Duchenne muscular dystrophy (DMD) patients present alteration of gastrointestinal motility and suffer from constipation, pseudo-obstruction, acute dilatation: although no attention was paid to investigate these processes, smooth muscle fibrosis was observed throughout the gastrointestinal tract [1]. Similarly, the mdx mice shared impairments in the intestinal contractility, linked to important abnormalities of the mucosal epithelial morphology normally associated to inflammatory state [2,3]. These evidences would suggest the contribution of alternative pathological pathways, other than the musculoskeletal one, in the disease pathogenesis. Skeletal muscle inflammation in mdx has a peak at 7 weeks and decreases at 12 weeks: cellular infiltrates involve both macrophage and pro-inflammatory CD4/CD8+ T-cells, whose amount remain elevated throughout the life of mice [4]. On the contrary, although it is known that mdx intestinal wall is inflamed, few is known regarding the contribution of different immune subpopulations: altered gut microbiota together with modified mucosal permeability could unbalance gut homeostasis favouring the expansion of inflammatory T-lymphocytes reactive against muscular proteins [5]. We suggest that dystrophic intestinal inflammation is dependent on the relationship between the intestine and its microbiota and that this axis is responsible for spreading the inflammatory cues throughout the muscles. For the first time, we have analysed the microbiota of mdx at 10-weeks and 9-months old (n=3 each) and we detected important differences among these mice. Thus, we demonstrated that following specific anti-inflammatory treatment mdx mice showed alteration of gut microbiota, especially in older ones. This way, we will unravel the role of microbiota in DMD aetiology and its contribution in determining immune system activation leading to muscle damage. If intestinal commensals promote the development of the disease, we will attempt to identify the microorganisms involved. As a result, we will employ different strategies to modulate the microbiota in mdx mice to counteract the development of intestinal inflammation and its effect on muscle degeneration. These proof-of-concept pre-clinical studies will lead to microbiota studies in DMD cohort and, if successful, will open to microbiota manipulation for therapeutic purpose in DMD patients.

References
P11 & FP - Gemcitabine chemotherapy shapes microbiota promoting pro-inflammatory state of pancreatic cancer xenografted mice.

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Background: Pancreatic ductal adenocarcinoma (PDAC) represents the fourth cause of cancer-related death.

Objective: We aimed to evaluate whether gemcitabine treatment shapes the gut microbiota in a model of PDAC xenografted mice.

Methodology: Pancreatic cancer xenograft mice were subjected to gemcitabine injection once per week for three weeks to assess the tumor volume as compared to control mice injected with normal saline solution. The composition of fecal microbiota, the activation of NF-κB pathway in cancer tissues and the serum metabolomics were further analyzed.

Results: Gemcitabine considerably decreases the proportion of Gram-positive Firmicutes (from about 39 to 17%) and the Gram-negative Bacteroidetes (from 38 to 17%) which are the two dominant phyla in the gut of tumor-bearing control mice. This downshift was replaced by an increase of Proteobacteria (Escherichia coli and Aeromonas hydrophila) from 15 up to 32% and Verrucomicrobia (Akkermansia muciniphila) from 5 to 33% in the gut of drug-receiving mice. An overall increase in inflammation-associated bacteria was observed upon gemcitabine. Consistently, activation of the NF-κB canonical pathway was found in cancer tissues from gemcitabine-treated mice. Serum metabolomics revealed a significant decrease of the purine compounds inosine and xanthine, and a decreasing trend for their metabolically-related molecule hypoxanthine.

Conclusions / Implications for practice: Understanding chemotherapy side effects may explain the lack of activity or the chemoresistant processes and it may help to set up strategies to improve the effectiveness of therapy.

References
P12 & FP - Metagenomic analysis of gut microbiota in Myasthenia Gravis and Multiple Sclerosis patients

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Background: Growing evidences support the role of gut microbiota in immune-mediated diseases, by influencing the immune system activation and controlling the pro- and anti-inflammatory balance. These aspects may play a role in autoimmune diseases, where predisposing genetic factors and environmental components could trigger pathogenic mechanisms, and an altered microbiota could contribute to disease progression (1). Data coming from animal models of autoimmune diseases and from patients demonstrated an alteration of intestinal microbial composition (2).

Objective: Aim of the present study was the characterization of the gut microbiota composition in myasthenia gravis (MG) and multiple sclerosis (MS) patients and in healthy donors - HD.

Methodology: The study was done on 49 MG, 31 MS patients, and 14 HD subjects, on voluntary bases. Bacterial DNA was isolated by combining classical lysis method and mechanical dissociation. To evaluate gut eubiosis, the ratio of Firmicutes and Bacteroidetes phyla was calculated by real-time qPCR. Metagenomic analysis was performed in 20 MG, 22 MS and in 7 HD subjects on hypervariable regions of bacterial 16S rRNA with the Ion Torrent PGM System platform.

Results: Firmicutes/Bacteroidetes ratio > 60, indicative of dysbiosis, was observed in 11/49 (22.4%) MG, in 5/31 (16.1%) MS patients, and in 4/14 (28.6%) HD subjects, without statistical significance (chi-square 0.97, p ns). 16S rRNA was performed; taxonomic analysis at the phylum level showed a decrease of Firmicutes proportion (MG 52.7%; MS 58.5%) compared with HD (72.6%; p<0.01) and increase of Bacteroidetes proportion (MG 29.3%; MS 26.5%) compared with HD (9.1%; p<0.01). Moreover, Actinobacteria slightly decreased in MG patients (3.5%) compared to healthy subjects (14.4%).

Conclusions: Our study shows differences in bacterial components of the gut microbiota in MG and MS patients compared with HD. Modifications of relative abundancies among microbiota may underly dysregulatory mechanisms affecting immune-inflammatory pathways that deserve further investigations. These studies may allow the identification of bacterial strains with immunoregulatory profile that could be tested in preclinical research.

References
P13 & FP – The postoperative gut microbiota impacts the weight loss and the general health improvement in obese patients

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Obesity is an abnormal or excessive fat accumulation, with life-threatening sequelae. The increased burden of obesity has inflated the request for bariatric surgery (1). Among the factors influencing the surgery-mediated weight loss, the gut microbiota seems to be pivotal (2).

Our aim was that of investigating the impact of postoperative gut microbiota on weight loss and health improvement in obese patients.

By Next Generation Sequencing, we conducted a prospective longitudinal study on 27 healthy lean subjects and 25 obese patients undergoing elective bariatric surgery (n° 12 Roux-en-Y Gastric Bypass, RYGB, and n° 13 Laparoscopic Sleeve Gastrectomy, LSG). Inclusion criteria for lean subjects were the absence of morbidity and age 18 to 65; inclusion criteria for obese patients complied with the international bariatric guidelines. Anthropometric and metabolic data, smoke habits, and stool samples were collected from lean subjects and from obese patients before, 3 and 6 months after surgery (T0, T3 and T6, respectively). A food preference questionnaire was administered both to lean subjects and to bariatric patients at T0, T3 and T6. Differences in microbial community composition were investigated using QIIME 1.9.1. The p values were corrected for False Discovery Rate.

