of D1a-D1b and 1.85 times higher number of irreversible carious lesions of D2-D4 compared to the other group. The mean value of pH for the group with incessant sugars' intake also is lower- 6.3 as a compared with the group with moderate carbohydrate consumption - 6.46. Concerning proteins' nutrition the group of children with pyelonephritis are characterized with limited intake in comparison to these without common health disorders (control group of 9 healthy children). The frequency of distribution of irreversible carious lesions (D2-D4) among children suffering from pyelonephritis is 2.95 times higher as a compared to the control group. Among the investigated group of children suffering from pyelonephritis (n=28) 82.14 % of them have irreversible carious lesions. In comparison among the control group 44.44 % have irreversible carious lesions.

Conclusions: Factors related to caries initiation and progression are the dietary factors and food choices, the common health status, oral hygiene and frequency of dental visits. In our

investigation we demonstrate the considerable impact of the common health status and the frequency of consumption of sugar-containing foods and drinks for caries prevalence in childhood. Low proteins' nutrition especially during the period of growth and development also has an impact upon the dynamics of the oral cavity and tooth structure. In the present study the investigated group of children suffering from the renal disorder pyelonephritis showed higher susceptibility to caries in comparison to the control group of children with no common health disorders. Understanding the influence of the diet, eating behaviors and the impact of the common health status is necessary for the efficient control and management of the oral health in children.

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Elevated Intake of High Amylose Wheat Improves Conception Rates and Increases Placental Size but not Fetal Weight in Mice

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Background and Objective: High amylose wheat (HAW) contains several nutritional attributes with the potential to improve reproductive outcomes, however, the effect of consuming elevated levels of HAW on reproductive parameters has not been investigated. This study aimed to determine the effects of consuming a diet rich in HAW on reproductive and pregnancy outcomes in female mice.

Methods: Female C57BL/6 mice (n = 20/group) were fed diets containing either ~63% (w/w) HAW or ~63% (w/w) standard amylose wheat (SAW) for four weeks prior to mating until gestational day (GD) 17.5, at which time pregnancy outcomes were assessed.

Results: Mice consuming the HAW diet exhibited higher food intake and body weight prior to mating, but not during pregnancy, compared to those consuming the SAW diet (p < 0.01), and spent longer in estrus and less time in diestrus over the course of the estrous cycle (p < 0.05). A greater proportion of females in the HAW group achieved pregnancy post-coitus (94% vs 61%, p = 0.043). At GD17.5, dams consuming the HAW diet had higher placenta weights (p < 0.0001) and a lower fetal-placenta weight ratio (p < 0.0001) compared to the SAW groups. The ratio of male:female fetuses was higher (61% vs 47%, p = 0.045) in the HAW group, however, the male fetuses in dams consuming the HAW diet were shorter and lighter compared to the SAW group.

Conclusion: While the observed improvement in progression from mating to pregnancy indicates improved reproductive success, further studies are required to investigate the short- and long-term impacts of maternal HAW consumption on the offspring.

Keywords: High amylose wheat, female, reproductive health, fertility, pregnancy

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Adolescents' Stress Reactivity and Emotional Eating during a Lab Stressor: Differences Depending on Chronic Stress and Overweight

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Introduction: The persistent coexistence of stress and paediatric obesity involves interrelated physiological mechanisms, which are believed to function as a vicious circle. Here, a key role is assigned to stress responsiveness and eating behaviour. This study investigated the difference in stress responsiveness and emotional eating among adolescents varying in weight and chronic stress levels. We believe that adolescents with elevated chronic stress and overweight are expected to express a stronger stress reactivity and emotional eating in response to an acute lab stressor. Additionally, we expect that high stress responsiveness and trait emotional eating directly or as moderator explain increased state emotional eating.

Methods: Adolescents [n=141, 50.4% boys, 6-18y] were recruited (from the general population or obesity treating hospital) and underwent the Trier Social Stress Test for children. Four BMI/chronic stress (based on hair cortisol, Perceived Stress Scale, Children's Depression Inventory CDI-2) groups were compared in stress responsiveness (salivary cortisol and alpha-amylase, heart rate variability, state emotions, self-reported stress), trait emotional eating and state emotional eating (intake of snacks after stressor: savoury to sweet and low to high fat). Repeated-measures-ANOVA, linear regression and moderation analyses were adjusted for age, sex, parental education, hunger and snack liking.

Results: The stress-induction was successful (time effect $p \leq 0.05$) and it increased food wanting. The group with overweight and high stress had significantly stronger relative salivary cortisol reactivity (than the normal weight groups), a weaker happiness recovery (i.e. further happiness decrease, compared to normal-weight&low-stress group), and higher fat&sweet food intake (g and kcal, compared to normal weight groups). Indeed, a significant association was seen between fat&sweet food and BMI z-score

($\beta=0.22$). Chronic stress level was positively associated with trait (but not state) emotional eating ($\beta=0.16$). Stress responsiveness was related to more state emotional eating: strong cortisol reactivity was linked to fat&sweet ($\beta=0.22$) and weak autonomic system recovery to high total and fat&sweet food intake ($\beta=0.2-0.3$). Moderation analyses further suggested that high stress reactivity and worse stress recovery might sometimes trigger more/unhealthier food intake after stress in at-risk groups.

