Topic: *AS01 PBI - The microbiota seeding (source of the microbiota: vaginal vs gut)*

GUT MICROBIOTA OF CHILDREN BORN TO OBESE MOTHERS.

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Background and Aims: To identify the features of the intestinal microbiota of children of the first year of life born to obese mothers

Methods: A longitudinal study of the intestinal cavity and parietal microbiota by the PCR method was carried out and the dynamics of physical development was assessed monthly for up to 1 year. We examined 22 children under 1 year of age, including 12 children born to mothers with obesity (BMI ≥ 30 kg / m^2), the comparison group consisted of 10 children born to mothers with normal body mass index (18.5-24.9 kg / m^2).

Results: Differences in intestinal microbiocenosis in children born to obese mothers up to 6 months were insignificant. After 6 months, an increase in the titer of opportunistic microorganisms, such as Staphylococcus_spp, Enterobateriaceae, Corinobacteriia, as well as a decrease in the titer of Lactobacillaceae, was more often observed in the main group. From 6 to 12 months in the main group there was an acceleration of growth rates, the frequency of children with SDS +2 was 26.7%, in the control group - 4.7%. ($p<0.05$). The number of children with increased weight gain, M + 2SD, is higher in the main group and is equal to 32.4%, in the control group - 6.8% ($p<0.05$).

Conclusions: The influence of maternal obesity on the intestinal microbiocenosis and physical development of the child begins to manifest itself from the age of 6 months. Further research is needed on the relationship between accelerated growth in children and changes in microbiota after complementary feeding.

Topic: *AS01 PBI - The microbiota seeding (source of the microbiota: vaginal vs gut)*

DECIPHERING THE VIABLE GUT MICROBIOTA TRANSFERRED FROM MOTHERS TO INFANTS

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Background and Aims: The infant microbiota is key for establishing a balanced host-microbiome symbiosis. The gut microbiota is shaped in the first months of life and metagenomic approaches have shown that the maternal gut is the main source of gut bacteria in healthy infants. However, a comprehensive assessment of viable and culturable gut microbiota transferred from mothers to infants is still uncovered.

Methods: To address this, a subsamples of 40 mother-infants pairs from the MAMI cohort were selected. Maternal-infant fecal and breast milk samples were collected from birth up to 6 months postpartum. In order to cultivate, isolate and identify the viable gut bacteria, an array of culture media and conditions coupled with complete16s rRNA gene Sanger sequencing were applied .

Results: The most abundant genera were Bifidobacterium (31%), Lactobacillus (15%), Enterococcus (14%) and Staphylococcus (14%) in 200 isolates from mother-infant feces (53% and 35%) and breast milk (12%). Interestingly, Bifidobacterium accounted for most of the bacteria, being Bifidobacterium longum the most abundant specie (42%) followed by Bifidobacterium breve (20%), Bifidobacterium animalis (14%), Bifidobacterium bifidum (8%) and Bifidobacterium pseudocatenulatum (7%) species. Furthermore, 5 Bifidobacterium isolates were present in samples from the same mother-infant pairs, matching 97% identity sequencing, suggesting vertical transmission.

Conclusions: Our work provides the foundation to identify the viable and cultivable bacteria transferred from mothers to infants. This results coupled with isolates whole-genome-sequencing and the gut metagenomes will allow the tracking of the vertical transmission of microbial strains from mother to infants over the time and its influence in infant microbiota development.

Topic: *AS01 PBI - The microbiota seeding (source of the microbiota: vaginal vs gut)*

THE TRAMIC PILOT STUDY – UNCOVERING THE PREGNANCY MICROBIOME

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Background and Aims: Not only the body and immune system of a woman changes during pregnancy, but so does her microbiome. This change in microbiomes might provide the best possible environment to the fetus in sense of changed metabolites or immune system responses or to vertically transmit the right microbes to the infant for colonization. To investigate those changes, samples from multiple body sites of pregnant women were collected before and after delivery: oral and vaginal swabs, urine and stool. Together with age-matched non-pregnant women who were recruited as non-pregnant controls, the microbiome of pregnant women in multiple body sides can be described and traced over the timeframe of pre-delivery to one month post-delivery. Post-delivery samples enable to describe how and to what extend the microbiome goes back to a non-pregnant state.

Methods: Microbial DNA was extracted and were amplicon sequenced with Miseq Illumina Sequencing. Raw reads were processed in Qiime2 and analyzed with multiple tools like MicrobiomeExplorer, Aldex2 and Sourcetracker2

Results: Substantial changes can be seen in the microbiomes of pregnant women pre-delivery, whereas one month after delivery, the microbiome already is more akin to the non-pregnant microbiome than to the pre-delivery samples. Those significant differences are consistent in taxonomy composition of the microbiomes on different taxonomic levels beyond all body sites. Metabolic flux analyses revealed strong changes of metabolites from pre-to post-delivery. Sourcetracker2 revealed a lowered contribution of urine as a source for the vaginal microbiome of mother_post.

Conclusions: The microbiomes of pregnant women changes strongly in multiple aspects across all investigates sample sites

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

THE MICROBIOTA PROFILE OF A NEWBORN WITH IVEMARK SYNDROME AND TWO EPISODES OF NECROTIZING ENTEROCOLITIS

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Background and Aims: Necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease that primarily affects preterm infants, but also term infants with congenital heart defects. Ivemark syndrome (IS) is a rare disorder characterized by the complex heart malformations and asplenia. The aim of the study was to assess the gut microbiota profiles associated with NEC IIA in a newborn with IS.

Methods: The feces were collected on the 7th, 14th, 22nd, 35th and 43rd days of life (DOL). On the 14th DOL, the newborn palliated with modified Blalock-Taussig shunt. Two episodes of NEC IIA were diagnosed on the 15th and 42nd DOL. The fecal microbiota was determined using NEXTflex™ 16S V4 Amplicon-Seq Kit 2.0 (PerkinElmer, Inc., USA). Taxonomy was performed with QIIME 2, based on SILVA v.138 NR 99 database (the 515F/806R primer pair).

Results: Totally, three phylum (Actinobacteriota, Firmicutes, Proteobacteria) were identified. On the 7th DOL g_Escherichia-Shigella and g_Veillinella were predominant, while on the 14th DOL the high proportion of g_ Clostridium sensu stricto I_Clostridium paraputrificum (56.1%) that was early found to be associated with acute colonic necrosis in patients with AIDS was noted. On the 22th DOL during antibiotic therapy, it revealed the shift of microbiota landscape, disappearance of Firmicutes and the pronounced increase of Gammaproteobacteria untill 99.8%. On the 35th and 43rd DOL the proportion of g_ Clostridium sensu stricto I_human gut reached 56.9% and 72.0%, respectively.

Conclusions: The presence of g_ Clostridium sensu stricto I in a gut microbiota of newborn with IS could be associated with the risk of development of NEC.

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

CHILDHOOD BEHAVIOR ASSOCIATION WITH THE GUT MICROBIOME COMPOSITION.

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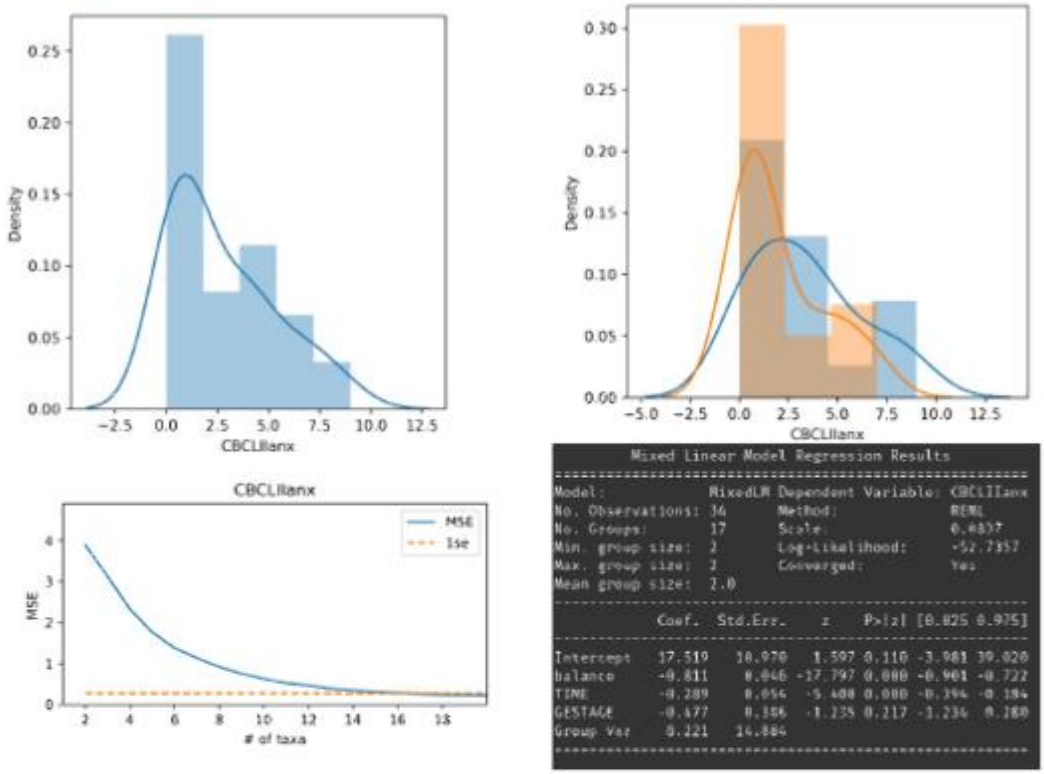
Background and Aims: The gut microbiome influences behavioral changes through epigenetic mechanisms,metabolites production,and altering brain structure and function(Sarkar et al., 2020).In order to explore and compare the taxa associated with the Child Behavior Checklist(CBCL)domains and scales,we performed a compositional balance analysis.