The gut microbiome of obese patients before surgery (T0) was not statistically different from that of the lean patients’ group. Instead, at T3 but not at T6 after RYGB, the gut pathogens Yokenella regensbergei (p=0.03) and Fusobacterium varium (p=4E-05) and the oral bacteria Veillonella dispar, Veillonella atypica, Streptococcus australis, and Streptococcus gordonii (p<0.05) were increased. Conversely, Akkermansia muciniphila (p<0.05) was permanently increased. In LSG, we observed a modified profile of the microbiota without significant changes. On the metabolic point of view, after RYGB, the microbiota composition was correlated to the excess weight loss and the improvement of hypercholesterolemia and hypertension. After LSG, the microbial variations were correlated to the changes in food preferences.

The bariatric surgery significantly impacts the microbiota composition depending on the surgical technique performed. RYGB implies significant microbial changes which are linked to the weight loss and the general health improvement. Probably, it depends on the anatomical rearrangement of the bowel after the procedure. Conversely, the LSG induces weight loss without significantly affecting the microbiota.

References
P14 & FP – Targeting bacterial adhesion: synthesis and on-cell NMR-binding studies of new FimH multivalent ligands

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Background: Bacterial adhesion is the first step in pathogen infection and bacterial adhesins are prime candidates as targets for antibacterial therapeutics such as specific-ligand-like inhibitors and vaccines [1]. Type 1 fimbriae of Escherichia coli are filamentous appendages that confer bacterial binding to glycoproteins with terminally exposed mannose [2]. Mannose-specific binding is mediated by a 30-kDa adhesive protein, FimH. FimH is a virulence factor and an attractive therapeutic target for urinary tract infection (UTI) and Crohn's Disease (CD) [3].

Objective: Aim of this work was the development of new multivalent FimH ligands able to target this adhesin thus preventing the D-mannose sensitive adhesion of pathogenic bacteria to mammalian cells. Moreover, we wanted to set up an NMR-based assay allowing a very rapid screening of FimH ligands and the structural characterization of their binding mode to bacterial cells expressing the protein on their surface.

Methodology: Monodispersed glycodendrimer based on pentaerythritol core and bearing a different number a FimH natural ligand, i.e. D-mannose, were synthesised through a convergent modular strategy exploiting Cu(I)-catalyzed azide-alkyne cycloaddition conjugation reaction (CuAAC, “click chemistry”). Compound ability to inhibit D-mannose sensitive adhesion was tested by yeast agglutination inhibition assay [4]. The STD-NMR experiment acquired on samples containing bacterial living cells [5] was exploited to develop a rapid and sensitive experiment allowing verifying compound ability to bind FimH on E. coli cell surface.

Results: We synthesised a small library of potential FimH ligands constituted by glycodendrimers functionalized with a different number of D-mannose units (Man4, Man6, Man18). STD-NMR experiments on living cells (E. coli CFT073, an uropathogenic strain expressing a high level of FimH) were set up to test compound ability to bind FimH on cell surface. A E. coli CFT073 ΔFimH strain was employed as negative control.

As expected, we found a strong correlation between the number of D-mannose units and compound affinity/inhibition potency. In particular, Man18 showed a MIC of 56 μM.

Conclusions / Implications for practice: The STD-NMR-based methodology and FimH ligands and inhibitors here described represent new tools towards effective therapeutic strategies against FimH-mediated bacterial adhesion, in particular for the treatment of UTI and CD. Moreover, our study demonstrates the versatility of the STD-NMR experiment, also exploitable for the characterization of recognition events involving other proteins expressed on bacterial cell surface.

References
P15 & FP – Effects of Probiotics and Prebiotics on Human Health

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Abstract: The last few years witnessed a major scientific breakthrough with the emergence of gut microbiota as a key player in host health maintenance and disease pathogenesis. It is well established that a stable and diverse gut microbiota is essential to various host physiologic functions such as immunoregulation, pathogen prevention, and metabolism. Beyond that, it is becoming clearer that an imbalance in the microbial community contributes to the pathogenesis of both intestinal and extra-intestinal disorders. Thus, approaches aiming to restore or prevent gut dysbiosis represent a new strategy to improve treatment efficacy and disease prevention. In this regard, we aimed to evaluate the probiotic potential of newly isolated *Lactobacillus* and *Bifidobacterium* strains and to assess the possibility to combine them with plant based carbohydrate prebiotics to support their growth and efficacy. The probiotic properties of *Lactobacillus acidophilus* LA, *Lactobacillus fermentum* LF, *Lactobacillus plantarum* LP, *Lactobacillus reuteri* LR, *Lactobacillus rhamnosus* LRh, *Bifidobacterium animalis* subsp. lactis BL and *Bifidobacterium longum* BLg strains were characterized in vitro by determining their tolerance to low pH and to bile salts, antibiotic sensitivity, antimicrobial activity and by considering their ability to modulate the inflammatory status of HT-29 cells. Moreover, carbohydrates were extracted and purified from plants using several extraction methods, and then their degree of polymerization (DP) was determined. Finally, their ability to support the growth of the probiotics strains was assessed. The bacterial strains, *Lactobacillus plantarum* LP, *Lactobacillus rhamnosus* LRh and *Bifidobacterium animalis* subsp. lactis BL showed good survival at low PH and were sensitive to all common antibiotics treatment. They were able to antagonize the growth of various pathogens with strong potential against *Pseudomonas aeruginosa* and *Escherichia coli* by secreting high amounts of acidic metabolites such as lactic acid. These strains were also able to modulate the release inflammatory cytokines such as IL-4, IL-10 and TNF-α and to increase the antioxidant potential in HT-29 cell line. Besides inulin and fructooligosaccharides (FOS) were extracted and purified at good yields by application of ultrasound-assisted method. In vitro fermentation revealed that mixtures of fructooligosaccharides of short degree of polymerization (DP<10) were highly fermented by these strains, while limited growth was observed on inulin with greater degree of polymerization (10 <DP> 60). These results suggest that these new strains show a good probiotic potential and their ability to grow on plant based carbohydrate source support the possibility of a pre/probiotics combination for an enhanced therapeutic efficacy.

References


P16 & FP – DNase I-Degraded Bacterial Genomic DNA Augments Activation of Human Toll-Like Receptor 9 by Synthetic DNA Oligonucleotides

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**Background:** Human Toll-like receptor 9 (TLR9), an endosomal pattern recognition receptor of the innate immune system, responds to specific DNA motifs, such as dimers of unmethylated cytosine and guanine (CpG), which are more abundant in bacterial than human genomes. Previous studies demonstrated that synthetic DNA oligonucleotides (ODNs) of length 20-24nt directly activate TLR9 *in vitro*, and that TLR9 activation by ODNs can be augmented by co-stimulation with CpG-containing short ODNs (sODNs) of length 2-7nt, which do not activate on their own.

**Objective:** We seek to determine and compare the role of genomic DNA from different bacterial species for human TLR9 stimulation, both as direct activators and as enhancers of TLR9 activation by other ligands.

**Methodology:** Genomic DNA of varying sequence-based predicted CpG concentrations from different bacterial species was isolated and treated with DNase I. Ramos Blue B cells were stimulated with combinations of synthetic oligonucleotides (ODN-2006, ODN-2006GC), short ODNs (TCGTT and TTTTT), and untreated or DNase I-digested bacterial genomic DNA. TLR9 response was measured as NF-κB induction via expression of the secreted embryonic alkaline phosphatase (SEAP) reporter gene.