Discussion: This research confirms that a combination of chronic stress and overweight increases stress vulnerability and highly palatable food intake after stress. Worse stress responsiveness seems to stimulate unhealthy/emotional eating. Hence, stress responsiveness and emotional eating might be targets to prevent stress-induced overweight.

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Crocus Sativus Bioactive Properties and Modulation of Glycol-Oxidative Stress

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Objectives: Plant secondary metabolites have been a source for many of our medicines. The major classes of plant phytochemicals are: phenolics, terpenoids, alkaloids. Previous studies have demonstrated that dietary phytochemicals exert bioactive properties and act on the human genome, either directly or indirectly, to alter specific gene expression, thereby influencing molecular mechanisms involved in development of human diseases. The effects of phytochemicals on gene expression in different tissues and cells have been of intensive research to elucidate mechanisms and novel targets of therapeutic nutrients. Diabetes is associated with glyco-oxidative stress due to hyperglycemia. Some polyphenolic phytochemicals influence expression of genes relevant for the development of type 2 diabetes, such as genes regulating glucose transport, antioxidant enzymes and inflammatory pathways. Among components of the cell signaling network implicated in pathogenesis of diabetes and many inflammation-associated disorders there are the redox-sensitive transcription factor nuclear factor-kappaB (NF- κ B), protein kinase B (Akt) and Sirtuins. Aim of the study was to investigate the modulatory roles of *Crocus sativus* phytochemicals on glycooxidative stress and the cell signaling network implicated in pathogenesis of diabetes and inflammation-associated human diseases.

Methods: The study was carried out in Adenocarcinoma Colon Cancer cells (Caco2) left to differentiate in absorptive cells in Transwell plate. The bioactive properties were studied in untreated cells and in cells incubated with methylglyoxal (MGO) as glyco-oxidative agent.

Results: Antioxidant activity of *Crocus sativus* phytochemicals was evaluated *in vitro* by ORAC and DPPH assays. A significant inhibition of the activity of enzymes involved in dietary carbohydrate (amylase, glucosidase) has been observed. Moreover, *Crocus*

sativus phytochemicals inhibited glucose uptake in differentiated intestinal cells. The effect was correlated to an inhibition of the expression of Glucose transporter 2 (GLUT2) and *Sodium-GLucose Transporter* (SGLT1). *Crocus sativus* polyphenols were also able to protect cells from glyco-oxidative stress induced by MGO. In fact, lower levels of advanced glycation end products (AGEs) and lower levels of intracellular reactive oxygen species were observed in cells incubated with MGO and *Crocus sativus* polyphenols. The protective effect was associated to a significant modulation of the expression of genetic markers involved in inflammatory pathways (caspase-3, NFκB, Akt and SIRT-1).

Conclusions: We confirm that *Crocus sativus* polyphenols exert antioxidant properties and anti-diabetic properties. Our results demonstrate also that *Crocus sativus* polyphenols modulate pro-inflammatory genes expression during glyco-oxidative stress. Our results suggest a potential use of *Crocus sativus* polyphenols as future functional ingredients either in food and pharmaceutical industry.

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Long-Term Differential Effects of Procyanidins on CCK Secretion in Young versus 21-Month-Old Female Rats under Cafeteria Diet

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The grape seed-derived procyanidin extract (GSPE) have satiating properties in part mediated through modulation of the anorexigenic enterohormone GLP1 and the orexigenic enterohormone ghrelin. Furthermore, in female rats fed a cafeteria diet GSPE treatment induces long-term effects on GLP-1 system. The GSPE effects on another key enterohormone that regulates food intake, CCK, are less clear. Using duodenal explants, we previously showed that GSPE acutely inhibits CCK secretion. To find out whether GSPE has long term effects on CCK secretion, we treated 2-month-old (young) and 24-month-old (aged) female rats with 500 mg GSPE/kg body weight for ten-days, followed by a cafeteria diet for 13 weeks to stimulate their food intake. Vehicle-treated controls and standard chow-fed controls were performed in parallel. Then, we obtained duodenal explants to measure their ability to secrete CCK under basal (non-stimulated) conditions and after peptone stimulation (50 mg/ml). Plasma CCK was also measured.

Our results revealed that ageing does not change basal CCK secretion, while GSPE-treated aged rats showed reduced CCK secretion thirteen weeks after having finished the treatment. In young rats, cafeteria feeding increased basal CCK secretion,

resulting in higher plasma levels. A GSPE treatment for 10 days previously to the cafeteria impeded this increase in CCK. In aged rats the effects differed, since cafeteria fed controls but not cafeteria fed GSPE-pre-treated animals showed reduced basal CCK secretion.

As CCK is a hormone released in response to food intake, we used a meat peptone to stimulate CCK secretion in the duodenal explants. Aged rats showed an increased CCK secretion after peptone compared to young animals. Pre-treatment with GSPE maintained this higher stimulated-CCK levels. Cafeteria feeding in young and aged rats did not affect peptone stimulation, as did not GSPE-pre-treatment.

Then we can conclude that basal but not stimulated CCK secretion is sensitive to cafeteria diet. Our results also point out a preventive effect of GSPE towards the effects of a cafeteria diet on CCK both in the young group and in the aged group.

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Grape-Seed Proanthocyanidin Extract Reverts Obesity-Related Metabolic Derangements in Aged Female Rats

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Obesity and ageing are current issues of global concern. Adaptive homeostasis is compromised in the elderly, who are more likely to suffer age-related health issues such as obesity, the metabolic syndrome and cardiovascular disease. The current worldwide prevalence of obesity and higher life expectancy call for new strategies for treating metabolic disorders.