Methods: The compositional data analysis creates log-ratio transformations(Aitchison, 1982;Pawlowsky-Glahn et al.,2015),allowing standard statistical methods.Behavioral assessment and sample collection occurred at 2 and 4 years of age, with the stool profiled by 16SrRNA V3-V4 sequencing.The CBCL assesses five problem domains and eight syndrome scales and is a gold standard for reporting symptoms associated with other diagnostic areas,such as Autism Spectrum Disorder(ASD)(So et al., 2013).The higher scores are closer to clinical range.17 children had the necessary information to perform analysis.

Results: The compositional balances model is significant in multiple scores of CBCL with overlay in the density graphs.Abundance of Firmicutes,particularly Clostridiales,existed in multiple scores(depression; autism;oppositional behavior;emotional behavior;anxiety symptoms;somatic symptoms;withdrawn behavior;sleep problems;aggressive behavior;and summative external symptoms).As well as abundance of Bacteroidetes,particularly from the family Barnesiellaceae,in anxiety,emotional,and Sleep scores and Desulfovibrionaceae at the top abundances in emotional and oppositional. We also detected reduced commensals of Paraprevotella,which is part of the Prevatellaceae family associated with anxiety.

CBCLIIanx:



TOP:

p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Actinomycetaceae; g__Actinomyces
p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__
p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__Eggerthella
p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__Slackia
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Porphyromonas
p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Carnobacteriaceae; g__Granulicatella
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptococcaceae; g__
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira
p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Megamonas
p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__
p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Clostridium

BOT:

p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium
p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__Collinsella
p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Barnesiellaceae]
p__Firmicutes; c__Bacilli; o__Turicibacterales; f__Turicibacteraceae; g__Turicibacter

Figure 1. Compositional analysis of the CBCL anxiety problems scale.

Conclusions: The overlap between ages displays stability of behavior throghout time.Even though our sample was not diagnosed with ASD,Clostridia,Bacteriodetes,Desulfovibrio are short-chain fatty acid producers associated with it(Johnson et al.,2020;Macfabe,2012).Beyond comparison between diagnosed versus non-diagnosed ASD,behavioral phenotypes differences should be considered in future research,aligned with possible mediators for change in the gut microbiome.No causal claims are proposed in this cross-sectional design with a small sample.

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

THE DIFFERENCE IN NEONATAL FECAL MICROBIOTA BETWEEN VAGINALLY BORN INFANTS AND INFANTS BORN BY CAESAREAN SECTION IS MINIMIZED WITHIN 2 MONTHS AFTER BIRTH.

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Background and Aims: The gut microbiome significantly affects the growth and the development of the newborn. In the first days the delivery type has a huge impact on the intestinal microbiome, while other factors affect more its later composition.
The aim was to assess changes in the composition of fecal microbiota in both breastfed naturally-born infants and infants born by Caesarean section from 2 days to 2 months after birth.

Methods: Real-time PCR with group- and species-specific primers. The quantitative assessment of each of the bacterial groups/species was calculated separately as a proportion of the total bacteria mass. In investigation 95 children were included: 2 days old - 22 children born naturally and 8 by Caesarean section; 2 months old - 36 children born naturally and 29 by Caesarean section. The nonparametric Mann-Whitney test was used to compare the results in different groups of children.

Results: The composition of the fecal microbiome of 2 days old naturally-born infants and children born by Caesarean section differed in the amount of Bacteroides (20% versus 0.1%, $p = 0.023$). By the age of 2 months children no longer had difference in the Bacteroides, but there was a significant increase in the amount of Bifidobacterium in both groups: infants born naturally had from 12.5% to 100% ($p < 0.000$), while infants born by Caesarean section had from 2.5% to 79.4% ($p = 0.001$).

Conclusions: Similar family conditions and feeding type in 2 months minimize the differences between the gut microbiome in naturally-born children and infants born by Caesarean section.

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

MICROBIOTA OF THE PROXINAL INTESTINE IN NEWBORN INFANTS WITH ENTEROSTOMY – PRELIMINARY RESULTS

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Background and Aims: Although gut microbiota has been recognized to play an important role in early life and have long-term health consequences, knowledge about intestinal colonization in infants with enterostomy is still scarce. The aim of the study is to characterize the microbiota of the proximal intestine in infants with enterostomy.

Methods: Samples of enterostomy effluent (≥ 2mL) from 29 infants were collected in two time-points: 1st post-surgery day and 21 days after the 1st collection. Clinical data were collected from medical records. Microbiota was analysed by RT-PCR (Universal, Bifidobacterium, Candida) and 16S rRNA gene sequencing. Statistical analysis was performed using SPSS® software and Graphpad Prism®.

Results: During study period, the total bacteria amount increased by 18 % ($p<0.05$), Candida amount decreased by 16 % ($p<0.05$) and Bifidobacterium amount did not change ($p=0.59$). The beta-diversity was different ($p<0.05$) between the two time-points. Total bacteria increase was positively correlated with several clinical factors, proximal small intestine enterostomy ($r=0.4067$, $p= 0.0353$), human milk intake ($r=0.4651$, $p=0.0192$), antacid drug therapy ($r=0.5618$, $p=0.0023$), and negatively correlated with sepsis ($r=-0.4351$, $p=0.0263$). Correlations of Candida decrease with anti-fungal therapy ($r=0.3851$, $p= 0.0473$) and sepsis ($r=-0.3979$, $p= 0.0488$) were also found. Total bacteria and Candida amounts were not correlated with antibiotic therapy.

Conclusions: These preliminary results describe the short-term evolution of microbiota profile after surgery, and its correlations with some clinical factors. A more comprehensive characterization of microbiota colonization after enterostomy may be important to guide the postoperative care in this population. Acknowledgements: CINTESIS, R&D Unit (reference UID/IC/4255/2013) and CHRC (UIDP/04923/2020).

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

GUT AND BREAST MILK BACTERIAL ANALYSIS IN COLOMBIAN WOMEN WITH GESTATIONAL DIABETES MELLITUS AND THEIR OFFSPRING

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Background and Aims: Human breast milk is a complex fluid that provides either nutrients critical for the infant development but also it is an important source of bacteria associated to infant homeostasis. Recent studies have been focused in the characterization of the human breast milk (HBM) microbiota, today we know it is composed by a rich community of microorganisms. It is considered that the human microbiome is not static, instead is a complex dynamic system shaped by several factors including metabolic disorders. Diabetes mellitus induces dysbiosis in the gut microbiome, however, how gestational diabetes mellitus (GDM) affects the HBM and neonate microbiota still remains poorly understood. In this study, we analyzed the bacterial diversity in GDM Colombian women and their children.

Methods: This descriptive study was approved by UAT Ethics Committee (001/2019/CEI). We collected stool and breast milk samples from 10 women and stool samples their children (<2 months old). Bacterial bacterial diversity was analyzed through the sequencing of the v3-v4 regions of the 16S rRNA gene.

Results: Firmicutes was the dominant phylum in GDM fed individuals, also we found a higher species richness (p=0.003) and significant beta diversity. However, at genus level we observed differential abundances of Bacteroides, Serratia, Sutterella, Ruminococcus, Veillonella, Streptococcus and Lactobacillus

Conclusions: Our results revealed that most abundant phyla in the collected samples are Firmicutes, Bacteroidetes and Proteobacteria. We found significant differences in the bacterial abundances between GDM and control samples that could be linked to vitamin K deficiency, inflammatory disease, immunomodulation and gut bacterial homeostasis in infants from GDM women.

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

EARLY LIFE FECAL METABOLOME AND MICROBIOTA MATURATION

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Background and Aims: Early life gut microbiota composition is important for immune system and metabolic development, and it has been suggested that metabolites produced in microbe-host interaction are essential in this process[PMID:27231050]. The diversity of gut microbiota ecosystem is increasing during early life and the colonization process is influenced by factors such as delivery mode, antibiotic treatments, and nutrition[PMID:30356187]. However, little is known of the developmental process in gut metabolites[PMID:33723382]. This study aims to explore the longitudinal patterns of targeted and untargeted gut metabolites, and how they are related to gut microbiota composition. Additionally, the study seeks to unveil how factors known to influence gut microbiota colonization relate to the metabolome maturation.

Methods: The samples were collected 2.5 (n=272), 6 (n=232), 14 (n=289), and 30 months (n=157) of age from infants in FinnBrain Birth Cohort Study. Next,16s rRNA sequencing was performed with Illumina MiSeq, and metabolome was analyzed with GC-TOF-MS and LC-MS. Microbiota maturation index was calculated based on random forest regression model[PMID:24896187].