**Results:** While stimulation with untreated bacterial genomic DNA induced less NF-κB than with ODN-2006, co-stimulation of even smaller amounts of ODN-2006, ODN-2006GC, and bacterial genomic DNA from different species with the sODN TCGTT enhanced TLR9 induction. In addition, DNase I-digested bacterial genomic DNA, which did not activate TLR9 on its own, augmented TLR9 activation by ODN-2006 at low concentrations. This “boost” effect of degraded DNA on TLR9 activation by ODN-2006 was dependent on the bacterial genomic CpG content.

**Conclusion:** The potential of microbial genomic DNA to activate human TLR9 may be dependent on a combination of direct stimulation by larger genomic DNA fragments and indirect enhancement of activation through other ligands by very short, degraded genomic DNA fragments.
P17 & FP – HPV-mediated cytological abnormalities and high-risk HPV genotypes associate with altered gut microbiota composition and function in cART-treated HIV+ males

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**Background:** HIV-infected individuals feature higher incidence of HPV persistence at both vaginal and anal level (1,2). Recently, the gut microbiota has been demonstrated to predict precancerous anal lesions (3), suggesting that some taxa featuring HIV-associated dysbiosis might fuel HPV persistence and pathogenesis.

**Objective:** We decided to explore whether the presence of HPV-related cytological abnormalities might be associated with bacteria functional modifications and with HIV-mediated gut mucosal dysfunction (i.e. increased gut permeability, microbial translocation -MT- and consequent immune activation -IA-) within the anal district of cART-treated HIV+ males.

**Methodology:** We enrolled 36 HIV+ males on cART (HIV-RNA<40cp/ml) and collected anal swab, blood and stool samples.

Normal cytology: absence of cytological abnormalities; altered cytology: presence of ASCUS, LSIL, HSIL. Lab analyses: (i) anal HPV genotyping and cytological evaluation; (ii) fecal microbiota composition (relative abundance, α-/β-diversity); (iii) bacterial metagenome prediction (PICRUSt); (iv) intestinal permeability (Calprotectin, I-FABP); (v) MT (sCD14, LPS, 16S rDNA); (vi) T-cell activation (CD8+CD38, CD8+CD38+CD45R0+). Mann-Whitney, with Bonferroni’s correction and Chi-squared tests were used.

**Results:** We identified 30/36 (83%) HPV+ patients, 24/30 harboring high-risk HPV genotypes (hrHPV). Of these, 18 had HPV-related cytological abnormalities w/o neoplasia (aHPV, Fig.1a).

aHPV patients showed a marked dysbiosis, with higher proportion of Prevotellaceae and lower Leuconostocaceae (Fig.1b). Interestingly, the presence of high-risk HPV genotypes, irrespective of cytological abnormalities, seemed to have a greater impact on gut dysbiosis, with hrHPV displaying higher proportion of Prevotellaceae and Veillonellaceae, but lower Bacteroidaceae, Lachnospiraceae and Rikenellaceae as compared to lrHPV (Fig.1c).

This shift in bacterial composition was accompanied by changes in predicted metabolic capacity (Fig.1d). Indeed, HIV+ patients with HPV-mediated cytological abnormalities and/or high-risk HPV genotypes showed increased abundance of genes related to immune system activation and to metabolic syndrome.

While the presence of HPV-mediated cytological abnormalities and high-risk HPV genotypes associates with gut dysbiosis, we failed to detect any difference in markers of intestinal permeability, MT and IA.

**Conclusions:** In cART-treated HIV+ males, the presence of HPV-related cytological abnormalities within the anal district is characterized by unique bacteria composition and functional metagenomic capacity, supporting a pathogenic link between gut microbiota and HPV. From a clinical standpoint, the observations of a Prevotellaceae-rich/Bacteroidaceae-poor profile, coupled with changes in metabolites involved in sustaining immune activation and co-morbidities seem to support the establishment of a pro-inflammatory environment that favors high-risk HPV genotype persistence and HPV-mediated cytological abnormalities.
References

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P18 & FP – Gut microbiota lipopolysaccharides: reverting the concept from bad to good

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The Gut Microbiota (GM) is an essential actor in the modern concept of human health driving many host physiological and pathological processes, including immune system modulation. Initial sensing of microbes by the host immunity is mediated by the recognition of microbial-associated molecular patterns, such as lipopolysaccharides (LPS), which are highly conserved among bacteria, thus shared by both commensals and pathogens. The LPS structure strongly influences the biological effects on the host immune system. Defined LPS structures can act as potent agonists on the immune receptors whereas others can operate as antagonists reducing or inhibiting the cytokine production otherwise induced by toxic LPSs.\(^1,2\) Thus, a crucial question to address is how the immune system distinguishes between permanently established commensal LPS and pathogen LPS. The elucidation of the structure and the immunological activity of LPS isolated from gut microbes will bear new advances in the medicinal chemistry and in the field of search of new molecules able to antagonize pathogens LPS effect, as well as of GM LPS-inspired molecules able to prevent uncontrolled host immune response against our microbiota. This will also shed light on the structure-activity relationship of LPS itself, which is an open question in immunology field. In particular, this will improve the knowledge of the still poorly investigated GM world, giving insights in the host-microbe interaction mechanisms both at intestinal and systemic level furnishing, in parallel, information about the elicitation/modulation of immune response triggered by pathogens and commensals LPS, thus improving the overall knowledge of the immune system.

In this communication, I will show some very recently elucidated GM LPS structures and their immunological properties that revealed to express unique and interesting features. Among others, I will discuss about the structure and activity of LPS from \textit{Bacteroides vulgatus} mpk, a commensal bacterium whose beneficial effects on health were clearly demonstrated.\(^3,4\) The full structure of such an LPS and of all the LPSs that will be presented, was defined by employment of a multi-technique approach comprising wet chemistry, NMR spectroscopy and mass spectrometry. The evaluation of the immunological properties of the above GM LPS has been investigated and will be also presented.

Insights gained from the structural and molecular analysis of GM LPSs might also help to chemically design novel inflammation-silencing drugs as a potential alternative therapeutic approach for the treatment of inflammatory disorders.

