
Abstracts of the 2nd European Summer School on Nutrigenomics

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Rosita Gabbianelli, Camerino
J. Alfredo Martínez, Navarra
Raffaele De Caterina, Chieti

Abstracts

1

Homocysteine-Induced Changes in Ferritin L Gene Expression Are Mediated by Akt Signalling Pathway in HUVEC Cells

Andzelika Borkowska^{1*}, Anna Herman-Antosiewicz², Jędrzej Antosiewicz¹

¹Department of Bioenergetics and Physiology of Exercise, Medical University of Gdansk, Poland; ²Department of Molecular Biology, University of Gdansk, Poland
E-Mail andzelika.b@gumed.edu.pl

Objectives: Low folic acid consumption or its poor absorption could be one of the reasons of hyperhomocysteinemia. It is a well-known independent risk factor for cardiovascular diseases such as ischemic heart diseases, stroke, peripheral vascular disease, atherosclerosis, and for dementia, notably Alzheimer's disease, brain atrophy and some other. In addition, elevated blood homocysteine (Hcy) has been demonstrated to be closely associated with iron-mediated ROS formation. However, the role of homocysteine in iron metabolism is not well understood at the molecular level. The purpose of this study was to determine whether Hcy may influence iron metabolism in HUVEC cells.

Methods: Human Umbilical Vein Endothelial Cells (HUVEC) has been used in this study. The HUVEC were cultured in Endothelial Cell Growth Medium (ECACC). The levels of ferritin L and H, Akt and P-Akt were determined by immunoblotting. siRNA transfection protocol was performed according to the supplier's instructions (Santa Cruz Biotechnology).

Results: We observed that Hcy (3 mM) treatment decreased Akt activity in HUVEC cells and subsequently increased ferritin L and H as well as intracellular iron level. The role of Akt in modulation of ferritin level was confirmed in cells with silenced expression of Akt by the specific siRNA which led to an increase in ferritin protein level. Changes in ferritin protein level were accompanied by changes in expression of ferritin L and H genes. Moreover, reactivation of Akt by insulin treatment reversed some effects of homocysteine.

Conclusions: In conclusion, our study for the first time demonstrates that homocysteine induced changes in iron metabolism in HUVEC cells are mediated by Akt signalling pathway.

2

In vitro Conjugal Transfer of Erythromycin Resistance from Enterococci Isolated from Omnivore and Vegetarian Subjects

Alice Litta-Mulondo^{1*}, Sonia Pasquaroli¹, Barbara Citterio²,
Carla Vignaroli¹, Francesca Biavasco¹

¹Department of Life and Environmental Sciences, Polytechnic University of Marche, Ancona, Italy; ²Department of Biomolecular Science, Biotechnology Section, University of Urbino 'Carlo Bo', Fano, Italy
E-Mail a.litta@pm.univpm.it

Objective: The consequences of the indiscriminate use of antibiotics in human health, agriculture, veterinary and aquaculture are catching up with us. Nowadays, there is increasing evidence that the human diet plays a central role in antibiotic resistance (AR) spread. Through the diet different bacteria, possibly carrying AR genes can enter the human gut, where they can undergo HGT events with both intestinal microbiota and transient pathogens. This study investigated the role of different diets, i.e., omnivorous, vegan or vegetarian on the spread of enterococcal AR genes from faecal isolates of subjects following the different diets.

Methods: 151 erythromycin (ERY) – resistant enterococci previously isolated from faecal samples of omnivore, vegetarian and vegan subjects were tested for Minimum Inhibitory Concentration (MIC) to assess their susceptibility to different antibiotics. Selected multiple drug-resistant (MDR) strains (1 *E. faecium*, 1 *E. faecalis* and 1 *E. hirae*) were used as donors in transfer assays to *E. faecium* 64/3, *E. faecalis* JH2-2, *L. ivanovii* 7842RF and *L. welshimeri* 11857RF. Resistance genes and species-specific genes were sought by PCR and plasmid content by the OMEGA bio-tek Plasmid DNA Kit.

Results: 11 isolates (1 from a vegan, 3 from vegetarians and 7 from omnivores) had MICs of erythromycin ≥ 256 mg/ml and were also found to be resistant to streptomycin and tetracycline. An *ermB*-carrying *E. faecium*, isolated from an omnivore subject was found to transfer ERY resistance to *E. faecium* 64/3, *E. faecalis* JH2-2, *L. ivanovii* 7842RF and *L. welshimeri* 11857RF with the following frequencies: 5.7×10^{-4} , 1.7×10^{-6} , and 4.3×10^{-7} and 8.5×10^{-8} , respectively. All ERY-resistant transconjugants tested positive for the *ermB* gene and carried two plasmids of the same size as detected in the donor. The resistance to streptomycin and tetracycline, was transferred as well. On the other hand, the two MDR enterococci (*E. faecalis* and *E. hirae*) isolated from vegetarian subjects failed to transfer antibiotic resistance to any recipient.

Conclusions: These findings further confirm that enterococci ingested by the diet may transfer their multiple ARs to intestinal microbiota or transient bacteria, also belonging to different species/genera, including (non-pathogenic) *Listeria* species that might in turn transfer the resistance to *L. monocytogenes*, a main food pathogen. Further experiments are needed to investigate whether the transfer may be more likely in omnivore than in vegetarian or vegan subjects.

3

Isothiocyanates, Compounds Present in Cruciferous Plants, Induce Autophagy in Cancer and Non Cancerous Cells

Anna Herman-Antosiewicz^{1}, Anna Pawlik¹, Jędrzej Antosiewicz², Joanna Brokowska¹, Aleksandra Hać¹*

¹Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdańsk, Poland; ²Department of Bioenergetics and Physiology of Exercise, Medical University of Gdańsk, Poland
E-Mail anna.herman-antosiewicz@biol.ug.edu.pl

Objectives: Naturally occurring isothiocyanates (ITC) are hydrolytic products of glucosinolates present in cruciferous plants. The reaction is catalyzed by myrosinases of plant or human gastrointestinal tract bacteria origin. ITC are extensively investigated due to their antimicrobial, chemopreventive and anticancer activities which are connected with modulation of gene expression and enzymes activities. It leads to modulation of numerous signal transduction pathways which results in cell cycle arrest and apoptosis of cancer cells while normal cells show greater resistance. It has been also shown that some ITC induce autophagy which is an evolutionary conserved, lysosome-mediated process for degradation and turnover of long-lived proteins and whole organelles. Defects in this process are observed in pathological conditions, such as cancer or neurodegenerative diseases as well as during aging. In case of cancer, decreased level of autophagy promotes first stages of carcinogenesis while its upregulation during progression stage allows cancer cells to survive harsh conditions, such as hypoxia or treatment with cytotoxic drugs. First report showing autophagy induction by ITC revealed that sulforaphane (1-isothiocyanato-4-methylsulfinylbutane, SFN) induced autophagy in prostate cancer cells, which preceded and delayed apoptotic cell death.