Results: Majority of the infants had stable increase in the gut microbiota maturity index that was positively related to alpha diversity. Short-chain fatty acids, except acetic acid, showed increasing trend over time. In the preliminary analyses the gut microbiota maturation was related negatively with antibiotic use, and alterations in metabolome were related to parent-reported stool consistency.

Conclusions: We show that short-chain fatty acids have generally increasing concentration. Moreover, we corroborate that antibiotic use is related to gut microbiota maturation, and additionally report novel findings in infants, such as association between stool consistency and metabolite composition.

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

BREAST MILK MICROBIOTA AS POTENTIAL SOURCE OF ANTIBIOTIC RESISTANCE GENES: RELEVANCE ON MATERNAL-INFANT GUT MICROBIOTA

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Background and Aims: Mode of delivery and breastfeeding practises are critical to driving the infant gut microbiota. the use of antibiotics during pregnancy and lactation would have a direct effect on the microbial development of the infant as well as on the resistome. Hence, little is known about the relevance of breastfeeding as potential route of antibiotic resistance genes (ARGs) and also, specific strains with antibiotic resistance. Our study aimed to understand the effect of antibiotic exposure and the potential antibiotic resistance genes transference during lactation on infant gut microbiota.

Methods: 54 paired fecal and mature milk from MAMI cohort collected within the first month post-partum were analysed. Maternal-infant fecal and milk microbiota profiling were analysed by 16S rRNA gene sequencing and also, ARGs (tetM, tetO, blaTEM and blaSHV) were quantified by qPCR. Specific milk strains belonging to Staphylococcus and Streptococcus genus were also isolated, identified by Sanger sequencing and tested for ARGs. Maternal-infant clinical data including mode of birth, intrapartum antibiotic and breastfeeding practises were recorded.

Results: Microbiota profiles were shaped by mode of delivery. Vaginal born infant microbiota was dominated by Bifidobacterium genus members and lower presence of ARGs. C-section and antibiotic exposition modulated the presence of Bifidobacterium genus. Breast milk is a potential transfer of ARGs from mother to infants as specific ARGs were identified. Those ARGs in breast milk were present mainly in Staphylococcus strains.

Conclusions: Further studies to clarify the potential impact antibiotic exposure early in life and the antibiotic resistance genes transfer during breastfeeding are needed.

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

PREBIOTIC EFFECT OF DIFFERENT PRESCHOOL CHILDREN MILKS ON CHILDREN'S GUT MICROBIOTA USING A MICROBIOME COLONIC MODEL

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Background and Aims: Lactose is the main carbohydrate in mammalian milks and has several positive health effects, which are mediated by the increased metabolic activity of the lactose-fermenting intestinal microbiota, thereby providing a prebiotic effect. The aim of the present study was to verify the prebiotic impact of 3 preschool children milks (PCM) with prebiotics GOS and FOS (4 g/L; 9:1) and different lactose contents (PCM1: 88.9%; PCM2: 79.1%; PCM3: 75%) on preschool children's gut microbiota using the Simulator of Human Intestinal Microbial Ecosystem (SHIME®).

Methods: Ascending colon (n=3) was inoculated with fecal microbiota from five children (3-5 years old). Microbiota composition was determined by plating and Prebiotic Index (PI), a quantitative score that describes the promotion of selective growth of specific beneficial bacteria in the presence of enteric competitors. Statistical analyses were carried out for all variables using two-way ANOVA followed by Tukey multiple comparisons test ($p<0.05$).

Results: Bifidobacterium spp increased 36.0, 31.9 and 26.1% and Lactobacillaceae family 36.6, 24.2 and 12.0% for PCM1, PCM2 and PCM3, respectively, when compared to control (before intervention, $p<0.001$), being PCM1 and PCM2 statistically higher than PCM3 ($p<0.05$) for Bifidobacterium spp and PCM1, with more lactose, higher than PCM2 and PCM3 ($p<0.01$) for Lactobacillaceae family. PCM1 showed also the highest PI (1.24; $p<0.05$) when compared to PCM2(0.99) and PCM3(0.69).

Conclusions: The higher lactose content in PCM1 showed the most potential beneficial effect as a prebiotic for children 3-5 years old, contributing to a higher bifidogenic effect, promoting a healthier microbiota.

Topic: *AS02 PBI - The microbiota early in life: the microbiologists comments for the clinician*

DELIVERY METHOD (VAGINAL OR CAESAREAN SECTION) DOES NOT IMPACT BACTERIAL LOAD OR DIVERSITY OF LOW-RISK TERM FETAL MEMBRANES

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Background and Aims: It is unknown whether the fetal membranes have a bacterial signature and whether it is influenced by mode of delivery. This research aims to determine if bacteria can be detected on low-risk term fetal membranes, and if the bacterial load and profile differ based on mode of delivery. It is important to understand this topic, as the fetal membrane microbiota may impact fetal health if bacterial transfer is prevented or enhanced during delivery.

Methods: The microbiota were investigated on combined amnion and chorion fetal membrane rolls from low-risk term elective caesarean section (ELCS; n=44) or spontaneous vaginal delivery (n=22) by amplicon sequencing of the V4 16S rRNA gene. Bacterial load was measured by BactQuant absolute qPCR of the 16S rRNA V3-4 gene region.

Results: 23% of fetal membranes displayed a detectable bacterial load, with no significant difference in copy number from ELCS (303.8 copies/ µl) and vaginal samples (300.3 copies/ µl, p=0.977). Samples had significantly greater copy numbers than negative controls (302.6 copies/ µl vs 124.8 copies/ µl, p=0.006). Vaginal samples had significantly greater abundance of Lactobacillus (p=0.001, FDR=0.023). No significant difference in diversity was detected between ELCS and vaginal samples, but bacterial communities differed between the fetal membranes overlaying the placenta and membranes taken from the zone opposite the placenta (α p=0.011, β p=0.001).

Conclusions: These findings suggest that detecting a bacterial signal in low-risk term fetal membranes is possible, but inconsistent, and delivery method does not impact bacterial load or diversity on low-risk term fetal membranes.

Topic: *AS03 PBI - The gut brain axis in early life*

GUT MICROBES AND MICROBIAL PRODUCTS FROM NON-HUMAN PRIMATES EXPOSED TO MATERNAL HIGH FAT DIET IMPACTS SEROTONIN IN ENTEROID MODELS

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Background and Aims: In a non-human primate model, we previously showed that persistent juvenile-onset anxiety and an altered gut microbial community are observed in offspring exposed to maternal high fat diet (mHFD). Next, we aimed to determine if mHFD-exposed microbes are sufficient to induce altered neurotransmitter activity at the level of the gut. We hypothesized that gut microbes from mHFD-exposed offspring will alter levels of intestinal serotonin in vitro via microbe-host interactions compared to maternal control diet.

Methods: Japanese macaques were fed mHFD or control diet during gestation/lactation then weaned onto a control diet. Stool was collected at 3 years (after 2.5 years of control diet feeding). Wild-type mouse jejunal enteroids (n=3) and adult human jejunal enteroids (n=2) were incubated with the macaque stool microbes. The enteroids were analyzed for serotonin secretion and expression of serotonin-relevant proteins.

Results: In mouse jejunal enteroids, stool treatment from mHFD animals decreased secreted serotonin levels (52ng/mL vs 250ng/mL, p=0.009), serotonin rate limiting enzyme expression (TPH1, 0.02 vs 0.66 fold change, p=0.03), and serotonin-producing cell marker expression (chromogranin A, 0.05 vs 0.65 fold change, p=0.03). However in human jejunal enteroids, stool treatment from mHFD animals increased TPH1 expression (1.24 vs 0.27 fold change, p=0.003).

Conclusions: Our results indicate that stool effluents from mHFD-exposed offspring induce changes in serotonin via host cells in the gut, even after 2.5 years of control diet feeding. These results were host species dependent. We speculate that interactions between microbes and the host are crucial to primate enteroneuroactivity and programmed development of the gut-brain axis.

Topic: *AS03 PBI - The gut brain axis in early life*

CHILDREN WITH ATTENTION-DEFICIT HYPERACTIVITY DISORDER OR AUTISM SPECTRUM DISORDER SHARE DISTINCT MICROBIOTA COMPOSITIONS

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Background and Aims: Studies have indicated a role of the gut microbiota in attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), although no bacteria have consistently been identified. ADHD and ASD coexists frequently with an overlapping heritability, thus raising the question of whether they also share gut microbiota signatures. We, therefore, aimed to investigate the gut microbiota of ADHD and ASD in parallel.

Methods: Fecal samples were collected from 95 children aged 5-17 years as follows: ADHD (n=32), ASD (n=12), and comorbid ADHD/ASD (n=11). Siblings to the patient groups (n=14, 5, and 11 for siblings to ADHD, ASD, and comorbid ADHD/ASD), or non-related children (n=17) were recruited as controls. The gut microbiota was assessed using 16S rRNA gene sequencing of the V4 region, while gastrointestinal inflammation and permeability were investigated through measurements of fecal calprotectin and plasma lipopolysaccharide-binding protein (LBP).

Results: While alpha diversity did not differ significantly between groups, the beta-diversity analyses revealed that children with ADHD and ASD possessed highly similar gut microbiota signatures, distinct from non-related controls. These were defined by an increased relative abundance of Streptococcus and a reduced relative abundance of Sutterella and Coprobacter. Finally, children with ADHD and/or ASD possessed higher concentrations of plasma LBP compared to non-related controls, whereas no differences were observed for fecal calprotectin.