References

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P19 & FP – Evaluation of bioinformatic methods to reveal drinking water microbial community

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The drinking water standards are set at levels necessary to protect the public from acute and chronic health risks associated with consuming contaminants in drinking water supplies. Although there are several indicators to evaluate treatment efficiency and product water quality, they do not allow to explore in depth the complex biological communities, an important field of research and public health, and the microbial population analysis is useful to optimize treatment systems and to verify the effectiveness of control and prevention actions as envisaged by the Water Safety Plan. Entire bacterial communities can be studied quickly and inexpensively using the environmental DNA metabarcoding approach, that it has increased dramatically in recent years for the detection of rare, elusive, invasive, or threatened wildlife species, helping us to routinely survey bacterial and fungal taxonomic communities, overcoming limits due to conventional culture-based methods. In this study, we compare two DNA metabarcoding bioinformatic pipelines to analyze HTS (High-Throughput DNA Sequencing) data for the monitoring of drinking water microbial community. In particular, we sequenced 84 samples in different steps of the potabilization belonging to process: raw water (groundwater), water passed through carbon filters and post-chlorination water. The sampling campaign lasted one year, with water samples collected monthly from a DWTP (Drinking Water Treatment Plant) located in Milan. Moreover, we had the opportunity to sample in parallel a second DWTP located in Milan for two months. We used the 16S ssuRNA molecular marker (V3-V4 hypervariable regions) for the diversity estimation of Bacteria. Sequences obtained with HTS technology were processed with two different pipelines designed with VSEARCH algorithm, calculating OTUs at a 97% of identity, a standard approach for Bacteria reads clustering, and the QIIME2 package, calculating ASV (amplicon sequence variants) with DADA2 algorithm. Reads were assigned with two different taxonomy classifier and two version of SILVA 16S database, a pre-clustered and curate database of 16S region with a formatted taxonomy. Our results showed the strength of samples’ molecular information describing the differences in community composition in the three main sites, they allowed us to describe the taxonomy information in microbial community composition of drinking water and, moreover, highlighted the main differences between pipelines and database versions. From this study, we conclude that metagenomic amplicon sequencing is an informative method to support current compliance-based methods and can be used to reveal bacterial community information for the evaluation of drinking water quality, in order to protect the public health.

References
P20 & FP – Investigation of Novel biomarkers and Definition of role of microbiome In Graves’ Orbitopathy (GO) (INDIGO): Microbiota analysis of patients at recruitment

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Background: In the past years, the microbiome of patients and animal models for inflammatory diseases and autoimmune conditions was extensively investigated. In the context of autoimmune thyroid diseases (AITD), the gut microbiota is thought to be implicated in the development of conditions, such as Hashimoto’s thyroiditis[1]. However, little is known about its role in Graves’ disease (GD), characterized by thyroid-stimulating auto-antibodies (TSAb) causing hyperthyroidism and in the concomitant eye disease Graves’ orbitopathy (GO). Since the balance between pro-inflammatory Th17 cells and anti-inflammatory T-reg cells seems to be compromised in the presence of specific pro-inflammatory bacterial strains[2], we hypothesized that the presence or the absence not only of pathogens, but also of symbiotic and commensal bacteria can favour an immune response more prone to inflammation and conducive to GD, and possibly sustain its progression to GO[3]. To address such hypothesis, we characterized the gut microbiota of GD (n=65) and GO (n=56) patients, enrolled within the EU INDIGO project from different centres across Europe, and compared to that of matched healthy controls (n=42).

Methodology: Metataxonomics (16S rRNA gene sequencing) was performed on total bacterial DNA extracted from both patients and controls’ faecal samples, targeting the V1-V2 plus bifidobacteria regions. QIIME pipeline was used to process the sequences, while statistical analysis was performed in R. A subset of faecal samples was also evaluated using traditional microbiology.

Results: The within-sample alpha and between-sample beta diversity indices were similar in patients and controls. However when considering the taxonomic composition, counts from the phylum Bacteroidetes were significantly more abundant in controls (38.5%) than in GD (24.2%) and GO (27.3%) (p=0.012), whilst Firmicutes were more abundant in GD (59%) and GO (60.5%) than controls (53.2%). Consequently, the Firmicutes:Bacteroidetes ratio was significantly higher in GD/GO than controls, but similar between GD and GO. At a genus level, Bacteroides was reduced in GD and GO patients with mild disease (p=0.012) compared to controls, but not between moderate-severe GO compared to controls. Reduction in the genus Bacteroides was observed also in two patients who developed GO during the study (BH adjusted p<0.0001), also confirmed using traditional microbiology techniques. Furthermore, Enterococcus gallinarum counts, a pathobiont reportedly involved in triggering autoimmunity[4], though low overall, were significantly higher in GD and GO than controls.

Conclusions: Our data illustrate substantial perturbation of the gut microbiota in GD/GO, which may be driven by hyperthyroidism. Reduction in the genus Bacteroides was also recently reported in the GO mouse model compared
to control mice\textsuperscript{[5]}. Future analyses will explore correlations between taxonomic profiles and TSAB auto-antibodies levels, thyroid function and GO disease severity and whether they are affected by treatment.

**References**


Supported by Marie-Skłodowska Curie Industry-Academia Pathways and Partnerships (IAPP) action, GA number 612116 project INDIGO.
P21 & FP – Phytochemistry and antibacterial activity of the essential oil of *Mentha suaveolens* Ehrh

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During an ethnopharmacological survey carried out in the Khénifra region, we have noted that the most widespread and most treated pathologies are the digestive disorders. Mentha Suaveolens Ehrh is one of the herbs used to relieve these infections. Indeed, this study aims to confirm the traditional know-how of the population surveyed through the evaluation of the antibacterial activity of the essential oil, the identification of the chemical composition and the isolation of the active ingredients responsible for this activity by chromatographic and spectroscopic methods (CCM, CG, SM, RMN $^1$H, RMN $^{13}$C et RMN-DEPT $^{13}$C).

Essential oil extracted from the aerial part of *Mentha Suaveolens* Ehrh, is obtained by hydrodistillation in a Clevenger type apparatus, its yield is 4.32% relative to the dry matter.

We selected nine microorganisms responsible for digestive infections to achieve the antibacterial activity of the essential oil of *Mentha Suaveolens* Ehrh, il is *Klebsiella pneumonia*1, *Escherichia coli* (Résistante et Sensible), *Staphylococcus aureus* BLACT, *Enterococcus faecalis*, *serratia fonticola*, *Acinetobacter baumannii*, *klebsiella oxytoca* and *Enterobacter aerogenes*, *P.aeuroginosa*. The essential oil of *mentha suaveolens* marked a strong activity with respect to *Klebsiella pneumoniae*, *Escherichia coli* (resistance and sensible), *Enterococcus faecalis*, *serratia fonticola*, *Acinetobacter baumannii* and *klebsiella oxytoca*, however, it is inactive against *Staphylococcus aureus* BLACT, *Enterobacter aerogenes* and *P.aeuroginosa*. The essential oil of *Mentha Suaveolens* Ehrh showed a very strong antibacterial power compared to the standard antibiotics Fox 30, TIM 58 and PRL 100.

The analysis performed by GC / MS has allowed us to identify the chemical composition of the essential oil extracted from *Mentha Suaveolens* Ehrh, the major components of this HE are *Piperitenone oxide* (75.50%), *piperitenone* (5.55%), *beta-caryophyllene* (2.02%), *limonene* (1.68%), *terpinen-4 -ol* (1.27%) and *pulegone* (1.05%).

The antibacterial activity of the marked *Mentha Suaveolens* Ehrh essential oil is related to its chemical composition, Indeed, this species was fractionated on an open column of silica, using an eluent (hexane / ether), of increasing polarity with a view to isolating *Piperitenone oxide* (75.50%) and *piperitenone* (5.55%).