Grape-seed proanthocyanidin extract (GSPE) is reported to be effective in ameliorating these pathologies, especially in young animal models. In this study we aim to test the effectiveness of GSPE in modulating obesity-related pathologies in aged rats fed an obesogenic diet. To do so, 21-month-old rats were fed a high-fat/high-sucrose diet (cafeteria diet) for 11 weeks. Two time points for GSPE administration (500 mg/kg body weight), i.e. a 10-day preventive GSPE treatment prior to cafeteria diet intervention and a simultaneous GSPE treatment with the cafeteria diet, were assayed.

Body weight, metabolic parameters, liver steatosis and systemic inflammation were analysed. GSPE administered simultaneously with the cafeteria diet was effective in reducing body weight, total adiposity and liver steatosis. However, the preventive treatment was effective in reducing only mesenteric adiposity in these obese aged rats. Our results confirm that the simultaneous administration of GSPE improves metabolic disruptions caused by the cafeteria diet also in aged rats.

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Live Biotherapeutic Probiotics Human Microbiomic: Machine Learning In silico Clinical Trials. Novel Practical Approach to Era of Personalized Probiotics Medicine

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Nutritional choices that are appropriate for one particular individual can't be healthy for another and we must understand the best food preferences for the ordinary person.

These Variation from person to person depend on their genomes, influencing the digestion, absorption and use of nutrients in the body. But the degree of difference between people goes beyond that. All of us have a common intestinal flora, known as the intestinal microbiota. Sometimes this is also named our second genomic.

Our microbiome, which collects 100 trillion germs and the microbes in the gut, skin, mouth and other body locations, is a major source of variations across people. The composition and function of everyone are special to both the microbiota, impacting our diet and influencing our reaction to nutrients. Throughout our metabolism and health this very diversity of ecological system plays a key role. It provides us, on the one side, many beneficial roles including the production of vital nutrients and vitamins, defense against bacteria that enter the body and cause infectious diseases and control of immunity and metabolism.

Changes in our nutritional human gutome microbiomic, on the other hand, can lead to numerous illnesses including obesity, diabetes, inflammatory conditions, digestive diseases, neurological disorders, and even cancer. The academic community is conscious of the prolonged state of microbial disequilibrium or diminished in the human gutome microbiomic, also called dysbacteriosis. probiotics novel approach and a promising strategy for restoring the microbiomic dysbacteriosis in the humane gutome. Probiotics have remarkable prospective in individualized diet and medication for improvement healthy nutrition. Probiotics are generally defined as human microbiota or mycobacteria isolates only that, when administered in - should be potent live forms, non-toxins, nonpathogenic, single pure strain or mixed strains, resistance to bile acids, Resistance to gastric acidity, have highly Competitive exclusion of pathogen binding, with Antimicrobial activity against potentially pathogenic bacteria with adequate amounts consumed. regularly affirm a medical advantage on people. There is impressive enthusiasm for probiotics for an range of diseases, such as gastrointestinal and non- gastrointestinal medical conditions and a vast number of individuals around the globe expend probiotics day by day for cut medical advantages. Clinical studies demonstrating that probiotics consumption regarding gastrointestinal health, acute diarrhea, pouchitis, cancer, atopic skin inflammation in kids, constipation, immunomodulation, *Helicobacter pylori*, liver disease and genito-urinary system diseases have been documented. Probiotics as biological medications for human beings selection and medicating are not the same in all conditions and the advantageous influences of each probiotic

strain should be personalized. It also proposes innovative understandings for a figure of valuable applications of probiotics as new bio-preparation probiotics design and advances in development of novel "biological drugs" probiotic-based managements and perceptive, protective, and personalized diets, as well as application of sophisticated Artificial intelligence system for gut molding "Insilco clinical trials" and deep imaging processing biocomputing techniques that can be approved in the near future by pioneering therapeutic professionals, justifying supplementary investigation and applied interpretation. Through increasing concern with the use of artificial intelligence (AI) and machine learning, how technology can have an effect on microbiome exploration in the future, in overcoming problems of big data. It's now very easy to sequence microbiome samples. We have enormous data if we merge that with a host's genetic composition, of course. Planning to look to integrate microbiome and genetic data, including eating habits and nutritional personal information with other parameters," So the data output is outrageous. "Deep learning and machine intelligence could, therefore, motivate others to use and make perfect sense of this knowledge and to find new interesting things." AI and natural language processing will become a major part of the microbiome's evolution' absolutely' The business sector would also have to incorporate natural language processing technology to help create personalized datasets and tips to create personalization of healthcare or diet. Such implementations represent many problems in genomics and in the field of machine learning, from demanding tension resolution in community databases to integrating large datasets for predictive machine learning. In this section, we give an insight into these issues by focusing on several technological fields and addressing advancement throughout microbiome research, personalized probiotics medicine approach and the potential developments in genomics and data analytics.

Keywords: Artificial intelligence system; Bio-computing; Deep imaging processing; Human Microbiota; Probiotic.

Assessment of the Relationship between Protein Consumption and Development of Glucose Metabolism Disturbances in Carriers of Some Common Single Nucleotide Polymorphisms in Gene BDNF (rs10835211)

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Objectives: The aim of this study was to estimate the association between BDNF single nucleotide polymorphisms (SNPs), dietary protein intake, and the risk of glucose metabolism disturbances.