Conclusions: Children with ADHD or ASD shared a distinct gut microbiota composition and increased gastrointestinal permeability, different from that of non-affected controls. This is interesting considering the high degree of comorbidity between the disorders and requires further investigation.

Topic: *AS03 PBI - The gut brain axis in early life*

NETWORK ANALYSIS AND COMPARISON FROM TODDLER TO PRESCHOOL.

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Background and Aims: Early life microbiome is gaining appreciation as a pivotal player for long-term health consequences.We analyzed and compared microbial networks of Very Low Birth Weight Infants between 2 and 4 years of age.

Methods: We performed a microbial network construction(NetCoMi) to see how the microbes interact and compare microbial networks at 2 and 4 years of age.Figure 1 shows only the 50 nodes with the highest degree. Clusters have the same color in both networks if they share at least two taxa.Seventeen children had data on both ages.

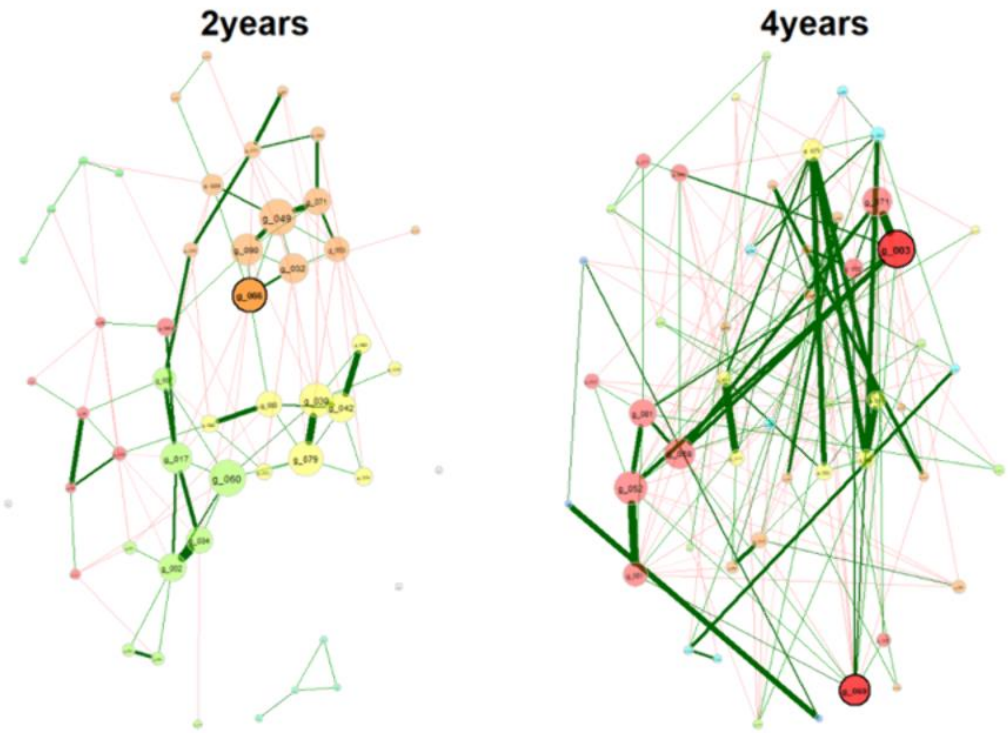

Figure 1. Comparison between 2 and 4 years old networks.

Results: The abundance of firmicutes,order Clostridiales,was detected on both ages.However,when analyzing for genus level,the two networks were not significantly related.This indicates key players differences in each network.Figure 1(comparison network)shows diversity in cluster nodes color at two years old that is overpowered by the red nodes at four years old.Among the identified bacterial family:while at two years old,the Lachnospiraceae family has the strongest eigenvectors, the Ruminococcaceae prevails at four.Enrichment of both bacterial families was associated with highly permeable metabolites,like cresol.Cresol changes gene expression and myelination resulting in social avoidance and depressive-like behaviors(Gacias et al.,2016).Ruminococcaceae was also associated with anxiety levels(Tengeler et al., 2020)and more abundant in persons with ADHD(von Rhein et al.,2015).

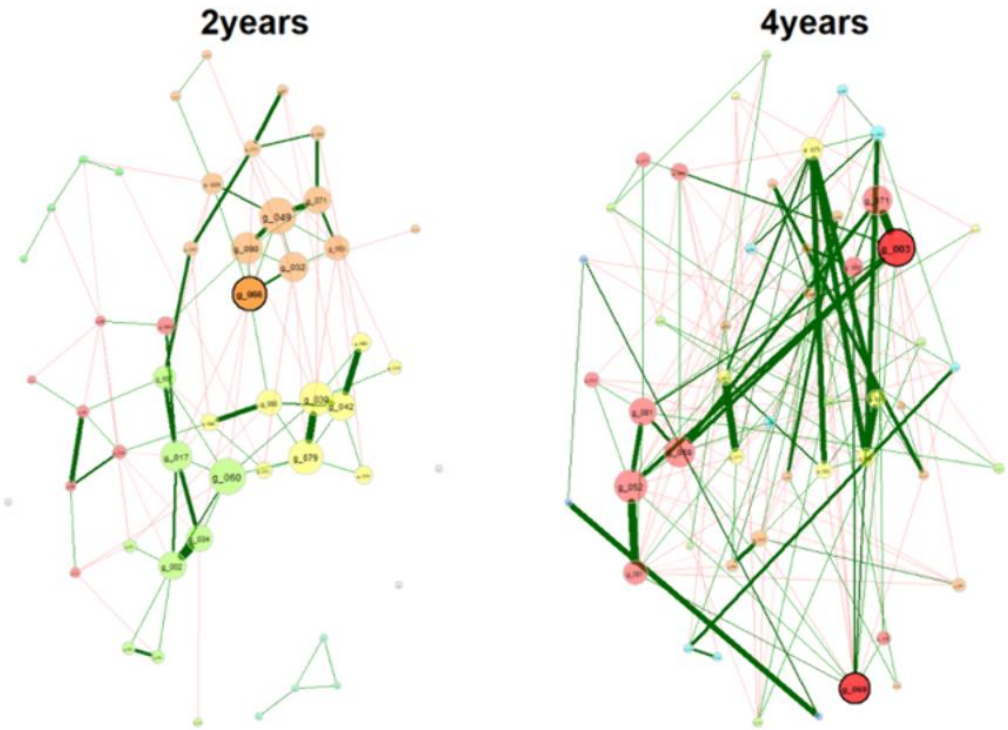

Figure 1. Comparison between 2 and 4 years old networks.

Conclusions: Results show a significant difference in the family and genus level between 2 and 4 years timepoints, displaying a shift from Lachnospiraceae abundance at two years old to Ruminococcaceae at four years old.Both family hubs are associated with behavioral changes.Further analysis should consider a cross-domain network with different domains of life,like fungi or viruses.

Topic: *AS03 PBI - The gut brain axis in early life*

THE INFLUENCE OF FECAL MICROBIOTA TRANSPLANTATION FROM CHILDREN WITH AUTISM SPECTRUM DISORDER ON FOOD INTAKE HORMONES IN MICE.

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Background and Aims: Autism spectrum disorder (ASD) is heterogeneous group of disorders characterized by deficits in social interaction and communication, and by restricted, repetitive behavior. Considering recent advancing research about microbiota-gut-brain axis, gut microbiota could take part in triggering ASD development in the existing genetic background. It has been found, that children with ASD have not only higher rate of GI disorders, than neurotypical controls, but they also frequently display the unusual feeding behaviors, as food selectivity, both of them associated with the severity of ASD symptoms. These “picky eaters” with ASD display different gut microbiota. Microbial metabolites and gut peptides are involved in homeostatic control of food intake through the hypothalamic and brainstem structures what emphasize the role of gut-brain axis in central food intake regulation. Gut hormones involved in homeostatic control of food intake, as ghrelin and leptin are altered in children with ASD.

Methods: We performed fecal microbiota transplantation (FMT) from ASD and control children to WT mice and evaluated the concentration of leptin, ghrelin and NPY, which are involved in food intake regulation.

Results: Ghrelin levels were significantly higher after FMT from ASD children compared to control FMT ($p=0.03$). Similarly, plasma leptin levels had trend to elevate ($p=0.07$) after FMT from ASD children. No significant differences in plasma levels of NPY were found. Interestingly, ASD FMT to mouse model of ASD SHANK3b-/- lead only to increase of plasma ghrelin concentrations (0.05).

Conclusions: These data support the hypothesis of gut microbiota role in food intake disturbances in ASD. APVV-20-0114, APVV-20-0070, APVV-20-0139, VEGA 1/0062/21

Topic: *AS03 PBI - The gut brain axis in early life*

ABERRANT GUT-MICROBIOTA-IMMUNE-BRAIN AXIS DEVELOPMENT IN PREMATURE NEONATES WITH BRAIN DAMAGE

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Background and Aims: Extremely premature infants are at high risk of suffering from brain injury and lifelong neurological impairments. The mechanisms underlying disease progression involve intestinal microbiota, but remain poorly understood, thereby limiting the development of novel therapeutic interventions. Here, we performed the first comprehensive, time-resolved analysis of the gut microbiota-immune-brain axis in a cohort of 60 extremely premature infants that were born before 28 weeks of gestation, weighing less than 1kg.