Key words: *Mentha Suaveolens* Ehrh, essential oil, chemical composition, antibacterial activity, antibiotic.
P22 & FP – NLRP3 lacking leucine-rich repeat domain can be fully activated by the canonical inflammasome pathway

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NLRP3 inflammasome is a multiprotein complex mediating inflammatory response in a variety of metabolic and degenerative diseases. Upon activation with diverse triggers NLRP3 oligomerizes, recruits adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD), and the effector protein pro-caspase-1. Inflammasome assembly leads to autoproteolytic activation of caspase-1, which processes IL-1β and IL-18 cytokines to their mature form and gasdermin D to a pore-forming protein that induces pyroptotic cell death. Although the role of NLRP3 in various pathologies has been described, not much is known about the molecular mechanism of NLRP3 inflammasome activation.

In order to define the role of particular domains of NLRP3 in inflammasome trigger sensing, assembly and autoregulation systematic truncation of NLRP3 and reconstitution of NLRP3 variants in NLRP3-deficient macrophages was performed. We demonstrate that LRR domain is dispensable for NLRP3 activation and self-regulation. A minimal NLRP3 truncation variant was found fully responsive to various canonical NLRP3 activators. Using bioluminescence resonance energy transfer we showed that further truncation led to the variant that still changed conformation in response to canonical NLRP3 inflammasome trigger yet failed to form a functional inflammasome, even when a disease-causing point mutation was introduced. Substitution of the pyrin domain of NLRP3 with the CARD domain of NLRC4 or ASC led to a constitutive activation, demonstrating that the pyrin domain restricts NLRP3 in an inactive conformation.

NLRC4 inflammasome is formed by self-catalytic polymerization of NLRC4 initiated with bacterial ligand/NAIP complex. We were interested whether similar process is involved in NLRP3 activation. We show that pathological mutations of NLRP3 failed to engage wild-type NLRP3 in a self-catalytic oligomerization, demonstrating that the activating signal is not enhanced at the level of NLRP3 oligomerization, representing an additional level of NLRP3 regulation.

These results contribute to the understanding of the molecular basis of NLRP3 inflammasome activation and demonstrate the versatility of recognition and regulation mechanisms of the innate immune receptors.
Development of a novel TNF-driven mouse model of gut & joint inflammation

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Abstract: The incidence of inflammatory diseases, including IBD and arthritis, have strongly increased in Western society (Bach et al., 2002). These diseases are associated with shifts in the microbiota ecological structure and complexity (dysbiosis), suggesting a contribution to disease development. It has been shown that the intestinal microbiota has profound effects on host’s physiology and immunity through the production of specific metabolites (Blacher et al., 2017), but the exact mechanisms explaining the link between microbiota dysbiosis, gut and joint pathology have yet to be found. Relevant mouse models which recapitulate the clinical features of human pathology which are influenced by the intestinal microbiota are pivotal to unravel these mechanisms. Previous studies have shown that mice which express increased TNF levels spontaneously develop intestinal inflammation and arthritis, and this phenotype is strongly impacted by the intestinal gut microbiota composition (Kontoyiannis et al., 1999; Schaubeck et al., Gut 2016). We wish to investigate diet-microbiota-immune networks in a TNF-driven genetic mouse model of gut & joint disease, using CRISPR/CAS9 gene editing technology. By targeting the AU-rich elements in the mouse tnf gene, we generated mice with more stable TNF mRNA. SPF-raised Tnf^{emΔARE} mice have increased TNF expression leading to inflammatory pathology in the intestine and joints. Initial analysis reveals that Tnf^{emΔARE} mice develop spontaneous ileitis and enthesitis as previously described (Kontoyiannis et al., 1999). In addition, bone marrow derived macrophages secrete increased levels of TNF in response to LPS stimulation. We recently established a new germfree and gnotobiotic mouse facility at Ghent University, and Tnf^{emΔARE} mice are currently being rederived germfree for host-microbiota interaction studies in gnotobiotic experiments. We have developed a new TNF/microbiota-driven mouse model of combined gut & joint inflammation, which can serve as preclinical platform to study arthritis and gut inflammation and to test novel therapies, including therapeutic strategies interfering with the microbiota composition.

References
P24 & FP – Lipid-based nutrient supplement fortified with prebiotics increase SCFA production and enhance bifidobacterial growth in moderately undernourished infants’ microbiota

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Background: The central role of gut microbiota in undernutrition, an underlying cause of 45% deaths in children under five in developing countries, is now recognized. Proof of concept studies using methods to produce results that can be extrapolated to human subjects are needed to document new therapeutics including gut health as now recommended.

Objective: The study aimed to assess the effect of lipid-based nutrient supplement fortified with inulin and FOS (LNSp) on moderately acute malnourished (MAM) infants’ colonic microbiota in a Simulator of the Human Intestinal Microbial Ecosystem (SHIME).

Methodology: Faecal samples were obtained from 3 MAM infants aged 10 months living in rural Burkina Faso, West Africa.

Faecal innocula were prepared and submitted to 3 treatment conditions during 13 days in a SHIME model: control (SHIME feed alone), LNS (SHIME feed +2g LNS) and LNSp (SHIME feed + 2g LNS + 1.5g native inulin +1.5g FOS)

Samples were taken daily and analysed for short chain fatty acids (SCFA) namely acetate, propionate, butyrate, branched chain fatty acids (BCFA) content, and to determine the microbial composition through Illumina sequencing. Differences in SCFA production between treatment conditions were analysed using linear mixed models. To assess the shift in microbial composition, a differential expression analysis were performed to identify operational taxonomic units (OTU) that were most affected by treatment conditions.

All statistical analysis were performed in R version 3.5.0. Alpha was set at 5% for all hypothesis tests.

Results: SCFA concentration was obtained for all samples (n=117). LNSp induced a significantly higher production of acetate, propionate and butyrate compared to control and LNS. When LNS were compared to control, all but acetate production were statistically similar. The production of BCFA was significantly higher in the LNS treatment group.

The Sequencing data was available for 114 samples. Results included 7373 OTUs belonging to 6 phyla and 84 genera. LNSp treatment resulted in a significant increase of Bifidobacteria counts by 7 to 9 fold compared to control and by 6 to 7 folds compared to LNS alone. Conversely, potentially harmful bacteria like veillonella, enterobacteriaceae and bilophila were downregulated by 4 to 8 folds.

Conclusions / Implications for practice: Fortifying LNS with prebiotics (Inulin and FOS) resulted in microbial community enrichment in Bifidobacteria, a decrease of potential pathogens and an increase in SCFA production in a SHIME model for MAM infants. These beneficial effects need to be confirmed through In vivo studies that would also investigate the repercussion of such effects on child growth and health.

References
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**P25 & FP – Design and feasibility of a multicentric study assessing blood microbiota and diet in relation to adenoma and colorectal cancer risk: a three-year project funded by AIRC**

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**Background:** Inflammation and immunity are inextricably linked to all phases of colorectal cancer (CRC) development. Gut inflammation leads to loss of epithelial barrier function, driving to the bacterial translocation from the gastrointestinal tract to bloodstream. In this context, an overrepresentation of bacterial cells in blood has been proposed as an indicator or a predictor of intestinal adenoma (IA) and/or CRC.

**Objective:** We designed a study to investigate whether bacterial translocation from gastrointestinal tract to bloodstream is associated to the risk of IA and/or CRC using an innovative metagenomic approach on blood samples. Diet will also be investigated in relation to bacterial translocation and to the risk of IA and/or CRC, with the aim to define dietary guidelines associated to a CRC risk reduction.