Methods: For this analysis, 490 participants from 1000PLUS Cohort Study were included. We selected 4 BDNF polymorphisms: rs6265, rs4923461, rs10501087, rs10835211, which we found to be in strong linkage disequilibrium, therefore we presented the results

for one of them- rs10835211. The blood glucose and insulin concentrations during an Oral Glucose Tolerance Test (OGTT) were analysed, as well as anthropometric measurements, body weight, total body fat content and body fat distribution analysis (subcutaneous and visceral fat content). HOMA-IR index was calculated from the formula: fasting glucose [mmol / L] $\times$ fasting insulin concentration [μ IU / ml] / 22.5. The SNPs were genotyped with TaqMan SNP technology from a ready-to-use human assay-library using a high-throughput genotyping system. Daily physical activity levels were evaluated using the International Physical Activity Questionnaire Long Daily macronutrient intake was analyzed based on three-day food diary.

Results: Our results show that carriers of GG genotype of rs10835211 had higher waist circumference ($p=0.021$), percentage of body fat content ($p=0.00025$), subcutaneous ($p=0.007$) and visceral fat ($p=0.013$) content, and BMI ($p=0.0016$), if more than 18% of daily energy intake was derived from proteins. We also observed higher fasting glucose ($p=0.048$), and insulin concentrations ($p=0.0016$), which resulted in higher HOMA-IR ($p=0.00084$). In homozygous AA and heterozygous AG genotypes carriers we did not observe these associations between protein intake and analyzed parameters.

Conclusions: Protein consumption above 18% of the daily energy requirement may be associated with higher BMI and body fat content, as well as with an increased risk of glucose metabolism disturbances in carriers of the GG genotype of rs10835211 BDNF gene.

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Caffeine Exposure and Folate Deficiency *in vitro* can Influence Chromosomal Translocation Events Associated with Childhood Leukaemia

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Leukaemia accounts for a third of all childhood cancers, with many thought to originate from chromosomal rearrangements *in utero*. Various abnormalities, including chromosomal translocations, have been retrospectively detected at birth. Although survival rates are increasing, the incidence of childhood leukaemia has also been increasing each year. Epidemiological studies have identified various environmental factors associated with increased risk of childhood leukaemia including maternal nutrition, smoking and air pollution. It is plausible these exposures *in utero* and early childhood could trigger initiating translocations. Maternal nutrition such as caffeine intake and folate deficiency have been shown to influence adverse pregnancy outcomes, and *in vitro* exposure has been shown to affect DNA repair and cellular processes.

We aim to investigate if environmental exposures, such as caffeine and folate deficiency, associated with an increased risk of childhood leukaemia, may trigger the induction of translocations associated with childhood leukaemia.

To identify the optimal conditions for increasing susceptibility to translocations, the leukaemic cell line NALM6 was exposed to physiological levels of caffeine and folate deficiency. Cells were exposed to caffeine for 48 and 96 hours or grown in folate deficient media for 96 hours, before extracting RNA. Reverse transcription PCR assays developed to detect the most common childhood leukaemia associated translocations were used to identify translocation events.

Translocation events were seen at medium (10 μ M) levels of caffeine for 48 hours and very high levels of caffeine (80 μ M) for 96 hours. Induction of translocation events also occurred with depleted (1nM) and physiologically normal (10nM) levels of folate over 96 hours.

This data suggests environmental factors associated with childhood leukaemia may induce initiating translocations, suggesting a biologically plausible mechanism for epidemiological associations. With incidence rates rising, limiting these exposures could reduce translocations with the aim to preventing childhood leukaemia.

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Extra Virgin Olive Oil Extracts Dampen the Inflammatory Response by Imprinting an Anti-Inflammatory Profile to Murine Dendritic Cells

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Objectives: Extra virgin olive oil (EVOO) represents one of the most important health-promoting food whose antioxidant and anti-inflammatory activities are mainly associated to its polyphenols content. To date, studies exploring the effect of EVOO polyphenols on dendritic cells (DCs), acting as a crosstalk between the innate and the adaptive immune response, are scanty. Therefore, the aim of this work is to study the ability of three EVOO extracts (cv. Coratina, Cima di Mola/Coratina, and Casaliva), characterized by different polyphenols amount, to regulate DCs maturation in resting condition or after an inflammatory stimulus.

Methods: The ability of the three EVOO extracts to reduce the inflammatory response was tested on bone marrow derived DCs (BMDCs) isolated from C57BL/6J mice (wild-type, WT). After differentiation, BMDCs maturation was studied in response to the treatment with 12.5 µg/ml of Coratina, Cima di Mola/Coratina, and Casaliva extracts using MetOH as a control, before the stimulation with 1 µg/ml of lipopolysaccharide (LPS) for 24 hours. ELISA, cytofluorimetric analysis, and Real-time PCR were used to study BMDCs modulation at protein and molecular level.

Results: Cima di Mola/Coratina and Casaliva extracts were demonstrated the most effective to modulate BMDCs toward an anti-inflammatory profile by a reduction of TNF and IL-6 secretion and CD86 protein expression, along with a down-modulation of *IL-1β* and *iNOS* molecular expression. Then, from factorial analysis results, especially 9 polyphenols were tentatively established to play a synergistic role in modulating BMDCs inflammatory ability thus reducing the risk of chronic inflammation.