Methods: MCF-7, T47D, MDA MB 231 and SKBR-3 breast cancer cell lines differing in receptors and p53 status have been used as well as PC-3 prostate cancer cells and HDFa human fibroblasts. The levels S6K or S6 and LC3-II, an autophagy marker, were determined by immunoblotting, and cells morphology – using microscopy techniques. Cell viability was assessed by MTT and SRB method or flow cytometry.

Results: We compared autophagy-inducing activities of SFN as well as its structural analogs, erucin (1-isothiocyanato-4-methylthiobutane) and sulforafen (4-isothiocyanato-1-methylsulfinylbut-1-ene) in different cancer as well as non cancerous cells. Our results indicate that ITC elevate autophagy in all tested cells which is connected with the inhibition of mTOR complex 1. In majority of cell lines ITC-induced autophagy plays pro survival role.

Conclusions: Autophagy induction by ITC is a general phenomenon. In case of pro survival autophagy in cancer cells inhibitors of this process might improve anticancer potential of ITC. Autophagy modulation by ITC in normal cells might be used in case of age related disorders.

4

The Hydroxyl and DPPH Radicals Scavenger Capacity of Natural SOD Vegetal Extract

Andreea-Roxana Lupu^{1*}, Cristina Cercel^{1,2}, Lidia Cremer¹

¹Immunomodulation Group, 'Cantacuzino' National Institute of Research, Bucharest, Romania; ²Faculty of Biology, University of Bucharest, Romania
E-Mail Idreea@gmail.com

Objectives: Natural SOD, a green barley extract patented in Romania by Cantacuzino National Research Institute, have been shown to have complex antioxidant properties: SOD-like and peroxidase-like activity, peroxy radicals scavenger activity. Our goals were: to evaluate the antioxidant ($\cdot\text{OH}$ and DPPH radicals scavenger) capacity of *Natural SOD* vegetal extract and to increase the complexity and thoroughness of the quality control protocols according to National and EU regulations. This objective is part of a project aiming to establish a complex and accurate control methodology for our product, as well as for other vegetal extracts with potential antioxidant capacity.

Methods: In our study we used Hydroxyl Radical Absorbance Capacity (HORAC) and DPPH methods to assess the antioxidant capacity (scavenging activity against $\cdot\text{OH}$ and DPPH radicals) of *Natural SOD* as follows:

- Samples taken at different time-points of the technological process – to identify potential variations of antioxidant capacity due to product manufacturing phases;
- Samples from 10 *Natural SOD* batches from 2015 production – to evaluate the necessity to test each product batch, due to the quality of the raw material;
- 10 batches from production corresponding to 2015 and 2016 – to establish HORAC and DPPH scavenging median values for *Natural SOD*.

We also aimed to establish the corresponding acceptance range for scavenger capacity of *Natural SOD* against $\cdot\text{OH}$ and DPPH radicals, compared with well characterized antioxidants – ascorbic acid and gallic acid respectively.

Results: The obtained results showed that $\cdot\text{OH}$ and DPPH radicals scavenging activity of *Natural SOD* were not significantly affected by the technological process (different steps of flow production or different production runs). Though, significant differences could appear between samples corresponding to successive years production. That is most probably due to plant quality, as a result of culture conditions and/or specific steps before obtaining the vegetal extract.

Conclusions: We conclude that HORAC and DPPH methods are suitable as quality control methods for antioxidant ($\cdot\text{OH}$ and DPPH radicals scavenger) capacity of *Natural SOD* product as well as for similar vegetal extracts. Moreover, the obtained results, correlated with the previous ones, contribute to a more valuable characterization of *Natural SOD* vegetal extract. Using an acceptability range as a reference for supplementary checking of the batches in the current production, the thoroughness of the quality control protocol will be increased.

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Investigating the Effects of Short-Term Probiotic Intake on the Saliva Microbiome by 16S rRNA Sequencing in a Two Stage Study Design

Francesco Asnicar^{1*}, Erik Dassi¹, Pamela Ferretti¹, Serena Manara¹, Annalisa Ballarini¹, Giuseppina Covello¹, HTM², Roberto Bertorelli³, Olivier Jousson¹, Michela Alessandra Denti¹, Veronica De Sanctis³, Adrian Tett¹, Nicola Segata¹

¹Centre for Integrative Biology, University of Trento, Italy; ²Students of the Course 'High-Throughput Technology', Master Degree in Cellular and Molecular Biotechnology, University of Trento, Italy; ³NGS Core Facility, Centre for Integrative Biology, University of Trento, Italy
E-Mail f.asnicar@unitn.it

Objectives: The oral cavity microbiome plays important roles in maintaining oral and systemic health. Probiotic products hold the promise to reproducibly modulate the human microbiome. Ongoing research is focusing on characterizing the effects induced by probiotic strains on the microbial ecology of the gut, but the potential effects on the oral microbiome remain uncharacterized. We thus aimed here at determining the effects of probiotics intake on the human saliva microbiome.

Methods: We profiled two independent cohorts with high-throughput 16S rRNA sequencing: an exploratory cross-sectional cohort of 12 subjects and a follow-up cohort of 21 subjects sampled longitudinally (n = 109). The volunteers followed the same diet during the sampling period and subgroups were subjected to a commercial probiotic product challenge.

Results: Several members of the Firmicutes and the Proteobacteria phyla (*Neisseria*, *Haemophilus*, and *Streptococcus* genera) dominated the saliva microbial community, consistently with other independent studies. In the exploratory cohort, we observed an increase in microbial richness in the probiotic group compared with the control one (p = 0.011). This was reinforced by the longitudinal design of the follow-up cohort, in which we found that subjects supplementing the diet with probiotics significantly enhanced the diversity of their microbiome. Using a sub-OTU level oligotyping approach, we further showed that some bacteria were present in both the probiotic product and the saliva of the probiotic group, but were missing in the control one.

Conclusions: Our experiment found and confirmed that there is a significant short-to-medium term effect of commercial probiotics on the saliva microbial diversity. The oral microbiome can thus be potentially modulated by probiotic intake, even though this effect might be transient. Ideally, this could open an opportunity for using probiotic products to improve and promote oral health. Despite the use of computational oligotyping, the adoption of the 16S rRNA sequencing approach limited the taxonomic depth of our analysis. Larger additional studies are needed to further validate our findings, and the adoption of shotgun metagenomics will provide a more *in-depth* understanding of the specific genes/strains promoted or depleted in the saliva by probiotics.