Methods: 16S rRNA gene-targeted qPCR and 16S rRNA gene amplicon sequencing: quantitative microbiome profilingv in premature infant stool. FACS & Luminex: T cell biology in premature infant blood. Metabolomics: quantification of short-chain-fatty acids in premature infant stool. Amplitude-integrated EEG and near-infrared spectroscopy: monitoring of neurophysiological development. cranial MRI: identification of severe brain damage.

Results: Infants suffering severe brain injury display a pro-inflammatory polarized T cell response, translating into suppressed maturation of electro-cortical activity. Elevated γδ-T cell levels, increased T-cell secretion of IL-17a and VEGF-A, and reduced secretion of neuroprotectives are key immunological elements in the pathogenesis of brain injury. Gastrointestinal Klebsiella overgrowth associates with pro-inflammatory tone, underlying disease progression, and is highly predictive for brain damage.

Conclusions: Our data suggest that Klebsiella overgrowth as well as associated alterations in the microbiome may exacerbate brain injury, perhaps by triggering changes in immunological development such as elevated γδ T cell levels. These immunological alterations, combined with the subsequent depletion of neuroprotective agents, may affect neurodevelopmental maturation. These findings suggest that novel therapeutic measures targeting the gut-microbiota-immune-brain axis may hold potential for protecting premature infants from severe brain injury.

Topic: *AS03 PBI - The gut brain axis in early life*

DOES A DYSBIOTIC NEONATAL MICROBIOME PROMOTE NEURODEGENERATIVE DISEASE (NDD) IN ADULTHOOD: A THEORY-DRIVEN DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHAD) HYPOTHESIS

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Background and Aims: Within seconds of birth, infants are seeded by millions of microbes. Infants born prematurely (commonly by c-section), at very low birth-weight (VLBW), are at increased risk of dysbiosis, because of immaturity, compromised mucosal lining, and inadequate innate immune responses. Our work shows that neonatal dysbiosis can lead to a diminished height trajectory, increased body mass index, and slowed neurodevelopment into toddler years. Longitudinal cohort studies of VLBW infants demonstrate higher incidence of atherosclerosis and other pathophysiological outcomes in adulthood. This study explored plausible neonatal origins of inflammatory-induced neurodegenerative disease (NDD) via the gut-brain-axis to generate a developmental origins of health and disease (DOHaD) theory for hypothesis testing.

Methods: Based on our previous research and our recently published review of the literature, we have hypothesized that sequelae stemming from the dysbiotic infant gut include risks for neurodegenerative diseases in adulthood.

Results: Developmental Origins of NDD Hypothesis proposes that a dysbiotic gut microbiome in early life promotes inflammation along the gut-brain-axis and the development of NDD in adulthood. It is hypothesized that premature VLBW infants experience higher incidence of NDD as adults compared to term infants.

Conclusions: This work proposes a testable Developmental Origins of NDD Hypothesis, based on evidence from the literature and preliminary studies. This theory and its related hypotheses should be tested through longitudinal observational cohort trials and epidemiological studies.

Topic: *AS03 PBI - The gut brain axis in early life*

CHILDHOOD ANXIETY AND OPPOSITIONAL BEHAVIOR ARE ASSOCIATED WITH INFANT GUT MICROBIOME ALPHA DIVERSITY

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Background and Aims: The Very Low Birth Weight (VLBW) infants' immaturity along with NICU events and procedures predispose these infants to gut dysbiosis. Microbial diversity is often decreased during intestinal dysbiosis favoring the proliferation of bacteria with pathogenic potential. We explored correlations between the VLBW gut microbiome richness, diversity, and Hill numbers and later childhood behavior.

Methods: Parents consented to a study of their infant with stool samples collected during their NICU stay and later assessments at 2 and 4 years of age. The Child Behavior Checklist (CBCL) scales were analyzed for correlation with alpha diversity indices including Chao, Shannon, Simpson, and Inverse Simpson(1-D). Additionally, we performed a canonical correlation analysis to simultaneously examine correlations between the infant microbiome and multiple measures of the Child Behavior Checklist.

Results: There were significant relationships between microbiome alpha diversity and anxiety and oppositional behavior domains.There was an inverse association between the CBCL domain of anxiety and earlier infant stool microbiome diversity(Chao rho= -.564,p= 0.01) and later infant diversity(Shannon,rho= -4.76,p= 0.025;Simpson,rho= -.469,p= 0.028;InvSimpson, rho= -.539,p=0.010). Later infant stool microbiome also showed an inverse association with oppositional behavior at 4 years old(InvSimpson, rho=-437, p=0.042).Canonical Correlation Analyses identified associations both between CBCL domains(eg, CBCL IV withdrawn and CBCL 3 autism),as well as between early infancy Hill values and the oppositional domain.

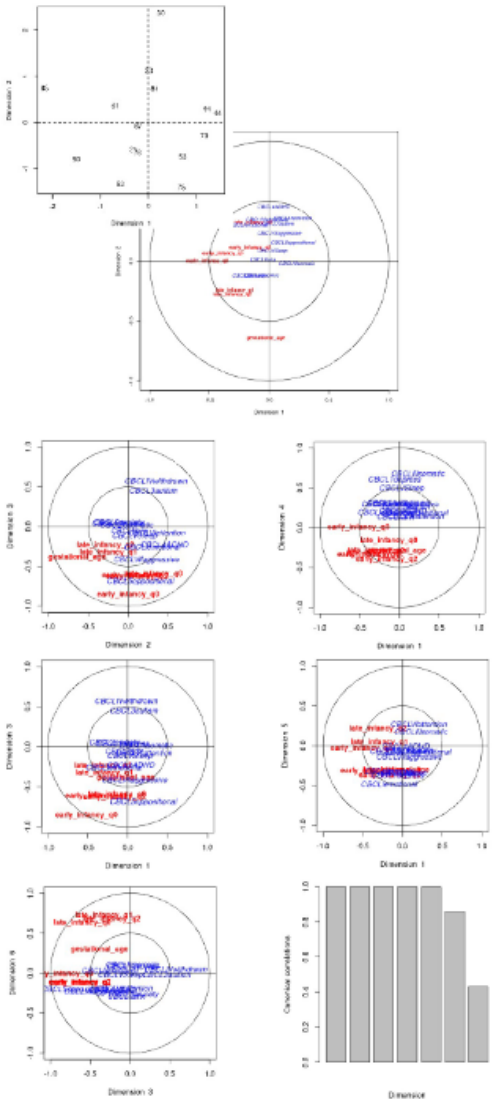

Figure 1. Canonical Correlation Analysis between early and later infant microbiome and behavioral domains and scales at 2 years of age.

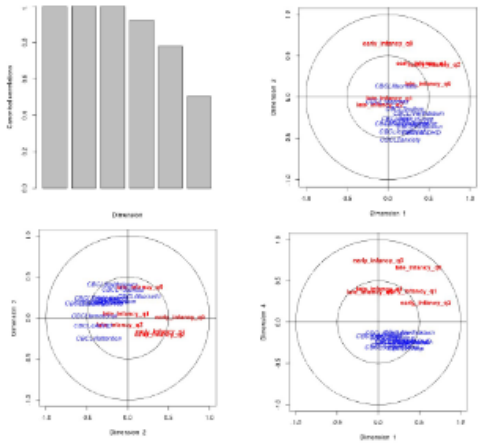

Figure 2. Canonical Correlation Analysis between early and later infant microbiome and behavioral domains and scales at 4 years of age

Figure 2. Canonical Correlation Analysis between early and later infant microbiome and behavioral domains and scales at 4 years of age.

Conclusions: These exploratory findings corroborate published findings that the infant microbiome is associated with subsequent behavioral outcomes(Loughman et al., 2020). Stool dysbiosis may alter the developing brain in infancy with potential long-term effects on behavior.

Topic: *AS03 PBI - The gut brain axis in early life*

A PRELIMINARY INVESTIGATION OF INTERACTIONS AMONG EARLY FEEDING PRACTICES, GUT MICROBIOME DIVERSITY, AND AMYGDALA GROWTH DURING THE FIRST YEAR OF LIFE

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Background and Aims: The human brain and the human gut microbiome undergo rapid change early in development, yet little is known about how they interact. Alpha diversity has been associated with outcomes related to amygdala function, and is impacted by early feeding practices (e.g., breastfeeding). We hypothesize that child feeding practices and gut microbiome alpha diversity between birth and 4 months old will be associated with rate of amygdala volume expansion between birth and 12 months old.

Methods: Bacterial constituents were identified using 16S rRNA amplicon sequencing from infant fecal samples (N = 33) using DADA2, and Chao1 alpha diversity was calculated for each sample. A linear regression model was used to examine the association between Chao1 and rate of amygdala volume growth.

Results: There was a significant association ($p = 0.0166$) between formula feeding (N=5) and slower right amygdala growth during the first year of life. This association was diminished in the left ($p = 0.0877$). There was no significant association between Chao1 and amygdala growth rate (left, $p = 0.9549$; right, $p = 0.0943$).