Conclusions: Results obtained in this study may provide new insight on how a specific combination of EVOO bioactive compounds could dampen the inflammatory response reducing the risk of chronic inflammation that supports the development and the ever-increased incidence of noncommunicable diseases nowadays.

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Redox vs. Nutrigenomic Activity of Ascorbic Acid and Its Derivatives

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Vitamin C, besides being an antioxidant present in fruits and vegetables, is also commonly used as a food additive. The term 'vitamin C' describes a group of compounds that exhibit the anti-scorbutic activity of L-ascorbic acid, thus it includes its oxidized form (called dehydroascorbic acid), as well as its sodium and calcium salts. Even though the stereoisomers of L-ascorbic acid, that is D-ascorbic acid and its sodium salt lack vitamin C activity, they have also been successfully implemented in the food industry as antioxidants. The aim of this study was to determine the relationship between redox properties of 6 different derivatives of ascorbic acid using electrochemical, chemical and biological approaches.

Potentiometric titration and differential pulse voltammetry were applied to establish the electrochemical parameters of tested compounds. The ABTS and DPPH tests were used to determine the stoichiometric n_{10} values implying the kinetics of radical scavenging. The cellular antioxidant activity was assessed using the CAA test. Protection against H₂O₂-induced DNA damage was investigated by the comet assay. Modulation of redox-related genes upon treatment with ascorbic acid derivatives was performed using microarray technology (Qiagen). All the biological

tests were carried out on human colon adenocarcinoma HT29 cells, a representative cellular model of digestive tract.

The results of colorimetric tests and the CAA assay showed a strong correlation with the electrochemical parameters, as long as high concentrations of ascorbic acid derivatives were considered. Physiological concentrations of investigated compounds did not show any protection against oxidative-stress induced DNA damage. Among the studied compounds, the strongest antioxidant was calcium ascorbate (CaA). However, strong redox activity of CaA was not reflected by changes in the expression of redox-related genes. More prominent response on the genomic level was exerted by a plant derivative of vitamin C, ascorbigen, whose redox properties differed from other investigated compounds. Results of this study imply that despite similar antioxidant activity, tested compounds may exert different nutrigenomic effects. As long as the physiological range of concentrations is maintained, there is no simple relationship between the redox properties and biological activity of ascorbic acid and its derivatives.

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Exploring the Anticancer Effects of the Food Bioactive Erucin: Focus on Cytoskeleton-Related Mechanisms

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Objectives: The consumption of cruciferous vegetables is associated with reduced risk of renal cancer, linked to the protective action of different phytochemicals, such as isothiocyanates. Erucin is an isothiocyanate that can be generated by *in vivo* reduction of sulforaphane or by enzymatic hydrolysis of glucorucinin. Contrarily to sulforaphane, limited studies have addressed the anticancer properties of erucin. Thus, this study aims to evaluate the impact of erucin on renal carcinoma cells by assessing cell features related to cancer progression and by exploring the underlying mechanisms of action.

Methods: The effects of erucin were assessed in 786-O and Vero-E6 cell lines, representative of human renal cancer and non-cancer kidney cells, respectively. Cytotoxicity was evaluated by MTT and PI staining, after exposing cells to erucin (1 -100 µM). Cell cycle distribution and cell death were analyzed by FACS after PI or FITC Annexin V/PI staining, respectively. Morphometric analysis was used to evaluate the effects on cell morphology. Collective cell migration and chemotaxis/chemoinvasion of cells treated with non-toxic concentrations of erucin, were assessed by the wound healing and transwell-based assays, respectively. Cell adhesion was measured after detachment and re-seeding of cells.

Several methodologies were used to explore the mechanisms underlying the observed effects. The intracellular levels of reactive oxygen species (ROS) were measured using the DCFH-DA. The activity of Lysyl oxidase-like 2 (LOXL2) was evaluated by the Amplex-UltraRed assay. Erucin effects on cytoskeleton were assessed by immunofluorescence and by the tubulin polymerization assay.

Results: Erucin induced a concentration-dependent decrease of cell viability, more pronounced in 786-O cells. Cell distribution along cell cycle was altered after cell exposure to the bioactive. Collective cell migration, chemotaxis and chemoinvasion abilities as well as cell adhesion were impaired in renal cancer cells upon treatment with erucin. Additionally, both vero-E6 and 786-O cells revealed concentration-dependent changes on their originally elongated form towards a smaller round conformation. Moreover, the bioactive significantly prevented appropriate tubulin polymerization. The activity of LOXL2 was slightly reduced, but only in the presence of very high concentrations of erucin. Intracellular ROS were reduced in Vero-E6 but not in 786-O cells.

Conclusion: Erucin may have promising effects against human renal carcinoma. Our results suggest that the impairment of tubulin polymerization may be a possible mechanism of action involved.

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Sour Taste SNP *KCNJ2*-rs236514 Associated with Mild Cognitive Impairment in an Elderly Cohort

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Objectives: Differences in sour taste thresholds have been identified in cognition-related diseases. Diet is a modulator of cognitive health, and taste perception influences dietary preferences and habits. Heritable genetics and polymorphisms in the *KCNJ2* gene involved in the transduction of sour taste, have been linked to variations in sour taste and non-gustatory functions. However, relationships between sour taste genetics, mild cognitive impairment and diet quality are yet to be elucidated. The objectives for this study were to investigate the associations between the presence of the *KCNJ2*-rs236514 variant (A) allele, diet quality indices, and mild cognitive impairment.