6

Nurr1 Gene Expression and Global DNA Methylation in Offspring from Permethrin-Treated Rats

Laura Bordoni^{1*}, Cinzia Nasuti², Maria Mirto³, Ilenia Cruciatà³,
Fabio Caradonna³, Rosita Gabbianelli²

¹School of Advanced Studies, University of Camerino, Via Gentile III da Varano, Camerino 62032, Macerata, Italy; ²School of Pharmacy, University of Camerino, Via Gentile III da Varano, Camerino 62032, Macerata, Italy;

³Department of Biological Chemical and Pharmaceutical Sciences and Technologies (STEBICEF, Unit of Cellular Biology), University of Palermo, 90128 – Palermo, Italy

E-Mail laura.bordoni@unicam.it

Objectives: Pesticides are largely used in agriculture and consequently are present in fruits and vegetables. The significant presence of pyrethroid metabolites in the urine of population over the world confirms that their presence in food is a global problem. Moreover, the wide presence of pesticide residues in breast milk underline the risk for the population, focalizing the long-term consequence of early life pyrethroid exposure.

In particular, it has been demonstrated that there is a correlation between the environmental exposure to pesticides and the development of neurodegenerative diseases.

Neonatal exposure to Permethrin (PERM), a member of the family of synthetic pyrethroids, can induce neurodegeneration (i.e. Parkinson's – like disease) and it can cause some alterations in striatum of rats, involving both genetic and epigenetic pathways.

The aim of this study was to evaluate if the rat offspring (F1 generation) from parents (F0) exposed to a low dose of PERM from postnatal day 6 to 21, presents alterations in Nurr1 gene expression as previously observed in early life permethrin treated male rats. Moreover, global DNA methylation was analyzed in untreated and early life exposed mothers as well as in their offspring (F1 generation).

Methods: Through Nurr1 gene expression analysis and global DNA methylation assessment in both PERM-treated parents and their untreated offspring, we investigated on the prospective intergenerational effect of this pesticide.

Results: 33% of progeny presents the same Nurr1 alteration as rats exposed to permethrin in early life. A decrease in global genome-wide DNA methylation was measured in mothers exposed in early life to permethrin as well as in their offspring, whereas untreated rats have a hypermethylated genomic DNA.

Conclusions: Intergenerational PERM-induced damage on progenies has been identified for the first time. On the light of these results, pesticide residues in the food could represent a risk factor for the health of children especially in early life when the brain is still in the developing phase. Further studies are needed to elucidate the molecular mechanisms associated with the damage.

7

Effect of *Lycium barbarum* Berries Cultivated in Umbria (Italy) on Human Hepatocellular Carcinoma Cells

Maria Rachele Ceccarini*, Samuele Vannini, Samuela Cataldi, Massimo Moretti, Milena Villarini, Bernard Fioretti, Elisabetta Albi, Tommaso Beccari, Michela Codini

Department of Pharmaceutical Science, University of Perugia, Via Fabretti 48, 06122 Perugia, Italy

E-Mail mariarachele.ceccarini@studenti.unipg.it

Objectives: The Solanaceae, one of the largest and most important families of flowering plants, includes *Lycium barbarum* (LB) species that is recorded in the Chinese Pharmacopoeia. The plant is commonly called Goji and is known to have health-promoting bioactive components. In this study the ability of LB berries, cultivated in Umbria (Italy) to modulate *in vitro* cell health status due to its antioxidant, antigenotoxicity and anticancer properties has been investigated.

Methods: The antioxidant properties of LB berries cultivated in Umbria were studied by Folin Ciocalteu and ORAC methods. The effects of LB extract on human hepatocellular carcinoma cells (HepG2) have been investigated by MTT, to evaluate their cytotoxic, and Comet assay, to test their genotoxic and potential antigenotoxic activity *in vitro*. The expression of 96 genes involved in oxidative stress, proliferation, apoptosis and cancer was performed by PCR-array.

Results: The berries from Umbria region display high antioxidant properties based on their high content of polyphenols (1278.247 ± 29.60 mgGAE/100 gr DW) and on values of ORAC assay (22507.03 ± 1402.02 μ molTE/100 gr DW). Moreover LB berry extract didn't change considerably cell viability and didn't show genotoxic effect on HepG2 cell line. The results obtained with the Comet assay showed, for the first time, that the berries extract had a protective effect on DNA damage. In fact LB extract is able to reduce significantly the DNA damage of 29.3%, if we consider the positive control as a 100% of DNA damage. Furthermore the berry extract is able to modulate the expression of five genes. In particular down-expression of genes involved in tumor migration and invasion (CCL5), in increased risk of metastasis and anti-apoptotic signal (DUSP1), in carcinogenesis (GPx-3 and PTGS1) together over-expression of tumour suppressor gene (MT3), suggested that Umbria LB berries could play an anti-cancer role.

Conclusions: In conclusion the overall results showed that the berries of LB plants, originally cultivated in East Asian, had Umbrian environment adaptability. Moreover the LB extract exhibits antioxidant, antigenotoxic and anti-cancer properties *in vitro*. These findings are of great importance for the use the LB extract in the diet or for the production of functional food.

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Antioxidant Activity and Gut Epithelial Regenerative Effect of Probiotics

Stefania De Marco^{1*}, Miranda Piccioni¹, Rita Pagiotti², Donatella Pietrella¹

Department of Pharmaceutical Sciences, ¹Unit of Biochemical Sciences and Health, ²Unit of Food Sciences and Nutrition, University of Perugia, Via del Giochetto, 06122 Perugia, Italy

E-Mail stefania.demarco@studenti.unipg.it

Objectives: Several beneficial effects have been attributed to probiotic bacteria strains: to maintain a proper balance of the intestinal microbiota, to modify immune responses and to manage inflammatory gut disorders. The most widely used probiotics in humans are bifidobacteria and lactobacilli, but other microorganisms such as the yeast *Saccharomyces boulardii* have been reported to have some beneficial effects.

In our previous studies it has been shown that metabolites produced by *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Lactococcus lactis* and by the yeast *Saccharomyces boulardii* were able to down-regulate the expression of IL-8 in HT-29 cells and to modulate IL-10 production by PBMC, suggesting an anti-inflammatory activity.

The aim of the present work was to assess the probiotic potentiality of the above-mentioned strains in terms of antioxidant activity, which could counteract oxidative stress in the host.

Successively, the effects of the probiotic strains on regeneration of colon epithelial HT-29 cell line have been tested. Prebiotics inulin, fructo-oligosaccharide (FOS) and isomaltose were added to the medium at a concentration of 1% to observe if they could promote probiotic activities.

Methods: Cell-free supernatants of probiotic cultures (10^6 CFU/ml), incubated overnight in presence or absence of prebiotics (1% w/v), have been tested in this study.