**Methodology:** A case-control study including 100 incident cases of histologically confirmed CRC, 100 IAs and 100 healthy controls is conducted. Participants are enrolled at hospital by involving outpatient or inpatient eligible subjects who have a colonoscopy appointment. Healthy controls and IAs are matched with CRC cases (1:1 by age +/- 5 years and sex). An interview is performed by trained interviewers through a valid food frequency questionnaire collecting dietary and lifestyle habits, besides socio-demographic information and anthropometric measures. Blood samples will be also collected by a nurse before the colonoscopy.

**Results:** Project received ethical committee approval and data recruitment started in May 2017 in two hospitals of Milan, with involvement of CRC regional screening program. One hundred fifty-one subjects, 71 women and 89 men were recruited, with a total of 25 three matched trios have been recruited to date. In order to keep the signal to noise ratio optimal and to reduce technical variability, possibly overcoming the biological variation between groups, all samples will be analyzed in the same time, with the same reagents batches and by the same manipulator at the end of data recruitment to perform DNA extraction, qPCR quantification and Metagenomic Sequencing of all samples in the same experiment.

**Conclusions / Implications for practice:** The project is designed to contribute original information to the on-going international scientific debate on the causes of CRC and its prevention. It will allow to evaluate whether early diagnosis of CRC may be defined by mean of metagenomic microbiota profiling in blood, with relevant implications on a public health level, specifically related to CRC high risk subgroups.
References

P26 & FP – Analysis of gut microbiota and immunological characterization in patients with Chronic Granulomatous Disease

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**Background:** Chronic granulomatous disease (CGD) is a primary immunodeficiency disorder of phagocytes, due to defect in the NADPH enzyme, resulting in impaired killing of bacteria and fungi. X-linked CGD is the most common genetic subgroup. In addition to phagocytes, the enzyme is expressed also in lymphocytes but its functional implication is still poorly characterized. Patients with CGD suffer from severe infections and deregulated inflammation. In particular, the mechanisms underlying the abnormal response of the intestinal immune system remain unclear.

**Objective:** To assess the link between microbiota and inflammation in condition of Nox-2 deficiency.

**Methodology:** PBMC of 13 CGD patients and 10 age-matched HD were analyzed by flow cytometry. The bacterial composition of fecal samples was determined, after DNA extraction, by 16S rRNA gene sequencing. Short Chain Fatty Acids (SCFAs) were quantified in fecal samples with a HPLC system. Fecal IgA were quantified by ELISA essay.

**Results:** Analysis at the enrollment showed diminished naïve CD4 and CD8 subsets and increased effector memory (CD45RA-CD27- and CD45RA-CCR7-) cells as well as a slight increase in the NKT subset. Despite normal B cell frequencies, the memory subsets (CD19+CD27+ unswitched and switched memory CD19+CD27+IgD-), were all below the normal range values. Analysis of intestinal microbiota composition revealed substantial differences between patients and age-matched healthy controls with a predominant skewing towards the inflammatory gamma-proteobacteria and enterobacteriaceae species. Preliminary analysis of SCFAs showed a reduced faecal concentrations of butyric, acetic and propionic acid, both in adult and pediatric patients and decreased level of fecal IgA.

**Conclusions / Implications for practice:** Our studies will help to shed light on the pathogenic mechanisms underlying the IBD-like disease in CGD. We expect to identify a correlation between the type of mucosal and systemic immune responses, the composition of intestinal microbiota, and the clinical severity of intestinal pathology in patients. Studies are underway to investigate also the presence of rare genetic variants associated with IBD and, eventually, use them as biomarker and prognostic factor associated with the development of inflammatory intestinal manifestations, to redirect therapeutic approaches and improve the life quality of these patients.

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https://microbiotami.com/
P27 & FP – Synergy between 15-lipoxygenase and sPLA₂ promotes chronic inflammation by formation of TLR4 agonists from extracellular vesicles

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Background: Sterile inflammation occurs under diverse pathological conditions, such as ischemia/reperfusion or trauma and it also underlies the pathologies of diseases with chronic inflammatory conditions including atherosclerosis, autoimmune diseases and aging-related pathologies. The innate immune signaling pathways (TLRs and inflammasomes are the most extensively studied) that sense infection also contribute to the sterile inflammation. Differences in the outcome of both conditions exist, but the mechanisms have not been fully determined yet.

Objective: Danger-associated endogenous molecules induce innate immune response, thus making sterile inflammation medically important. During oxidative stress conditions, stress-derived EVs (stressEVs) were found to activate Toll-like receptor 4 (TLR4) with a gene profile different from lipopolysaccharide (LPS). Along with understanding of oxidative stress mechanisms and their role on TLR function we want to identify endogenous ligands, which drive inflammation in chronic diseases. Additionally, some enzymes, among them lipoxygenases (LO) and secreted phospholipase A₂ (sPLA₂s), are induced by stress and contribute to the inflammation presumably by the formation of DAMPs and their role will be exploited.

Methodology:
- StressEVs were isolated from HEK293 cell stimulated with 12mM A23187.
- 15-LO was immobilized to magnetic beads. SynEVs, single PLs and lysoPLs were incubated with 15-LO.
- SynEVs or oxidized synEVs (composed of 30% AAPE and 70% POPC) were incubated with porcine sPLA₂-IB, rh sPLA₂-IIA, rh sPLA₂-X or synovial fluid.
- qPCR on macrophages or dual luciferase test on HEK293 were performed for measurement of TLR4 activity.

Results: Here we show that stressEVs in comparison to LPS activate different transcription factors resulting in activation of different immune response genes. Additionally, synergistic activity of 15-LO and sPLA₂ is needed for the formation of TLR4 agonists, which were identified as lysophospholipids (lysoPLs) having oxidized unsaturated acyl chain. Hydroxy, hydroperoxy and keto products of 20:4 lysophosphatidylinositol (lysoPI) oxidation were determined by mass spectrometry and they activated the same gene pattern as stresses. Furthermore, sPLA₂ activity, which was also detected in the synovial fluid from the rheumatoid arthritis and gout patients, promoted formation of the TLR4 agonists.

Conclusions: We revealed the mechanism of endogenous ligand formation through activity of 15-LO and sPLA₂, which contribute to sterile inflammation in chronic conditions like RA with differences to classical inflammation on the signaling pathway as well as cytokine level, thereby giving option to design specific inhibitors that will limit sterile inflammation but will not globally affect systemic innate immunity.
Lipopolysaccharides (LPS) represent one of the most important glycoconjugates found on the outer membrane (OM) of Gram-negative bacteria cell wall, covering around 75% of its surface. They are crucial for bacterial survival, contributing significantly to the integrity and stability of the OM and protecting the bacterium from the external milieu stress factors. These amphiphilic molecules are divided into three genetically, biologically and chemically distinct domains—a hydrophobic glycolipid portion called lipid A, a repeating glycan termed O-polysaccharide and a core oligosaccharide connecting the two domains[1]. More interestingly, the LPS are classed as PAMPs (Pathogen Associated Molecular Patterns) since they are able to trigger hosts innate immune responses. The key event in the signalling is the recognition of LPS by the TLR4/MD-2 receptorial complex, triggering the activation of immune defences, and stimulating the production of inflammatory cytokines[2]. If the TLR4/MD-2 driven activation of the innate immune response is beneficial to combat the infection, its over-stimulation leads to sepsis and finally life threatening septic shock. Nonetheless, modifications in the LPS structure, and more precisely in the lipid A region, affect its immunostimulant properties, including reduction of TLR4/MD-2 activation and even inhibition of signalling caused by agonistic molecules[3]; hence, the search of LPS possessing inhibitory activity is a high importance and interest topic.