Methods: This secondary cross-sectional study analysed data from the Retirement Health & Lifestyle Study conducted on the Central Coast of NSW, Australia. Data from 524 elderly Australians (≥ 65 y) were analysed using standard least squares regression and nominal logistic regression modelling, with demographic adjustments applied. Cognitive impairment was evaluated by the Mini-Mental State Examination (MMSE). A previously validated food frequency questionnaire provided data for diet quality estimates.

Results: Results showed that the presence of the *KCNJ2*-A allele is associated with increased proportions of participants scoring in the range indicative of mild or more severe cognitive impairment (MMSE score of ≤ 26) in the total cohort, and males. These associations remained statistically significant after adjusting for age, sex and diet quality indices. The absence of association between the *KCNJ2*-A allele and cognitive impairment in women may be related to their higher diet quality scores in all indices.

Conclusions: The potential link between sour taste genotype and cognitive impairment scores may be due to both oral and extra-oral functions of sour taste receptors. Further studies are required on the role and relationship of neurotransmitters, sour taste genotypes and sour taste receptors in the brain, and dietary implications, to identify potential risk groups or avenues for therapeutic or prophylactic interventions.

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Could Nucleic Acids Oxidatively Modified during Thermal Processing of Red Meat be Components with Potentially Carcinogenic Properties?

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Objectives: Processed red meat is classified by the International Agency for Research on Cancer to the Group 1 - “carcinogenic to humans”. Oxidative properties of haem iron in red meat contribute to the formation of several modified food components, which are known risk factors in the process of carcinogenesis. The aim of this study was to verify whether oxidized dietary nucleic acids, that might represent so far not recognized potentially carcinogenic modified food components, could arise during heat treatment of red meat.

Methods: The oxidation of DNA isolates (50-100 bp) was carried out in the presence of bovine methaemoglobin as a source of haem iron and phospholipids, which are main prone to oxidation lipid components of cellular membranes. Both methaemoglobin and phospholipids are abundant components of red meat. Additionally, to accelerate the induction of oxidation of DNA and phospholipids, ascorbic acid was added in some experiments. Ascorbic acid is an antioxidant used by meat industry, however it is also known to maintain Fe^{2+} level and thereby to promote

Fenton reaction producing ROS. The kinetics of the phospholipids oxidation and the influence of different concentrations of DNA on it were followed spectrophotometrically.

Results: Preliminary experiments have shown that lower concentrations of DNA (up to 0.1 mg/mL in reaction mixture) seem to accelerate the kinetics of phospholipid oxidation. Addition of ascorbic acid depending on concentration further increased oxidation rate (between 30–50 μ M) or slowed it down (above 70 μ M). In contrast, higher concentrations of DNA (0.25 – 0.50 mg/mL) seemed to prevent oxidation of phospholipids as indicated by lowered, almost inhibited, rate of methaemoglobin oxidation.

Conclusions: Nucleic acids exhibited high susceptibility to oxidation in the presence of haem iron. Further investigations are needed to determine if such oxidized dietary nucleic acids may have carcinogenic effect for the consumers. One indirect indication that such harmful mechanism might exist is the increased activity of MTH1 protein in the alimentary tract. The role of this enzyme is to hydrolyse triphosphates of oxidized purines to prevent their incorporation into host's DNA. Additional conclusion suggested by the results of this study points to the need of greater attention to the composition of meat products, so that such components like ascorbic acid do not contribute to the oxidation mechanisms during red meat processing.

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Biological Activity of Tea-Chokeberry and Chokeberry Fermented Beverages

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Fermented beverages, such as kombucha are gaining more and more popularity due to their beneficial effects on human health. These products are obtained by the fermentation of tea infusion using so called tea fungus. This microbial consortium can also be used to prepare fermented fruit beverages. Therefore, the aim of this study was to determine the changes in the antioxidant profile of tea-chokeberry and chokeberry beverages during fermentation carried by tea fungus. Additionally, the ability of beverages to inhibit the activity of the amylolytic enzymes was also determined.

Changes in content and profile of antioxidants during fermentation were determined by HPLC-DAD-MS analysis. The antioxidant activity of the compounds was determined using by HPLC postcolumn derivatization with ABTS. Furthermore, to determine the inhibition of amylolytic enzymes, spectrophotometric methods were used.

The results revealed that the composition of antioxidant compounds and the ability to inhibit α -amylase and α -glucosidase enzymes depended on the duration of beverage fermentation. In addition, it has been shown that the tea-chokeberry beverage has a more abundant composition of compounds capable of neutralizing free radicals and reactive oxygen species, compared to the fermented chokeberry beverage.

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Digestive Tract Diseases and the Microbiome: How to Functionally Integrate Host Transcriptomics and Metabolomics

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The inflammatory bowel diseases, Crohn's disease and ulcerative colitis, are chronic disorders which affect millions of people worldwide. Although, the incidence of inflammatory bowel disease seems to be stabilizing in Europe, an estimated 2.5 million people are affected by the disease with a direct healthcare cost of 4.6–5.6 bn Euros/year. They are complex diseases and heterogeneous at the clinical, molecular, genetic and microbial levels. Recent years some significant research questions have been arisen such as how to track gut microbiota alteration in the disease and how it contributes intestinal inflammation and how to effect human health. To answer all these questions, we need to analyse multi-omics of the gut microbial ecosystem. With the better understanding of this research questions, we can explore not only prevention and treatment of the inflammatory bowel disease but also other gut-microbiota related diseases.