Conclusions: However, there was a significant interaction ($p = 0.0396$) between Chao1 and formula feeding for right amygdala growth rate, suggesting that in formula fed infants lower alpha diversity is potentially related to slower growth of the right amygdala. Investigations using a larger sample size with more balanced early feeding practices and more detailed descriptions of relative abundances of gut microbiota are needed to confirm or refute these preliminary findings.

Topic: *AS04 PBI - How to interpret the preterm microbiome -Preterm birth: microbial ethology or not*

POSTNATAL GUT MICROBIOME DEVELOPMENT INFLUENCED EARLY CHILDHOOD GROWTH IN PRETERM INFANTS

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Background and Aims: Gut microbiota dysbiosis has been implicated in the growth deviance of preterm infants. The timing of acquisition as well as abundance of bacterial genuses that support appropriate growth in preterm infants is unclear. Our study examined the relationship between early microbiome development and childhood growth patterns in preterm infants.

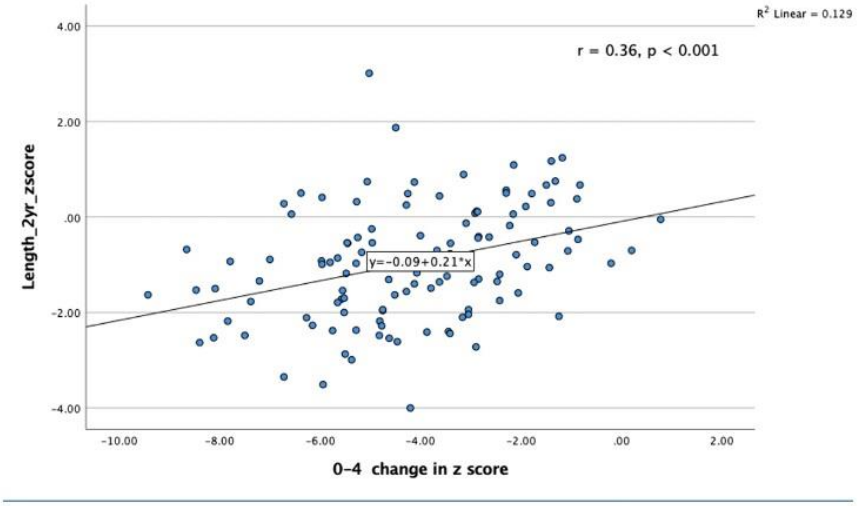
Methods: This is a retrospective study of infants who were born <35 weeks between 2012-2018 at a level III urban NICU and had at least one well visit during 2-5 years of age. Growth outcomes were categorized as early or late catch-up growth; both stages of growth measured by change in weight and length z scores as well as childhood body mass index (BMI). In a subset of infants, stool microbiome data from 16S rRNA sequencing was analyzed along with growth pattern.

Results: There were 160 infants included in the growth analysis with birth weight and gestational age of 1149 ± 496 grams and 28 ± 3 weeks. At 2 years, most of the infants (71%) had a healthy BMI. During the first month after birth, increased Bacteroidales and decreased Gammaproteobacteria were associated with better early catch-up growth (Table 1). Early catch-up growth was associated with better linear growth at 2 years ($p < 0.001$) (Figure 1).

Table 1: Early catch-up growth correlates with microbial abundance over time

Time	Abundance	P value
0 – 14 days of life	Increased Bacteroidales	0.024
14 – 28 days of life	Decreased *Proteus*	0.04
> 28 days of life	Decreased *Gammaproteobacteria*	0.018

Figure 1:



Conclusions: The results suggest that gut microbiota development during the first month after birth was predictive of early catch-up growth and childhood BMI of preterm infants. Our findings add to the understanding of post-natal microbiome acquisition in the context of preterm growth patterns.

Topic: *AS04 PBI - How to interpret the preterm microbiome -Preterm birth: microbial ethology or not*

THE ASSOCIATION BETWEEN VAGINAL MICROBIOTA COMPOSITION AND PRETERM BIRTH IN PEMBA ISLAND, TANZANIA

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Background and Aims: Preterm birth (PTB) has multiple contributing factors including infection, inflammation, and the vaginal bacterial ecosystem (vaginal microbiota, VMB). VMB are often dominated by lactobacilli, which further increase in abundance during pregnancy. When dysbiotic, VMB can associate with PTB, whose burden differs significantly across geographical regions and populations. Women of African ancestry appear to bear a disproportionately high PTB risk, as well as carry a diverse non-Lactobacillus dominated VMB, frequently associating with vaginal dysbiosis.

Methods: In this retrospective, biobank-based study, VMB from Pemban women who experienced PTB (n=27) or at term births (n=40) were characterized. Samples were collected using FLOQSwabs vaginal swabs (Copan Italia) in the first and second trimester of pregnancy and preserved in eNAT buffer (Copan Italia).

Results: VMB were analysed by IS-pro approach, a method that characterizes bacteria by species-specific length polymorphisms of the 16S-23S rDNA interspace region. In the second trimester, VMB were mostly L. iners-dominated both among women experiencing PTB (37%) and at term birth (50%). In these groups, women carrying diverse VMB were 26% and 23%, respectively; while 59.3% and 60% respectively had a combined relative abundance of pathobionts (commensals with pathogenic potential) >20%. In the third trimester, VMB were mostly L. iners-dominated among women experiencing PTB (53%) and at term birth (50%), with both groups carrying diverse VMB (7%); while 46.6% and 48.4%, respectively, had a combined relative abundance of pathobionts >20%.

Conclusions: These preliminary results show a limited difference in VMB composition and pathobionts presence between the two groups. Further testing is ongoing.

Topic: *AS04 PBI - How to interpret the preterm microbiome -Preterm birth: microbial ethology or not*

DOES PROBIOTICS SUPPLEMENTATION IMPROVE FEEDING TOLERANCE IN VERY PRETERM INFANTS?

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Background and Aims: Given that probiotics reduce the risk of necrotizing enterocolitis (NEC), the European Society for Paediatric Gastroenterology, Hepatology and Nutrition recently published a Position Paper supporting its use. Thereafter, the NICUs in Stockholm/Sweden implemented probiotics as standard of care for very preterm infants. We hypothesized that probiotics supplementation would improve feeding tolerance and reduce risks of neonatal morbidities.

Methods: Observational retrospective cohort study including very preterm infants (28+0 – 31+6 weeks) born in Stockholm 2019-2021, before and after implementation of probiotics supplementation. Primary composite outcome of death, infection and NEC. Secondary outcomes: duration of parental nutrition; time to full enteral feeding; length of stay; abdominal x-ray; number of days on antibiotics; and breast milk feeding at discharge.

Results: Among 349 very preterm, 209 received no probiotics and 140 received probiotics, with similar perinatal characteristics. As compared with no probiotics supplementation, rates and adjusted RR of infants received probiotics supplementation were for the composite outcome (death, infection and NEC) 4.3 % versus 10.5%, 0.37 [0.14 – 0.96]; abdominal x-ray 17.9% versus 28.2%, 0.51 [0.30 – 0.89]; breast milk feeding at discharge (exclusive and partial) 80.0% versus 70.0%, 1.78 [1.05 – 3.01] and number of days on antibiotics 5.8 versus 7.5, -1.96 [-3.52 – -0.41]. Among infants without the composite outcome, probiotics was still associated with less need of abdominal x-ray, less use of antibiotics, more breast milk feeding at discharge and shorter time to full enteral feeding.

Conclusions: Very preterm infants benefit from probiotics supplementation by reduced risks of neonatal morbidities and improved feeding tolerance.

Topic: *AS04 PBI - How to interpret the preterm microbiome -Preterm birth: microbial ethology or not*

INCREASED FETAL MEMBRANE BACTERIAL LOAD LINKED TO HISTOLOGICAL CHORIOAMNIONITIS IN PRETERM BIRTH

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Background and Aims: Histological chorioamnionitis (HCA) is inflammation of the fetal membranes. Understanding the fetal membrane microbiota linked to inflammation and bacterial infection is incomplete. This research aimed to increase current knowledge of the fetal membrane microbiota in HCA, including bacterial profile, bacterial load and correlation to inflammatory response.

Methods: A retrospective cohort study was employed on fetal membranes from patients with preterm spontaneous labour and HCA (n=12), or preterm (n=6) and term labour without HCA (n=6), plus low risk term patients (n=58). To investigate bacterial profiles, 16S rRNA Illumina sequencing was performed. Bacterial loads were assessed by 16S rRNA BactQuant qPCR, with bacterial loads also correlated to inflammatory marker data.

Results: Bacterial loads were significantly greater from HCA patients (5013.066 copies/µl) compared to preterm (288.873 copies/µl) and term without HCA (254.819 copies/µl). Increased bacterial load was positively correlated with maternal inflammatory staging, and the expression of five inflammatory markers. Non-HCA patients, low risk term patients and negative controls did not display distinct bacterial loads (200-300 copies/µl). A trend for increased Prevotella with increased inflammation was detected.

Conclusions: Inflammatory HCA involves increased bacterial load in a dose response relationship, with an association to increased Prevotella. Bacteria is not acquired in utero on fetal membranes without an inflammatory condition.