Here, I will present the structural characterization of the lipopolysaccharides extracted from two different bacteria, Acetobacter pasteurianus and Phaeobacter gallaeciensis. A. pasteurianus is an acetic acid bacterium used in production of traditional Japanese black rice vinegar, kurozu. The beverage is believed to carry several health benefits. Phaeobacter gallaeciensis BS107 is a bacterium living in a particular ecological interaction with algae. After isolation and purification of the cell wall components, the LPS components were separated and the structure of three domains was obtained using a combination of chemical, spectrometric and spectroscopical methods; furthermore, their inflammatory and inhibitory activity was also evaluated.

References
NLRP3 inflammasome is a multiprotein complex which forms within cells in response to various stress associated triggers. It consists of oligomerised NLRP3, ASC and pro-caspase-1 proteins. Caspase-1 activates pro-inflammatory IL-1β and IL-18 cytokines which drive inflammation through recruitment of neutrophils and other immune cells. Chronic protein or crystal deposits in diseases such as neurodegenerative diseases or atherosclerosis and NLRP3 gene mutations in cryopyrinopathies act as triggers of the NLRP3 inflammasome which contributes to the disease progression.

The mechanism of NLRP3 inflammasome activation including the stoichiometry of NLRP3 oligomerisation is not yet fully elucidated, which hinders the design of effective and specific NLRP3 inflammasome inhibition strategies. Objectives of our research were to determine minimal NLRP3 oligomerisation state necessary to initiate inflammasome complex formation and to design specific peptide based inhibitors of NLRP3 inflammasome.

In order to define the minimal activating NLRP3 oligomerisation state, we prepared retrovirus-transduced stable macrophage cell lines which express NLRP3PYD bound to various oligomerisation domains (dimerisation and trimerisation) under the doxycycline control and observed that trimerisation of NLRP3 was the minimal oligomerisation stoichiometry that promoted inflammasome activation.

To inhibit inflammasome formation at different stages we designed a set of putative inhibitory peptides which could potentially interfere with the protein interactions within the complex. The design was based on the crystal structures of the PYD and CARD interaction domains and on the pathological mutation hotspots in the NLRP3 NACHT domain. We identified peptides that were capable of inhibiting the activation of caspase-1 and the release of IL-1β from myeloid cells. Inhibitory effects were observed on macrophages as well as on microglial cells. The inhibitory effect of the peptides was independent of the type of NLRP3 inflammasome trigger. Furthermore, we found that some of the peptides specifically inhibited the NLRP3 inflammasome. Peptides also effectively inhibited inflammasome NLRP3 activation in cell lines with NLRP3 mutations linked to cryopyrinopathies.

We also explored potential applications with one of the identified inhibitory peptides in therapy of neurodegenerative diseases by equipping it with peptide sequence to allow its transfer through the blood-brain barrier. Peptide P3 was localised inside the cells as well as within the brain of mice after intravenous injection showing its potential implications as an NLRP3 inflammasome inhibitor in a neurological setting.

Designed peptides provide an insight into the mechanism of NLRP3 inflammasome assembly. Together with the identified minimal oligomerisation state they provide the basis for the development of novel anti-inflammatory strategies.


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**Abstract**

**P30 & FP – Airway mycobiota in severe asthma: isolation from the exhaled breath condensate**

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**Background:** There is current evidence to demonstrate a close association between fungal sensitisation and asthma severity. This can be due simply to exposure to high levels of fungal spores where they have been implicated as a cause of severe exacerbations. Exhaled breath condensate (EBC) collection potentially offers a less invasive alternative for lung mycobiota sampling.

**Objective:** This study was carried out to investigate the clinical and immunological features of severe asthmatics with a fungal colonization detected in EBC.

**Methodology:** We enrolled 27 subjects with a diagnosis of severe asthma. They underwent spirometry, fractional exhaled nitric oxide (FeNO) analysis, skin prick tests, total IgE and blood and sputum count cell collection. Selected EBC were plated directly onto fungal specific culture and fungi were identified by morphology and species identity was confirmed by DNA sequencing.

**Results:** A fungal sensitisation to at least 1 fungal allergen in the EBC of 66.7% of enrolled subjects with severe asthma was detected. The most common species were *Cladosporium* spp (72.2%) followed by *Aspergillus* (50%), *Penicillum* (44%) and *Alternaria* (22%) species. Fungal colonization was higher in female, non-atopic and obese severe asthmatics with an eosinophilic inflammation and a fixed airflow obstruction.

**Conclusions / Implications for practice:** The association of a positive EBC culture in severe asthmatics with an eosinophilic inflammation, without atopy and with fixed airway limitation support the hypothesis that a chronic fungal airway colonization might have an important role in the pathogenesis of this severe asthma phenotype and encourages the use of the analysis of exhaled mycobiota in these subjects.

**References**

P31 & FP – The involvement of microbiome in hair disorders: the example of Alopecia areata

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Background: Some authors reported evidence on the link between the gut microbiome and Alopecia areata (AA)1,2 but poor information are currently available as regards microbial communities on the scalp3,4. Since its unique features scalp is expected to harbor a specific microbiome, which is expected to play a peculiar role in scalp conditions related to hair growth5.

Objective: Investigate if in AA subjects there is an imbalance of bacterial communities of the scalp and if this disequilibrium extends till subepidermal compartments of the scalp.

Methodology: Bacterial communities in healthy and AA subjects, were investigated by mean of RT qPCR and 16S sequencing. Samples of superficial epidermis have been collected by mean of swab procedure. 4mm punch have also been collected from the scalp in order to investigate the distribution of bacterial communities in the subepidermal compartment of the scalp.

Results: The analysis of bacterial distribution at the genus level highlighted an increase of Propionibacterium in AA subjects alongside a general decrease of Staphylococcus. Analysis of log Relative abundance of main bacterial species inhabiting the scalp showed a significant increase of Propionibacterium acnes in AA subjects compared to control ones. AA is also associated with a significant decrease of Staphylococcus epidermidis relative abundance while no significant changes were found for Staphylococcus aureus. Therefore data from sequencing profiling of the bacterial population strongly support a different microbial composition of different area surrounding hair follicle from the epidermis to hypodermis, highlighting differences between normal and AA affected scalp.

Conclusions / Implications for practice: Our results showed the presence of a microbial shift on the scalp of patients suffering from AA and gives the basis for a larger and more complete study of microbial communities involvement in hair disorders.

References

https://microbiotami.com/
Is microbiota linked to bergamot cardiovascular protection?