The antioxidant activity of probiotic supernatants (1, 5 and 10% v/v) was measured using the DPPH (25 µg/ml) assay. Regeneration of colon epithelial HT-29 cells was assessed by determining cell proliferation after treatment with 0.1, 1 and 5% v/v cell-free supernatants (Via-Light, Lonza).

Results: The DPPH radical scavenging activities of the 5 strains increased in a dose-dependent manner, with the exception of *S. boulardii* supernatants that did not show any antioxidant activity.

The effects of lactic acid bacteria on HT-29 cell proliferation varied from strain to strain: cell-free supernatants of all tested strains, except for *L. reuteri*, significantly increased the growth rate of cells after 48 hr of incubation.

Conclusion: Our results indicated that probiotic supernatants could effectively scavenge free radicals and stimulate cell proliferation. Moreover, it has been observed that the addition of prebiotics did not promote probiotic activities.

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The Changes in Profile of Dietary Nucleic Acids after Thermal and Other Culinary Processing of Meat

Joanna Głazowska*, Urszula Stankiewicz, Agnieszka Bartoszek

Department of Food Chemistry, Technology and Biotechnology, Gdansk University of Technology, Gabriela Narutowicza 11/12 Street, 80-233 Gdańsk, Poland

E-Mail glazowska.joanna@gmail.com

Objectives: Nucleic acids are one of major components of raw and low-processed food products, where they constitute approximately 0.5–10% of dry weight. It is known that dietary nucleotides play a significant role, i.a. in regulation of immune and gastrointestinal systems in humans. The role of nucleic acids as nutritional and functional components is still very poorly understood despite constant presence in food. The molecular biology experiments show that food processing does not prevent the obtainment of DNA of plant or animal origin in sufficient quantity and quality to enable for example amplification by PCR techniques. However, it is not sufficiently recognized how food-processing techniques affect the structure and fragmentation of nucleic acids, which may influence both digestibility and rheological properties of food products. The aim of this study is to determine the profiles of DNA fragments present in food after processing, and to visualize the present nucleic acids in processed food slices.

Methods: Meat samples were processed thermally following traditional recipes: cooking, steam cooking, roasting, oven baking, microwave processing with grilling, drying, curing and grilling. After each treatment the samples for paraffin embalmed sections were collected and the remaining meat portions were frozen at -80°C . Nucleic acids were isolated with Qiagen DNeasy Blood & Tissue kit, following the manufacturer's instructions, from the frozen samples and the isolated DNA was separated by capillary electrophoresis. The paraffin-embalmed samples were cut into 8 μm thick sections and the nucleic acids were stained with fluorescent dyes.

Results: The nucleic acids appeared to remain in the processed meat, also after thermal and microwave treatment. In the case of unprocessed, cooked and grilled meat, the nuclei of the muscle cells showed no significant damage and stained well with common fluorescent dyes. Dried meat samples showed the presence of high molecular mass nucleic acids.

Conclusions: These studies show that not only low-processed and raw food products contain well preserved cell structures like nuclei and nucleic acids, but also meat after thermal processing can contain nucleic acids in non-degraded form. This gives important information about the occurrence of these basic food components and the impact of food-processing on their nutrition potential.

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Long-Term Supplementation of Dietary Inulin and Short-Chain Fatty Acids (SCFAs) Suppress High-Fat Diet-Induced Obesity in C57BL/6J Mice

Karolin Weitkunat^{1*}, Sara Schumann¹, Michael Blaut², Susanne Klaus¹

¹Research Group Physiology of Energy Metabolism, ²Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
E-Mail Karolin.Weitkunat@dife.de

Objectives: The role of dietary fibers and their bacterial fermentation products, short-chain fatty acids (SCFAs; mainly acetate, propionate, butyrate) in obesity development is controversially discussed. On one hand, SCFAs can serve as an additional energy source, but on the other hand they increase satiety by acting as metabolic regulators. From our previous short-term studies we hypothesize that the acetate:propionate ratio (Ac:Pr) is important for the effect on obesity development, since higher cecal propionate production is accompanied with a reduced lipogenesis. Our aim is now to evaluate the effects of dietary fibers and SCFAs on energy metabolism and obesity development in long-term feeding trials.

Methods: C57BL/6J mice were fed a high fat diet (HFD) supplemented either with 10% dietary fibers (HFC: 10% cellulose; HFI: 3% cellulose + 7% inulin; HFG: 3% cellulose + 7% guar gum) or 5% SCFAs with different Ac:Pr ratios, a high acetate (HAc; 10:1 Ac:Pr) or high propionate diet (HPr; 1:2.5 Ac:Pr) for 30 weeks. Control mice received a HFD without SCFAs.

Results: As expected, in the HFD control groups (HFC and Con) body weight and fat gain was highly increased compared to LFD. This was greatly reduced by inulin (HFI) and completely prevented by SCFA supplementation. This is consistent with a reduced adipose tissue weight (subcutaneous and gonadal adipose tissue) after 30 weeks of intervention. In addition, feeding of inulin and SCFAs (but not of guar gum) led to lower hepatic triglyceride concentrations. Gene expression analysis and fatty acid synthase (FASN) activity in liver indicate that hepatic lipogenesis was reduced by acetate. Oral glucose tolerance test in week 20 revealed no differences in glucose clearance, while insulin sensitivity was increased after inulin and SCFA consumption.

Conclusion: The present data show that guar gum and inulin, two fermentable dietary fibers, have different effects on diet-induced obesity. In the long term, inulin attenuates HFD-induced lipid accumulation and insulin resistance while guar gum has no effects. SCFA supplementation has similar effects on HFD induced obesity as inulin, but there are indications that acetate and propionate act differentially on lipid metabolism. While acetate seems to decrease hepatic lipogenesis, propionate rather appears to induce fatty acid oxidation. The molecular mechanisms underlying these effects will be further elucidated.

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Induction of Phase II Detoxification Enzymes by Fruit Extracts Rich in Anthocyanins

*Izabela Koss-Mikołajczyk**

Department of Food Chemistry, Technology and Biotechnology, Chemical Faculty, Gdańsk University of Technology, Narutowicza 11/12, 80-233 Gdańsk, Poland

E-Mail iza.koss88@gmail.com

Objectives: There is much evidence that elevated tissue levels of phase II detoxification enzymes are associated with decreased susceptibility to chemical carcinogenesis. These enzymes, which include quinone oxidoreductase (NQO1), glutathione S-transferases (GST) and others are able to convert harmful chemicals into hydrophilic metabolites that are readily eliminated from the body. Phase II enzymes are highly inducible. One of the most potent inducers of these enzymes are compounds from isothiocyanate group. Also some evidence of induction of mentioned enzymes by anthocyanines has been recently mentioned in literature.