Topic: *AS05 PBI - The microbiome-immune system crosstalk in the first 1000 days*

MATERNAL MICROBIOTA ARE CRUCIAL FOR FETAL ANTI-VIRAL IMMUNE RESISTANCE AGAINST ZIKA VIRUS DURING PREGNANCY

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Background and Aims: Zika virus (ZIKV) is known to cross the placenta and infect the fetus, causing congenital disease. We have previously demonstrated in a germ-free mouse model that the absence of microbes in pregnant mice increases vertical transmission. Here, we hypothesized that beneficial microbes are essential for normal immune function and development, including against viral infections. Our aims were to profile 32 cytokines of strong biologic relevance in germ-free, recolonized, and control animals challenged with ZIKV.

Methods: Timed pregnant specific-pathogen-free (SPF) or gnotobiotic germ-free (GF) Swiss-Webster mice were mock injected or received 4 doses of $1x10^4$ PFU first passage ZIKV: SPF mock (n=44), GF mock (n=36), SPF + ZIKV (n=13), GF + ZIKV (n=33), adult recolonized + ZIKV (n=11), SPF + ZIKV + anti-IFNαR (n=7), and GF + ZIKV + anti-IFNαR (n=18). Mouse placentas were extracted via sterile Cesarean, homogenized, and then analyzed via Luminex Assay.

Results: All but four cytokines (GM-CSF, IL-3, IL-4, MIP-2) were of significance in relation to the increased risk of congenital ZIKV infection in the absence of bacteria. A total of 17 of the 32 cytokines including IL-1α, IL-2, and IL-13 ($p<0.0001$, Kruskal-Wallis with Dunn's multiple comparisons test) showed significantly increased concentrations in GF mock infected compared to SPF mock infected mice, highlighting the profound impact of maternal microbiota on fetal immune responses in general. Interestingly, postnatal "rescue" of bacterial recolonization did not restore natural microbial anti-viral immune resistance.

Conclusions: Overall, our findings suggest that developmental exposure to microbes is essential to later in life anti-viral immunity in pregnancy.

Topic: *AS05 PBI - The microbiome-immune system crosstalk in the first 1000 days*

DISTINCT IMMUNOMODULATORY EFFECT OF SHORT CHAIN FATTY ACIDS AND COMMENSAL BACTERIA DERIVED METABOLITES ON IMMUNE CELLS

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Background and Aims: A symbiotic relationship between microbes and host ensures the maintenance of homeostasis in the gut, which is a complex co-ordination between the microbiota associated products and various immunological mediators. Short chain fatty acids (SCFAs), derived from bacterial fermentation of dietary fibers, and metabolic products from commensal bacteria induce immune mediators on the mucosal surface and regulate the mucosal immunity. LL-37, an antimicrobial peptide ubiquitously expressed on gut epithelial surface, has broad spectrum antimicrobial and immunomodulatory activities. We aimed to investigate the efficacy of a SCFA butyrate (PBA) and the metabolites from different Lactobacillus, Lactobacillus reuteri (LR) and Lactobacillus rhammnosus (LGG) on the expression and production of LL-37, IL-18, IL-8 and IL-6 from HT-29 epithelial cell line and monocyte derived macrophages.

Methods: PBMCs were stimulated with PBA or cell free supernatants (CFSs) from LR and LGG. Real time-PCR and ELISA-based methods were used to analyse gene expression and protein production respectively.

Results: In vitro stimulation with PBA and the CFSs from the lactobacilli induced LL-37 gene expression and protein production by both HT29 cells and macrophages. Differential expression of the IL-18 gene was observed for PBA and CFSs in HT-29 cells and macrophages, while only CFSs from LR and LGG elicited IL-6 and IL-8 production from the macrophages.

Conclusions: These findings suggests that the metabolic products from gut commensal bacteria modulate the immune response differently and could play a role in the innate immune response to fight pathogenic microbes.

Topic: *AS05 PBI - The microbiome-immune system crosstalk in the first 1000 days*

A PILOT STUDY: CYTOKINE PATTERNS AND GUT MICROBIOTA IN CHILDREN BORN WITH VERY-LOW- BIRTH-WEIGHT

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Background and Aims: Gut dysbiosis frequently occurs in infants with Very-Low- Birth-Weight (VLBW) and is related to the risk of infection or inflammatory diseases in early childhood. Our previous study showed VLBW infants have significantly different microbiome composition and pathways compared to term infants, which could result in a risk of infection and inflammation. Cytokines are one of the biomarkers to measure inflammation, however, there are currently no studies examining the relationship between inflammatory cytokine and gut microbiota for children born with VLBW. Here, we measured inflammatory cytokine levels collected from dried blood spots (DBS) and the gut microbiota for children between the ages of 2-4 years old, born with VLBW.

Methods: 17 of VLBW children's parents consented to follow-ups at ages 2 and 4 years. We measured cytokines from DBS samples. In order to identify the ASVs significantly associated with the cytokine levels, mixed model regression employing 'Negative binomial and zero-inflated mixed models' implemented in R package 'NMZIMM' was utilized.

Results: Il-TAC, INF-a, IL-4, and IL-6 showed a significant negative correlation to butyrate-producing bacteria in genus levels. Furthermore, Bacteroides ovatus, related to inducing a serum antibody response in inflammatory bowel diseases, showed a negative correlation with IL-6 ($t=-2.310$, $p=0.010$). Streptococcus Luteciae, known to induce diarrhea in children, was associated positively with I-TAC ($t=2.920$, $p=0.011$). Ruminoccoccus gnavus, known to exacerbate symptoms of Crohn's disease, was positively corrected to IL-4 ($t=2.931$, $p=0.012$).

Conclusions: Our pilot study suggests relationships between the gut microbiota and cytokine levels in children born with VLBW infants at ages 2-4 years old.

Topic: *AS05 PBI - The microbiome-immune system crosstalk in the first 1000 days*

MAPPING INTESTINAL HOST-MICROBIOTA COMMUNICATIONS AT BIRTH, AND THEIR POTENTIAL IMMUNOLOGICAL IMPACT

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Background and Aims: The interactions between the gut microbiota and the immune system at birth influence long-term immunological development and the long-term risk of asthma, allergies, and other inflammatory diseases. One of the major factors that determine neonatal gut microbiota composition is delivery mode, and numerous studies have demonstrated dysbiotic microbiome composition in cesarean-section (CS)-born infants. Here, we aim to understand how intestinal colonization by microbiota at birth shapes the immune system and impacts health and disease throughout life. Moreover, we aim to determine whether delivery mode-related alterations to neonatal microbiota composition in humans disrupt early-life intestinal responses, and to identify molecular mechanisms that control disease susceptibility throughout life.

Methods: To address these aims, we developed an intestinal gut organ culture system for the murine embryonic gut. Intestinal tissues were dissected from E19 mice embryos (fully developed, yet still germ-free) and were colonized on-a-chip with microbiota collected from human babies, born by vaginal or CS delivery.

Results: We found that ex-vivo colonization of the embryonic gut elicits rapid and distinct transcriptional responses to the microbiota, that display birth mode-specific features. I.e., CS-derived microbiota, but not vaginal birth-derived microbiota, induced colonic proinflammatory gene expression. We further identified specific bacterial strains that mediate the effects induced by whole-microbiota colonization.

Conclusions: Collectively, we suggest that birth-mode related differences in microbiota composition trigger distinct intestinal responses at birth, which potentially mediate long-term immunological development and diseases susceptibility.

Topic: *AS05 PBI - The microbiome-immune system crosstalk in the first 1000 days*

IMPORTANCE OF THE TIMING OF MICROBIAL SIGNALS FOR PERINATAL IMMUNE SYSTEM DEVELOPMENT AND ITS MODULATION BY LIMOSILACTOBACILLUS REUTERI TREATMENT IN EARLY LIFE

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Background and Aims: Perinatal immune development prepares the infant for the various microbial and environmental antigens it will encounter throughout life and microbial products from the maternal and neonatal gut microbiomes are known to influence immune system development. However, we are only beginning to understand the importance of the timing of microbial signals, either prenatal, preweaning or postweaning, for immune system development and whether probiotic bacteria could be used to redress alterations in host development due to neonatal gut microbiome perturbations.

Methods: To determine the relative importance of these events, we characterized immune cell populations in mice born to conventional dams (CONV) and mice that were born to germ-free dams and colonized with a complex gut microbiome either immediately after birth (early colonized; EC) or one week after weaning (delayed colonized; DC).

Results: We found similar proportions of nearly all examined immune cell populations in CONV and EC mice, while DC mice showed alterations in the proportions of several splenic immune cell populations, including a greater proportion of dendritic cells and regulatory T (Treg) cells compared to EC mice. Early life treatment of DC mice with L. reuteri restored the proportions of some splenic immune cell populations, including dendritic cells and Tregs, to what was observed in EC mice.

Conclusions: Our findings indicate that microbial signals in the preweaning period are more important than prenatal microbial signals for perinatal immune development and that probiotic microorganisms may be useful to rectify some of the deleterious effects of gut microbiome perturbations on neonatal immune development.

Topic: *AS05 PBI - The microbiome-immune system crosstalk in the first 1000 days*

EFFECTS OF EXPOSURE TO PARTICULATE MATTER ON RSV BRONCHIOLITIS IN INFANTS

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Background and Aims: Bronchiolitis caused by respiratory syncytial virus (RSV) is one of the main causes of hospitalization in infants. The severity of RSV infection is only partially explained by known risk factors. Exposure to particulate matter (PM) has been demonstrated to cause dysbiosis of the upper respiratory microbial community, which might in turn affect the antiviral immune defense. Our hypothesis is that exposure to PM might alter the inflammatory cascade that brings to RSV infection, thus influencing host responses to RSV in infants.