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Background: One of the most popular teas in the world, Earl Grey, is scented by bergamot essential oil, extracted from Citrus bergamia Risso et Poiteau, an endemic plant of the Calabria. The traditional use of bergamot is currently being rediscovered due to its characteristic pattern of polyphenols almost unique in nature. This peculiar composition revealed antioxidant, hypoglycemic and hypolipidemic activities, with positive modulation of Metabolic Syndrome (MetS) and protection against cardiovascular disorders. We have now applied to bergamot polyphenolic extract our lecithin food-grade delivery system (phytosome®), a dispersed state of phytocomplex more readily absorbed.

Objective: exploration of Bergamot phytosome® (Vazguard™) on human microbiota in order to demonstrate the importance of microbiota modulation in cardiovascular health.

Methodology: A simulated gastric and duodenal human digestion of Bergamot phytosome® was performed in vitro before adding it to the batch culture system. Fecal samples were obtained from 3 healthy women aged 45–53 years. They had not used antibiotics in the previous 12 months. Fecal slurries (1% w/v) from each individual were used to inoculate the batch-culture system containing basal nutrient media and the digested Bergamot phytosome® (1000mg/L). Batch culture system without Bergamot phytosome® were also included in the experiment as controls. After 16h of incubation at 37°C in anaerobic condition samples were centrifuged and DNA was extracted. 16S Amplicon barcoded library were prepared and run on the MiSeq (Illumina Inc.). A paired t-test was used to compare microbial profiles in the batch culture systems.

Results: in this experimental model we have observed a significant increase of Blautia (a sp correlated with the improvements in glucose and lipid homeostasis, Tong 2018), and Ruminococcus (key symbionts of the gut ecosystem, La Reau 2018). In parallel, a significant decrease of Corynebacterium (nosocomial infections ) was measured. Furthermore a decrease of Desulfovibrio (correlated with IBD, Lennon 2014) , and Granulicatella (correlated with MetS, Si J 2017) were registered with a trend that approached significance.

Conclusions: For the first time, the interaction with bergamot phytosome® and microbiota was addressed showing a possible link between positive modulation of microbiota and cardiovascular health.

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P33 & FP – Age-dependent effect of whey protein on body weight, lipid metabolism and gut microbiota in high-fat diet fed mice

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Background: Whey proteins are present in the serum during milk manufacture. They are considered as a dietary solution to the obesity problem thanks to their beneficial effect on energy balance and lipid metabolism [1]. However, the mechanism of action of these proteins is poorly understood.

Objective: Determine the impact of duration of WPI intake on intestinal mechanisms and adipose tissue metabolism related to energy balance.

Methodology: Three-week-old C57BL/6J mice were housed four per cage and were divided into 6 groups. Groups 1 (n=16) and 2 (n=16) were provided with 45% energy high fat diet containing 20% whey protein isolate (HFD-WPI) or casein (HFD-CAS), respectively, for 5 weeks. Groups 3 (n=16) and 4 (n=16) were provided with 10% energy low fat diet containing 20% WPI (LFD-WPI) or CAS (LFD-CAS), respectively, for 5 weeks and then their diets were switched from LFD to HFD for another 5 weeks (tot=10 weeks) (HFD-Later WPI and HFD-Later CAS groups). The control groups, 5 (n=8) and 6 (n=8), received LFD-WPI and LFD-CAS, respectively, for 10 weeks. During the trial, body weight and energy intake were recorded weekly. At 5 and 10 week time points, faecal samples were collected and organs were harvested to carry out shotgun metagenomic analysis and gene expression using qPCR (ileum, liver and adipose tissue).

Results: The HFD-WPI group showed a significant decrease in body weight compared to the HFD-CAS group, after 5 weeks. Conversely, no differences in body weight were observed between the HFD-Later CAS and WPI groups. Despite this, both HFD-WPI and HFD-Later WPI fed mice intake significantly more energy than the respective HFD-CAS groups.

In the epididymal adipose tissue, we observed a decrease in expression of the gene encoding for leptin (ob) and an increase in the expression of two genes involved in fatty acids β-oxidation (cpt1a and ucp2) in the HFD-WPI group, relative to the HFD-CAS group. These differences did not persist in the HFD-Later groups.

From the shotgun metagenomic analysis, higher proportions of Lactobacillus, and the related species Lactobacillus murinus, were evident in the HFD-WPI fed mice compared to the HFD-CAS groups. Again, this difference was no longer observed in the HFD-Later groups.

Conclusions / Implications for practice: These results suggest that the effect of whey proteins on body weight, adipose tissue and intestinal related mechanisms depend on the stage of life of the mice and on diet duration. Whey proteins influence the gut microbiota composition, which might orchestrate the observed metabolic and physiological modification.

References

https://microbiotami.com/
P34 & FP – The more the better: effect of cell concentration on the persistence of four probiotic strains in the human intestine

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Background: Reportedly, daily consumption of at least one billion viable probiotic cells is recommended for potential effect on the host. Nonetheless, more precise recommended dosages for probiotic intake are not established. Investigation on the outcome of the intake of different probiotic dosages is therefore required, especially for multi-strain formulations, in which a possible inter-microbe interference is postulated.

Objective: This study aimed to evaluate the influence of cellular dosage on the ability of four probiotic bacteria in a multi-strain blend to persist in human gastrointestinal tract.

Methodology: Forty healthy adult volunteers participated to a single-blinded, randomized, parallel group pilot trial. Each subject consumed daily a capsule containing 7 or 70 billion CFU of a probiotic blend composed of *Bifidobacterium animalis* subsp. *lactis* Bl-04 (10.7 or 9.7 log_{10} CFU per formulation), *Lactobacillus acidophilus* La-14 (10.1 or 9.1 log_{10} CFU), *Lactobacillus plantarum* SDZ-11 (9.4 or 8.4 log_{10} CFU) and *Lactobacillus paracasei* SDZ-22 (8.8 and 7.8 log_{10} CFU). Quantitative-PCR with strain-specific primers was used to detect and quantify the probiotic strains in fecal samples collected during the administration (2 weeks) and follow-up (2 weeks) phases. Fecal samples from the last day of probiotic administration were also used for strain-specific semi-quantitative estimation of viable probiotic cells.

Results: Strain Bl-04, which was present in highest amount in the formulations, was recovered from all volunteers. On the contrary, La-14 and SDZ-11 were recovered from all subjects receiving the 70 billion formulation, and from 90 and 80% of subjects in the 7 billion treatment group respectively. Finally, strain SDZ-22, which was the least abundant in the formulations, was recovered from 90 and 20% of the subjects in 70 and 7 billion treatment groups respectively. On average, the detection of probiotic strains was anticipated of about 1 day, ended 3 days later and resulted 3 days longer. The viable recovery at the end of the administration period was successful in all fecal samples for all strains in the 70 billion group, whereas 11% of samples in 7 billion group resulted negative. The number of viable cells recovered was also significantly higher in 70 vs 7 billion treatment group.

Conclusions: This study demonstrates that different strains, belonging to diverse taxa, may co-exist and be selectively quantified upon ingestion in a multi-species probiotic formulation. Moreover, we highlight that higher dosages of bacterial cells in probiotic formulations may allow a higher, anticipated and longer recovery of the probiotic strains in the feces of healthy adults.