The aim of this study was to compare the anthocyanin profiles of different fruit varieties (raspberries, grapes, mulberries and currants) and to examine their impact on antioxidant and biological activity of tested plant extracts.

Methods: The composition of anthocyanins was characterized using HPLC-DAD-MS, total antioxidant activity was assessed by spectrophotometric tests (ABTS, DPPH, FC and FRAP), antioxidant profiles were obtained using HPLC with post-column derivatization. Biological activity of extracts was characterized by assessing their ability to induce phase II detoxification enzymes (GST and NQO1).

Results: The highest content of anthocyanins was observed in black mulberry extract followed by extracts from red raspberries, black and red currants and red grapes extracts. In extracts from white mulberries, grapes, currants and yellow raspberries no pigments from anthocyanins group were detected, but they are rich source of other biologically active compounds such as ascorbic acid or chlorogenic acid. The strong relationship between anthocyanins content and antioxidant activity was observed. Tested extracts had different effect on phase II detoxification enzymes (GST and NQO1). The strongest ability to induce activity of detoxification enzymes after 6 h incubation of cells with tested extracts showed red grape extract, while in the case of 24 h incubation of cells the strongest inducer turned out to be yellow raspberry extract.

Conclusions: This study showed, that anthocyanins content and composition has a strong and predictable influence on biological properties of studied plants.

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Neutrophils in Obesity and Low-Grade Systemic Inflammation

Louise Madsen*

Institute of Veterinary Disease Biology, University of Copenhagen,
Denmark

E-Mail sft321@alumni.ku.dk

Objectives: This project is split into a human and murine study. The objective of the human study is to investigate how obesity affects low-grade systemic inflammation (LGSi) by examining granulopoiesis in obese subjects with features of the metabolic syndrome. The objective of the murine study is to examine how the fat tissue may affect granulopoiesis in high fat diet (HFD) induced obese mice.

Methods: qPCR to investigate gene expression in RNA isolated from epididymal fat tissue; optical projection tomography to visually localise the presence of neutrophil and CD8 T cells; determination of possible granulopoietic factors, IL-1 β , GM-CSF and G-CSF by ELISA in conditioned fat media (CFM) from mice and stimulation of bone marrow stem cells with CFM to investigate the granulopoietic potential of the fat tissue from the obese mice evaluated by flow cytometry. Lastly, flow cytometry was performed on murine spleen cells to investigate markers of neutrophils.

Results: The human study showed a correlation between BMI and neutrophil numbers, indicating that obesity correlated with increased neutrophil numbers in the blood. Furthermore, the cell size and granularity of neutrophils correlated. The next step is to investigate the correlation between neutrophil cell size and markers of different developmental stages to investigate whether cell size is linked to the maturity of the neutrophils.

In the mouse study, we saw a significant weight increase of the fat pads from mice on a HFD at day 30 and 37 after initiating HF feeding.

In the spleen, the amount of neutrophils was increased at day 3 in mice compared to mice on a control diet, followed by a decrease of neutrophils. The spleen is considered a filter of the blood and may reflect the content of the blood. Thus, indicating an increased recruitment of neutrophils to the circulation.

The qPCR showed a significantly reduced expression of haptoglobin (hp) in the fat pads of the HFD-induced obese mice. We have previously shown that hp is a suitable marker for immature neutrophils in the blood. Therefore, these results may reflect a decrease in the amount of immature neutrophils. At day 23 after initiating the HFD, the TLR4 expression in the fat pads became slightly elevated in the HFD-induced obese mice. Simultaneously, we saw a transient increase in the expression of IL-1 β . IL-1 β has been suggested to induce myelopoiesis. These results indicate myeloid infiltration of the fat tissue in obese mice.

Conclusions: The preliminary results indicate an increased amount of neutrophils in the circulation of human obese subjects and myeloid infiltration in murine fat tissue early after the commencement of a HFD, which may be the first sign of LGSi.

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Antioxidant Power Series as a Tool for Rational Design and Assessment of Health-Promoting Properties of Functional Foods Based on Antioxidant Phytochemicals

Monika Baranowska, Klaudia Suliborska, Jacek Namieśnik, Wojciech Chrzanowski, Agnieszka Bartoszek*

Faculty of Chemistry, Gdansk University of Technology, Poland
E-Mail monbaran1@student.pg.gda.pl

Objectives: The aim of the project is to organize antioxidants relevant for human health into Antioxidant Power Series (APS) based on their reduction potentials in relation to glutathione, which is a major physiological antioxidant. Bioactive phenolic compounds, main dietary antioxidants, support endogenous antioxidant defense system of human organism against oxidative stress. Excess of reactive oxygen species (ROS) is suggested as one of the causes of civilization diseases. It is still unknown if stimulation or inhibition of genes involved in oxidative stress and antioxidant defense is related to reductive potential of dietary antioxidants or is rather associated with their chemical structure. Neither impact of antioxidant phytochemicals on gut microflora is fully understood. APS will be constructed as a basis for planning subsequent experiments aimed at verification of its usefulness in the prediction of antioxidant activity of substances under physiological conditions.

Methods: The first group of examined redox active compounds was flavan-3-ols. Chemical studies performed in cell-free models included determination of reduction potentials, as well as antioxidant capacity by spectrophotometric tests ABTS, DPPH and FC. Furthermore, measurements of lipophilicity using RP-HPLC enabled predictions of bioavailability of compounds studied. The biological tests embraced cytotoxicity measurements, comet assay and CAA test.

Results and Conclusion: The statistical tools employed to establish the correlation between the chemical properties and biological potential of flavan-3-ols suggest the role of these phytochemicals in oxidative stress prevention. Studies provide information about relationships between redox properties of antioxidant compounds and their biochemical and biological behavior in situation of ROS challenge. The results create foundations to propose a set of methods, which combined with APS would help to predict the chemopreventive value of antioxidants and would allow rational design of nutraceutical foods, supplements as well as therapeutic strategies.

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Protection with Electrolyzed Reduced Water on Gut Microbiota in Rats Exposed to Permethrin during Postnatal Development

Cinzia Nasuti^{1*}, Donatella Fedeli¹, Dennis Fiorini², Ivan Dus³,
Rosita Gabbianelli¹

¹School of Pharmacy, ²School of Bioscience and Biotechnology, University of Camerino, Italy; ³Head of Research for Chanson Water Company, Taiwan

E-Mail cinzia.nasuti@unicam.it

Objectives: Electrolyzed reduced water (ERW) is a functional drinking water with antioxidant activity due to its negative oxidation reduction potential (ORP = -300 mV) that release electrons by hydrogen (400 ppb).