The present study aim to make a host-transcriptomics and metabolomics analysis of a real dataset taken from the Inflammatory Bowel Disease Multi-Omics Database. The dataset consists of integrated longitudinal molecular profiles of 132 participants with Crohn's disease, ulcerative colitis disease and healthy. In the first stage, host transcriptome data which is given as RNA-seq transcript counts across samples were analysed. After detecting differentially expressed genes, we have made pathway analysis. We identified genes that were significantly differentially expressed in patient biopsies taken in inflamed locations of the ileum (from individuals with Chron's disease) and rectum (both Crohn's disease and ulcerative colitis) compared to individuals without IBD. This analysis identified 901 and 598 genes that were differentially expressed in the ileum and rectum, respectively for Crohn's disease. Additionally, 21 and 5400 were differentially expressed in the ileum and rectum, respectively for ulcerative colitis disease. Both analyses have been performed with FDR<0.05 and |log2 Fold Change|>1. Enrichment analysis testing for overrepresentation of WikiPathways among differentially expressed genes also confirmed strong representation of immune-related and inflammatory response pathways. In metabolomics part, metabolic alterations from metabolomic data were calculated by using the data to calculate the metabolites that show different levels in patients with IBD and in healthy individuals. We then looked at the host pathways that showed up in the host transcriptomics analysis described above and checked whether any of these metabolites appears in these pathways. It is clearly seen from the experimental results enriched host pathways includes altered metabolites. Besides, it is also worth to mention that the data confirm that Chron's Disease is primarily an Ileum disease, but effects in rectum are visible. While the Ulcerative Colitis effects are smaller but concentrate in Rectum as expected.

Difference in Genetic Susceptibility to Cancers among Asians and Caucasians: A Literature Review

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Two third of data used in GWAS come from people of predominantly European ancestry, even though they account for only a fifth of the global population. A pharmacogenetic report has recently concerned that raised significant concern about metabolic differences between Asian and Caucasian population, where clinical managements of drug administration predominantly come from European ancestry. To the best knowledge, few studies have explored the difference of cancer susceptibility on the metabolic function-related genes among Asians and Caucasians. Understanding the difference in genetic expectation among Asians may develop a precise cancer management plan for this population in the future.

Objectives: To understand the difference in genetic susceptibility to cancer among Asians and Caucasians, the author selects three genes including Cytochrome P450 1A2 (CYP1A2), alcohol dehydrogenase 1B (ADH1B), and patatin-like phospholipase domain-containing 3 (PNPLA3) that are well reported to metabolic function and carcinogen bio-activation elsewhere. The different cancer susceptibility of these selected genes between Asians and Caucasians is under consideration by reviewing current meta-analysis or systematic review studies.

Methods: A literature search on Cumulative Index of Nursing and Allied Health Literature (CINAHL) and PubMed using the following search terms: Asia* and each of relevant gene names or abbreviation (i.e., alcohol dehydrogenase 1B or ADH1B). Eligible publications are meta-analysis or systematic review articles with a full-text in the English language on the last 20 years.

Results: The initial search identified 23 meta-analysis and review articles on these genes, and then only six articles are included in this review. There are three well-identified polymorphisms in relation to the difference in cancer susceptibility between Asians and Caucasians including CYP1A2 rs762551, PNPLA3 rs738409, and ADH1B rs1229984. Stratified analysis by ethnicity demonstrated that CYP1A2 rs762551 and PNPLA3 rs738409 polymorphisms are not significantly related to cancer risks among Asians, but are more likely to be at high risk in Caucasians. Contrastingly, ADH1B rs1229984 polymorphism is more cancerous in Asians than Caucasians.

Conclusions: The present review does not aim to draw a firm conclusion, but the finding has reflected the difference of cancer susceptibility on these selected genes between Asians and Caucasians. Notably, Asian participants in these included articles are small compared to their Caucasian counterparts. Further investigation in a large-scale Asian population is necessary to fill an existing gap of diversity in genetic research.

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MiRNA in Raw Cow Milk: Any Contribution to the Nutritional Quality?

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Objectives: Cow milk is a nutrient-dense food and may act as an epigenetic regulator of gene expression of the milk recipient. In particular, miRNAs from cow milk may influence human genes expression since they are identical to human analogues and recognize the same target mRNAs. Furthermore, being resistant to heat treatments, bovine miRNAs may contaminate human food chain. The aim of the present study was to investigate the expression of inflammatory miRNAs (miR-148, miR-29 and miR-21) in raw cow milk, intended for sale either as beverages or dairy products, in association with other parameters of milk nutritional quality, i.e., total bacterial cell count (TBC), somatic cell count (SSC), antioxidant activity, fatty acids profile.

Methods: 7 farms from Italy were selected and 10 samples of raw milk (from different cows) for each farm were analysed. Data about the daily milk production per farm (kg/head), as well as TBC and SSC in milk were collected. Total RNA (including miRNAs) was extracted from each sample and retrotranscribed; miR-148, miR-29 and miR-21 were quantified by qPCR. The antioxidant activity of raw milk was calculated according to the Oxygen Radical Absorbance Activity (ORAC) test, while gas chromatography was used to evaluate the lipid profile of each milk sample.