Here, the effect of ERW was investigated in an animal model developing neurodegenerative disease after exposure to permethrin pesticide (PERM) in early life. In particular, the impact of ERW on gut microbiota and permeability was studied.

Methods: Rats were treated in early life with permethrin (PERM), vehicle (control), PERM + ERW. At adolescent age (60 days old), feces of rats were collected and submitted to microbiota identification by 16S rRNA gene-amplification (MiSeq Illumina). In addition, fecal short chain fatty acids (SCFA) were measured by GC.

In order to measure intestinal barrier permeability, rats were gavaged with a permeability tracer, FITC-dextran 4 Da (500 mg/kg) and 2 h later, blood was withdrawn.

Results: PERM group showed an increase of intestinal permeability, a lower abundance of bacterial species and changes of bacterial composition in the feces compared to CONTROL group.

The co-treatment with electrolyzed reduced water could not prevent at all the abnormal intestinal permeability induced by PERM but could attenuate the leakiness of the intestinal barrier.

Gut microbiota analysis shows that the co-treatment with ERW was effective to increase the bacterial diversity. Higher diversity in bacterial OTUs is advantageous because there are less dominant species who monopolize most of the resources at the expense of the others.

Analyzing the composition of bacteria phyla, we observed that ERW increased *Firmicutes* (55.33%) at the expense of *Bacteroides* (43.06%), whereas CONTROL and PERM groups did not differ from each other.

The co-treatment with ERW was effective to prevent the negative effect of PERM by increasing the abundance of many members of the *Lachnospiraceae* family.

ERW had a positive effect on fecal metabolites with respect to CONTROL group.

Conclusion: ERW is an alkaline (pH = 9) and hydrogen molecule-rich water with ROS-scavenging activity. A moderate consumption of alkaline water predisposes to bowel reducing environment promoting the growth of *Lachnospiraceae* family. These bacteria family have been associated with a low grade intestinal inflammation state which prevents gut barrier alteration and disease development.

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Effect of Electrolyzed Reduced Water in an Animal Model of Parkinson-Like Disease

Cinzia Nasuti¹, Donatella Fedeli¹, Laura Bordoni², Maura Montani³, Ivan Dus⁴, Rosita Gabbianelli¹

¹School of Pharmacy, University of Camerino, Italy; ²School of Advanced Studies, University of Camerino, Italy; ³School of Biosciences and Veterinary Medicine, University of Camerino, Italy; ⁴Head of Research for Chanson Water Company Taiwan

Objectives: Early life environmental factors, life style and diet have a profound impact on the organism's later development and subsequent onset of age-related diseases such as neurodegenerative diseases. In previous studies, we developed an animal model where the early life exposure (from postnatal day 6 to 21) to low dose of pesticide permethrin (PERM, 34 mg/kg) induced Parkinson-like neurodegeneration in rats characterized by decreased dopamine, Nurr1 and glutathione levels in the basal ganglia, altered immune responses and gut disbiosis. The animal model was exposed to a neurotoxin PERM that is an insecticide widely used for indoor and outdoor applications (i.e. carpets, kitchen worktops other treated wood furniture, lawn, mosquito control). The presence of its metabolites in the urine of 98% of population makes this insecticide a reliable environmental risk factor to health.

The present study aims to test the effect of electrolyzed reduced water (ERW) on this animal model developing neurodegenerative disease after exposure to PERM pesticide in neonatal age.

Methods: The effect of ERW is determined by cognitive tests, dopamine and Nurr1 levels measured in basal ganglia and Tyrosine Hydroxylase (TH) immunostaining in the Substantia Nigra pars compacta (SNc) in 60-day-old rats.

Results: When working memory is assessed in a T-maze, PERM group has a worst performance compared with healthy controls, whereas the performance of PERM+ERW is similar to control group. The same trend is observed for the average of perseverative errors.

With regard to dopamine levels measured by HPLC in basal ganglia, decreased levels are observed in PERM group compared to control group, whereas the co-treatment with PERM+ERW protects against the damage induced by the pesticide even increasing the levels of dopamine. Similar results are obtained with regard to Nurr1 levels measured by western blot in basal ganglia.

Immunohistochemical analysis with anti-TH antibody marking dopamine neurons in SNc shows a reduced number of TH-positive neurons in PERM rats compared to control rats, whereas a slight increase (not statistically significant) was observed in the PERM+ERW group with respect to PERM group.

Conclusion: The use of ERW as functional water could be helpful as a therapeutic tool in the prevention of neurodegenerative diseases.

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Carnitine Supplementation and Skeletal Muscle Function in Aging

Angelika Sawicka¹, Damian Sadowski¹, Piotr Aschenbrenner², Anna Platta³, Robert A. Olek^{1*}

¹Department of Bioenergetics and Nutrition, ²Department of Biomechanics, Gdansk University of Physical Education and Sport, ³Gdynia Maritime University, Poland
E-Mail robol@awf.gda.pl

Objectives: A fundamental cause of, and contributor to disability in aging is the involuntary loss of muscle mass, strength and function (sarcopenia). Healthy humans muscle samples analysis, showed a drastic reduction of carnitine and acetyl carnitine in older subjects. Due to a strong reverse correlation between age and muscle carnitine levels, age related carnitine deficiency has been suggested as the potential cause of muscle fatigue, weakness and sarcopenia. Recently, it has been demonstrated that muscle carnitine content can be elevated by dietary intervention. Therefore, the aim of our study is to determine the effect of carnitine supplementation on muscle mass and strength in aged humans.

Methods: Twenty two female volunteers over 65 years old participated in the study. The subjects were randomly assigned into two groups: carnitine (n = 12) and placebo (n = 10). Both groups consumed gelatin capsules every day throughout the 24-week intervention period. Body weight and composition were determined using InBody720, before, at the mid-point and at the end of the supplementation period. Moreover, the dominant leg knee extensor peak torque, total work and average power were determined for all subjects on Biodex System 4 Pro in isokinetic and isometric exercise tests.

Results: Knee extensor average power and total work tended to be higher in the carnitine group compared to placebo after 24 weeks of supplementation. However, there were no differences in peak torque. Moreover, carnitine supplementation did not affect either body mass or body composition (skeletal muscle mass, body fat mass, protein mass).

Conclusions: Further studies are necessary to analyze the effect of carnitine supplementation on function in aged human skeletal muscle.

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Less Capability to Excrete Food Pesticide Residues in ASD Children

Mattia Orazi^{1*}, Maria Cavaliere¹, Valentina F. Domingues²,
 Marco Piangerelli³, Marina Marini⁴, Alessandro Ghezzi⁴, Paola Visconti⁵,
 Rosita Gabbianelli¹

¹School of Pharmacy, University of Camerino, 62032 Camerino, Italy;

²Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, 4200-072 Porto, Portugal; ³Computer Science Division, School of Science and Technology, University of Camerino, 62032 Camerino, Italy; ⁴DIMES, School of Medicine, Università di Bologna, 40126 Bologna, Italy and Don Carlo Gnocchi Foundation ONLUS, 20162 Milan, Italy; ⁵Institute of Neurological Sciences-Bologna, 40126 Bologna, Italy

E-Mail mattia.orazi@studenti.unicam.it

Objectives: Pyrethroids are widely used in agriculture as pest control and their residues are present in fruits and vegetables. Moreover the main pyrethroid metabolite, the 3-phenoxybenzoic acid (3-PBA), was detected in people's urine from different countries.

The objective of this study was to evaluate if there is a correlation between pyrethroid exposure and the incidence of Autism Spectrum Disorder (ASD). To reach this aim the level of 3-PBA in urine from ASD and Control (CTR) children was analyzed by GC-MS.

Methods: A total of 40 children were recruited in this case-control study. All were admitted to the Child Neuropsychiatric Unit of the Bellaria Hospital of Bologna (Neurological Sciences Institute IRCCS-Bologna). In these, 21 (17 males and 4 females) had a diagnosis of ASD, and 19 (15 males and 4 females) were Control children (CTR). In the ASD group, mean age was 6.9 years (SEM = 0.509); in CTR group, mean age was 7.4 years (SEM = 0.485).

ASD diagnosis was made according to the Diagnostic and Statistical Manual of Mental Disorders IV (DSM IV TR criteria, Autism Diagnostic Observation Schedule (ADOS) and Childhood Autism Rating Scale (CARS). CARS was between 31.5 and 47 (mean value = 39.265, SEM = 0.873).

The analytes were separated from the matrix by means of solid-phase extraction using a reversed-phase/strong cation exchange column. Separation and quantitative analysis was carried out by capillary gas chromatography and mass selective detection. The detection limits was 0.0038 µg per ml urine. The relative standard deviations of the within-series imprecision were always less than 10%.

Results: The average values in urine of each population were 1.48 µg/ml in ASD (Variance 4.94) and 0.63 µg/ml in CTR (Variance 0.22). A significant increase of 3-PBA with the age in CTR children was found ($R^2 = 0.2196$; $p = 0.043$), while no age-related change could be observed in ASD children ($R^2 = 0.0661$; $p > 0.05$). No correlation between CARS total score and 3-PBA in urine was observed ($R^2 = 0.0539$; $p > 0.05$). In two children (both male and 6 years of age) in ASD group we found very high values of 3-PBA, reaching values from 3 to 6 times higher than the average of the sample, respectively.

Conclusions: We could hypothesize that the increased level of 3-PBA in ASD children compared to CTR might be due to different factors like increased absorption (i.e. higher gut permeability) and/or differences in metabolization of 3-PBA (i.e. polymorphisms). Studies devoted to this aim might be object of future investigation.

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Influence of Cabbage Phytochemicals on the Formation of Oxidized Phospholipids in Meat Products

Karol Parchem, Agnieszka Bartoszek*

Department of Food Chemistry, Technology and Biotechnology, Gdansk University of Technology, Gabriela Narutowicza 11/12 Street, 80-233 Gdansk, Poland

E-Mail parchem.karol@gmail.com

Objectives: Phospholipids (PLs) are low or medium molecular weight substances, which are often characterized by high biological activity. PLs not only form lipid bilayer membranes but also impact their fluidity and permeability. Furthermore, membrane PLs act as precursors of numerous messenger substances in cell signaling, such as eicosanoids derived from fatty acids of n-3 family, anti-inflammatory prostaglandins of 5 series and leukotrienes of 3 series. Unsaturated fatty acids (UFA) present in the phospholipid structure are particularly vulnerable to oxidation, which leads to the formation of oxidized derivatives (OxPLs). On the one hand oxidative changes in membrane PLs can lead to disruption of functioning of cellular and organelle membranes, especially mitochondrial membranes. On the other hand OxPLs may lead to pathological conditions in cells by aberrant regulation numerous genes implicated in cell proliferation, differentiation, cellular stress, inflammation and lipid metabolism. Transcription factors activated by OxPLs include PPARs, Nrf-2 or ATF4. There are mechanisms capable of replacing oxidatively changed PLs by deleting the damaged fatty acids by the enzymes of phospholipases A₂ family. However, in such cases, it is important to provide dietary UFA especially in the form of phospholipids. Another approach is to limit ingestion of OxPLs by designing foods to be less conducive to oxidative processes. The aim of this study is to determine the effect of cabbage phytocomplex on oxidation of phospholipids present in the meat products.

Methods: Lipid fraction of combined meat/cabbage products containing cabbage phytocomplex was extracted using Folch method. The PLs composition was determined qualitatively and semi-quantitatively by thin-layer chromatography (TLC) and ³¹P nuclear magnetic resonance spectroscopy (³¹P NMR). Primary products of phospholipid oxidation (OxPLs) were separated by normal-phase TLC, visualized with N,N-dimethyl-p-phenylenediamine.

Results and Conclusion: Our research suggest that addition of cabbage phytocomplex exhibiting antioxidant properties can inhibit the oxidation of lipid compounds, including phospholipids, especially in the meat products exposed to elevated temperatures. Animal fat is characterized by low content of endogenous antioxidants and consequently readily undergoes oxidation. Therefore, the addition of phytochemicals to the meat products, especially heat processed may limit delivery of OxPLs with foods to the human body.

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Effects of Yerba Mate on Mitochondrial Biogenesis

Marcelo L. Ribeiro^{1*}, Tanila Wood dos Santos¹, Juliana C. Santos¹, Ana Aiastui²

¹Sao Francisco University, Bragança Paulista, SP, Brazil; ²Cell Culture Platform, Biodonostia Health Research Institute, Donostia-San Sebastian, Spain

E-Mail marcelo.ribeiro@usf.edu.br

Objectives: The purpose of this study was to determine the effect of yerba mate (YM) in mitochondrial biogenesis *in vitro* and *in vivo*.

Methods: We evaluated, using a C2C12 cells, the effects of YM (50; 100; 200; and 300 µg/ml) in the metabolic flux to understand cellular metabolism, and the measurement of oxygen consumption rate using the Seahorse XF Analyzer. Additionally, mtDNA content and expression of mitochondrial energy metabolism-related genes were investigated. A high-fat diet-induced mice model of obesity was used to evaluate the effects of YM (1 g/kg⁻¹) on muscle mitochondrial biogenesis after 8 weeks of intervention. Indirect calorimetry and food intake were assessed; oxygen consumption (VO₂), CO₂ production (VCO₂), respiratory quotient (RQ) and resting energy expenditure (RER) data were obtained at 6th week. Muscle biopsies were used to determine mtDNA content and gene expression.