Results: Milk from 3 farms showed significantly higher levels of miR-148 and miR-21, while miR-29 levels did not statistically differ among the milk collected from different farms. A positive association between TBC and miR-148/miR-21 levels was found. TBC directly correlated also to the milk antioxidant activity, which instead showed an inverse relation with SSC. TBC was also negatively associated to the average daily milk production. Lastly, the lipid profile was correlated to inflammatory markers.

Conclusion: Our preliminary data show that the variability in the expression of pro-inflammatory miRNAs may reflect cows' health status and milk nutritional quality. Remarkably, even though all analysed samples are within microbiological safety ranges, bacterial infections are associated with reduced lactation performances and higher levels of inflammatory miRNAs in milk. Further studies are warranted to define if, because of their epigenetic burden, pro-inflammatory miRNAs may lower the nutritional value of milk and dairy derivatives

Walnut-Derived Peptide in a Model of Low-Grade Inflammation: Any Nutrigenomic Effect?

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Objectives: Epigenetic plays a major role in the regulation of inflammation, which, on the other hand, promotes the onset of chronic complex diseases. Bioactive components and nutrients contained in foods can modulate epigenetic pathways. This study investigates potential nutrigenomic properties of a walnut-derived peptide (YVLLPSPK), with high antioxidant activity, against lipopolysaccharides (LPS)-induced low-grade inflammation in human macrophages (THP-1) cells.

Methods: Cell viability for THP-1 was measured by the MTT assay. THP-1 cells were seeded at the concentration of 1×10^5 cells/well into a 96-well plate and were incubated with 5 ng/mL PMA, and YVLLPSPK (6.25, 12.5, 25, 50, 100, 200 μ M) for 24 h, 48 h and 72 h. Cells were exposed to 20 ng/mL LPS for 24 h after the treatment with 5 ng/mL PMA for 48 h. The effect of the peptide YVLLPSPK (100 μ M) was tested according to two different experimental schemes: 1) the peptide was added before the exposure to LPS (as a pre-treatment with PMA for 72 h); 2) the peptide was added as a co-treatment with LPS (total exposure for 24 h). The expression of genes involved in the control of inflammation and the epigenetic homeostasis (IL6, MCP-1, IL1 β , DNMT1, DNMT3A, DNMT3B, TET1, TET2, TET3) was tested by qPCR. The relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Results: The results on cell viability showed that the concentrations and time of YVLLPSPK had no significant impacts on cell viability compared with the control ($p > 0.05$). Analysis of the expression of inflammation genes revealed that the pre-treatment with YVLLPSPK significantly suppresses IL6 and MCP-1 in LPS-induced low-grade inflammation ($p < 0.05$). However, no significant modulation of IL1 β gene was detected. Among genes involved in the regulation of epigenetic homeostasis, only TET2 was significantly modulated by the peptide ($p < 0.01$).

Conclusion: These preliminary data suggest an anti-inflammatory effect of the walnut-derived peptide, YVLLPSPK. No significant changes of DNMTs expression were detected, while a potential modulation of TET2 activity in this LPS-induced low-grade inflammation model is hypothesized. Further ongoing studies will investigate the effect of YVLLPSPK peptide on DNMTs activity. Elucidating the molecular mechanisms underpinning the beneficial effect of this peptide will provide the basis for its application in functional foods in the future.

Effect of Capsicum and Cinnamon on Cellular Metabolism and Immunoregulation-An *In vitro* Study with Targeted Metabolomics

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Background: Numerous clinical and in vivo studies revealed the antidiabetic and anti-obesity properties of cinnamon and capsicum. However, the antidiabetic/anti-obesity effects of individual said spices at cellular levels are largely unknown and yet to be investigated

Objective: This work specifically aimed to disclose the effects of capsicum and cinnamon polyphenols on the cellular metabolism and immunoregulation of overweight/obese (OW/OB) human peripheral blood mononuclear cells (PBMC) using the targeted metabolomics.

Methodology: Blood was drawn from the OW/OB participants ($n = 3 + 3 = 6$) to isolate PBMC to be incubated with designated concentration (15 – 4500 μ g/mL) of cinnamon and capsicum. Cell culture without the extracts were used as controls. Following the designated time of incubation (48h), the supernatants were isolated, samples were prepared and used for targeted metabolomics using Liquid Chromatography – Triple Quadrupole Mass Spectrometer (LC/QqQ-MS). Data was analyzed using vendor-based software and significantly altered metabolites were selected using multivariate (VIP > 1 and p -value ± 0.5) and univariate statistical analysis (corrected $p < 0.05$).

Results: Cinnamon and capsicum significantly altered the cellular metabolism as revealed from targeted metabolomics of supernatant from control-to-treated cell cultures. In total 25 significantly varied metabolites were observed in comparison from control-to-treated cell cultures. Most of these significantly varied metabolites are short-chain organic acids, degradation products of amino acids, and intermediate products of glycolysis and TCA cycle. Pathway enrichment analysis showed that capsicum and cinnamon treatments significantly affected nearly 30 pathways in these cell cultures among which citrate cycle (TCA cycle), glyoxylate and dicarboxylate metabolism, pyruvate metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, alanine, aspartate and glutamate metabolism were the most affected pathways.

Conclusion: This study investigated the effects of cinnamon and capsicum on cellular metabolism of PBMC by employing the targeted cell-culture profiling approach. The spices extract greatly affects the metabolic pathways related to glucose and protein degradation.

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