Results: Our data shows that YM, at the highest concentrations (200 and 300 µg/ml), were able to increase the spare respiratory capacity. We also observed that YM increased the mtDNA content, which together suggests an increase in mitochondrial biogenesis. Complementarily, mRNA data indicated a significant increase in mitochondrial biogenesis-related genes, such as *Ampka2*, *Cd36*, *Creb1*, *Mttfa* and *Nrf1* after treatment with YM (50 µg/ml). We also observed an up-regulation on thermogenesis-related genes *Ucp1* and *Ucp3*. The high-fat diet-induced mice model of obesity indicated that the regular ingestion of YM extract significantly decreased the final body weight (48.7 ± 6.70 g), when compared with those fed the high-fat diet (55.7 ± 2.73 g). The weight loss was not related to a reduction in food intake. The mice in the high-fat diet–YM group had significantly less epididymal fat than the mice in the high-fat diet group. Indirect calorimetry showed that YM intervention significantly increased VO₂, VCO₂, RQ and RER in high-fat diet–YM group, compared with high-fat diet group. The muscle mtDNA content were significantly increased after YM treatment. Moreover, our data showed a downregulation on genes related with energy metabolism *Cd36*, *Mttfa*, *Creb1*, *Pgc1α*, *Sirt1*, *Ucp1* and *Ucp3*, in the high-fat diet group. The present study also showed that those mRNA levels were recovered after YM treatment.

Conclusions: In summary, the data presented in this study suggests that the YM is able to modulate bioenergetics mechanisms, which significantly improves energy expenditure, reflecting directly in weight control and body fat reduction.

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The Inherited Microbiome: Towards a Map of Microbial Strains and Functions Vertically Transmitted from Mother to Infant

Serena Manara^{1*}, Francesco Asnicar¹, Christian Milani², Leonardo Mancabelli², Gabriele Lugli², Sabrina Duranti², Francesca Turrone², Chiara Ferrario², Marta Mangifesta³, Alice Viappiani³, Pamela Ferretti¹, Valentina Gorfer⁴, Anna Pedrotti⁴, Federica Armanini¹, Adrian Tett¹, Marco Ventura², Nicola Segata¹

¹CIBIO, UNITN, Italy; ²Life Sciences Department, UNIPR, Italy; ³GenProbio, Parma, Italy; ⁴Azienda Provinciale Servizi Sanitari, Trento, Italy
E-Mail serena.manara@unitn.it

Objectives: The infant gut microbiome is established in the first days of life and rapidly evolves during the first few months. Elucidating how this process occurs and what are the primary sources of the colonizing microbiome could unravel how dysbiotic conditions arise and possibly how to prevent them. However, a comprehensive strain-level understanding of which infant-associated microbes are acquired from the mother is currently lacking. We aimed at assessing which active strains and microbial pathways are vertically transmitted from the gut and breast-milk microbiome of the mother to the infant gut.

Methods: We collected and shotgun sequenced stool samples obtained at multiple time points from mothers and their children during the first year of life. We also sequenced samples of maternal milk to investigate its role in shaping the gut microbiome of breast-fed infants. Metatranscriptomics was applied to investigate the differential expression profiles of the vertically transmitted bacterial strains in the gut of mothers and their children.

Results: A number of species were found to be shared between mothers and their children. For many of them the same exact strain was present in mother/infant couples while being different in other couples, strongly suggesting vertical transmission. At early time points, shared strains mainly consisted of *Bifidobacteria* and *Escherichia coli*. We observed the development of the post-weaning microbiome toward a more adult-like structure, with additional sharing of several Lachnospiraceae. A rapid change was observed in the functional potential of the post-weaning microbiome, where pathways highly represented in children approached lower adult-like levels. Metatranscriptomics further highlighted differential transcriptional patterns of vertically transmitted strains in mothers and children, with genes and pathways exclusively expressed in either one or the other, suggesting intriguing transcriptional adaptation patterns.

Conclusions: By combining mother/infant longitudinal microbiome sampling and strain-level computational profiling, we showed that it is possible to track the vertical transmission of members of the microbiome. Applied to larger cohorts, this will permit compiling a catalog of strains that are naturally transmitted from mothers to infants that can be a source of probiotic strains for non-physiological births and nutrition regimes.

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Microbioma Italiano: Preliminary Analysis of the Italian Gut Microbiome Composition

*Eleonora Sattin¹, Barbara Arredi¹, Andrea Telatin², Diego Bacchin¹,
Giorgio Malacrida¹, Fabio Piccini³, Barbara Simionati¹*

¹BMR Genomics srl, Via Redipuglia 21A, 35121 Padua, Italy; ²CRIBI Biotech Center, University of Padua, Via Ugo Bassi 58/B, 35121 Padua, Italy;

³Medicine Department, University of Marche, Square Roma 22, 60121 Ancona, Italy

E-Mail Eleonora.sattin@bmr-genomics.it

Objectives: More than ten years of research highlighted that human microbiome is a complex field, in which the reliability of the results is strongly influenced by the sample size [Qin et al. (2010)]. Many studies showed that gut microbiome is very different across populations and countries [Yatsunenko et al. (2012)], thus some large studies have focused on the microbiome of particular countries (i.e. American Gut or British Gut projects). Microbioma Italiano (Italian Microbiome Project) is a citizen science project that aims to map the Italian lifestyle with its microbiome, defining the typical gut composition for this Country. We've set an online platform (www.microbiomaitaliano.it) through which people can order their own kit for stool sampling and to receive their analysis. Participants' lifestyle surveys connected to sequencing data will build an open source database. Moreover our aim is to develop a home-service to help Italian people to monitor their health and eventually improve it.

Methods: Since September 2015 we have sequenced 98 samples with the connected survey. We extracted DNA using Mobio powerfecal kit and sequenced V3-V4 16S hypervariable regions with Illumina Miseq platform with 300PE approach. Bioinformatic analysis were performed by means QIIME 1.9.1.

Results: We obtained an average of 281141 reads (minimum 52467; maximum 584004) that showed an average of 448 OTUs (minimum 146; maximum 1142). Here we are presenting the preliminary analysis on diverse microbiome composition according to different diets and lifestyle habits.

Conclusions: We have set up an innovative science project based on active collaboration with citizen using the most cutting-edge Next Generation Sequencing technologies. The sequencing depth we are obtaining is allowing us to well characterize Italian microbiome in relation to different diets and lifestyles. By means this approach we'd like to turn scientific knowledge into concrete advantages for Italian citizen and contribute to a better worldwide understanding of this micro-macro-world.

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