Methods: We recruited children younger than one year old with different RSV bronchiolitis severity degree. The estimated levels of daily PM_{10} and $PM_{2.5}$ concentrations obtained from Open Data Lombardy Region, Italy, were assigned to each subject from the day before the enrolment to one month before. To explore nasal microbiota composition, 16S analysis on V3-V4 regions on nasal swabs sampled from case and control children were used.

Results: We observed a strong positive association between the severity of RSV bronchiolitis and PM exposure levels. Investigating the effect of PM on children nasal microbiota composition, we identified Haemophylus as most significant differential genus: its relative abundance was increased in RSV bronchiolitis and with PM concentration. Specie-level analyses determined that Haemophylus influenzae was the dominant specie colonizing the airways of children.

Conclusions: This approach aimed to help unveiling the link between PM exposure and the severity of RSV bronchiolitis in infants, suggesting respiratory tract dysbiosis as an important trigger.

Topic: *AS05 PBI - The microbiome-immune system crosstalk in the first 1000 days*

IGA - MICROBIOTA EARLY IN LIFE: THE IMPACT OF BREASTFEEDING ON IMMUNE-MICROBIAL INTERACTION

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Background and Aims: Recent studies suggest that secretory IgA (sIgA) is a modulator of intestinal microbiota and also, it would contribute in the development of the newborn's immune system, until the neonate is capable of independently producing its own antibodies in the intestine. This work is aimed to identify the role of IgA-specific microbial pattern recognition in the infant gut microbiota and the impact of specific IgA-bacteria on the host response.

Methods: We separated IgA-coated bacteria from 1-month-old infant gut microbiota using magnetic beads (Dynabeads) and used IgA+ and IgA- fractions for targeted microbial isolation, focused mainly on Bifidobacterium and Lactobacillus strains. In binding assays using purified IgA from human colostrum the ability of isolated strains to be opsonized with IgA was assessed. The immunomodulatory effect of these strains was evaluated in THP-1 macrophages.

Results: IgA binding was strain- and concentration-dependent, particularly B. bifidum and B. pseudocatenulatum showed very high binding to IgA (> 80%), while in B. longum subsp. infantis less than 10% of bacteria were opsonized with IgA. In Lactobacillus strains, we identified L. rhamnosus R1 as highly coated with IgA (94.3%). Co-incubation with THP-1 macrophages showed that most bifidobacteria when pre-associated with IgA enhanced IL-1β secretion and reduced IL-6 and IL-10, especially in B.bifidum, while low IgA binding of B. infantis was not able to induce significant differences when compared to IgA-unassociated B. infantis.

Conclusions: Our work has identified strains that are highly coated with IgA and suggests that IgA opsonization of commensal bacteria elicits a differential immunomodulatory response in the host.

Topic: *AS06 PBI - Other*

IDENTIFICATION OF NOVEL INTERACTIONS BETWEEN INFANT GUT MICROBIOTA COMPOSITION AND FUNCTION, ENVIRONMENTAL EXPOSURES AND HEALTH OUTCOMES

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Background and Aims: Many early life events have a known effect on the infant gut microbiota development; for example, the effects of antibiotics and birth mode have been extensively studied. Nevertheless, a large majority of infant gut microbiota variation sources remains unknown.

Methods: In the Finnish Health and Early Life Microbiota (HELMi) birth cohort, over 50 000 longitudinal questionnaires were collected from over 1000 families during the first 2 years of child's life in parallel to stool samples, providing a diverse and extensive source of pre- and post-natal variables and outcomes to explore and evaluate their temporal importance. In the ongoing analyses 16S rRNA gene amplicon sequences from over 10 000 fecal samples collected from the first two years from the infants and spot samples from both parents are coupled with the extensive lifestyle and health data collection, and with technical variables e.g. on fecal sample storage and consistency. The most important microbiota co-variates revealed in amplicon analyses will be extended to metagenomic data analyses from 400 samples to further examine their associations to microbiota functions.

Results: Previously known variables, such as delivery mode and antibiotics stand out as major contributors to overall gut microbiota composition, but we also identified less studied variables like the household size or skin contact after birth playing a role on early microbiota composition.

Conclusions: Our comprehensive analysis can identify novel co-variables for future gut microbiota studies, raising new interesting biological questions and identifying important confounders, including the effects of different technical variables.

Topic: *AS06 PBI - Other*

THE DANISH MATERNAL AND OFFSPRING MICROBIOME STUDY (DANMOM): A STUDY PROTOCOL FOR A LONGITUDINAL PROSPECTIVE COHORT

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Background and Aims: The human gut microbiome has a great influence on human health and is established early in life. Several factors have been described that influence how establishment occurs, including mode of delivery, breast feeding, use of antibiotics, and even prenatal factors. Disturbances in some of these factors may be associated with a dysbiotic gut microbiome and disease development. Most studies are cross-sectional, disabling the possibility to identify temporal changes in the microbiome prior to adverse events. Longitudinal prospective cohort studies are therefore vital. Here we present the study protocol for an ongoing longitudinal prospective Danish mother-child cohort.

Methods: Pregnant women are enrolled at gestational weeks 11-14. Offspring are enrolled at birth. We aim to include 300 women. Samples for microbiome and biochemical analyses are collected from the women at gestation weeks 11-15, 19-20, and 34-37. Follow-up samples from the mother and child are collected within 3 days after birth, at 2 weeks, 6 months, 1 year, 2 years, 3 years, and 5 years postpartum. From the mother we collect feces, urine, blood, saliva, vaginal fluid, and breast milk. Feces and a blood spot are collected from the offspring. In addition, we collect a large amount of demographic and medical data using medical charts, registers, and questionnaires.

Results: We have currently included 130 women and 38 children. Recruitment runs from June 2019-December 2022.

Conclusions: We expect the DANMOM cohort to provide us with valuable information on the role of the gut microbiome in human disease.

Topic: *AS06 PBI - Other*

EVALUATION OF NOMADIC AND NICHE-SPECIALIST LACTOBACILLI AS POTENTIAL VAGINAL PROBIOTICS

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Background and Aims: The aim of this study is the development of a multi strain probiotic gel to promote lactobacilli-dominated vaginal microbiota in pregnant women and to establish a proper eubiosis on the new-born. Nomadic lactobacilli, mainly isolated from food sources, were screened for functional characteristics and the capability to inhibit Streptococcus agalactiae and Candida albicans, which may lead to adverse pregnancy-related outcomes.

Methods: Ninety-five strains were screened for hydrophobicity, auto-aggregation, hydrogen peroxide production, and lactic acid isomers quantification. Cell-free supernatants (CFSs) of the candidate strains were co-inoculated with vaginal pathogens for high-throughput inhibition screening. Aiming to evaluate the reduction of the expression of genes involved in the inflammatory cascade the best performing strains were investigated in vitro alone and in combination.

Results: The production of hydrogen peroxide was strain dependent, with the highest concentrations found for Lacticaseibacillus paracasei. Lactiplantibacillus plantarum produced both isomers of lactic acid, while Lb. paracasei produced only L-isomer. Hydrophobicity and auto-aggregation characteristics higher than 50% were observed respectively for 25% and 86% of all the strains. S. agalactiae was strongly inhibited by a wide range of CFSs in different modes of action, whereas C. albicans inhibition was less frequent.

Conclusions: Overall, L. plantarum had the highest pathogen inhibition score and the best functional traits. Two of the best performing strains showed a reduction on the expression of genes involved in the inflammatory cascade.

Topic: *AS06 PBI - Other*

LARGE-SCALE METAGENOME INSIGHTS ON EARLY LIFE MICROBIOME AND HEALTH

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Background and Aims: A prospective Finnish Health and Early Life Microbiota (HELMi) birth cohort (NCT03996304) has been set up to identify environmental, lifestyle and genetic factors that modify the early intestinal microbiota development, and to study how these relate to the child's health and well-being. The cohort consists of 1055 term infants born in 2016-2018 in Helsinki metropolitan area, and their parents.

Methods: In the context of the Million Microbiomes from Humans Project, deep metagenome data (over 70 Tb) was generated from over 5000 stool samples, mainly from infants at ages 3, 6, 12 and 24 months in parallel to spot samples from the parents. The cohort has exceptionally rich metadata as over 50 000 longitudinal questionnaires were collected during the first 2 years of child's life, providing a unique collection of exposure and health outcome data.

Results: This presentation will focus on the following key questions relating to early life microbiota colonization and later life health 1. The impact of delivery mode, early nutrition, probiotics, antibiotics and other medications on microbiota development and maturation 2. Association of the microbiota development with child well-being and health patterns 3. Vertical transfer of bacteria and their genes from both parents to the infant

Conclusions: Selected results and insights based on a deep and frequent longitudinal metagenome data from a well-characterized birth cohort will be presented.

Topic: *AS06 PBI - Other*

UTERINE MICROBIOME IN RECCURENT PREGNANCY LOSS AND ITS INFLUENCE ON ENDOMETRIUM RECEPTIVITY

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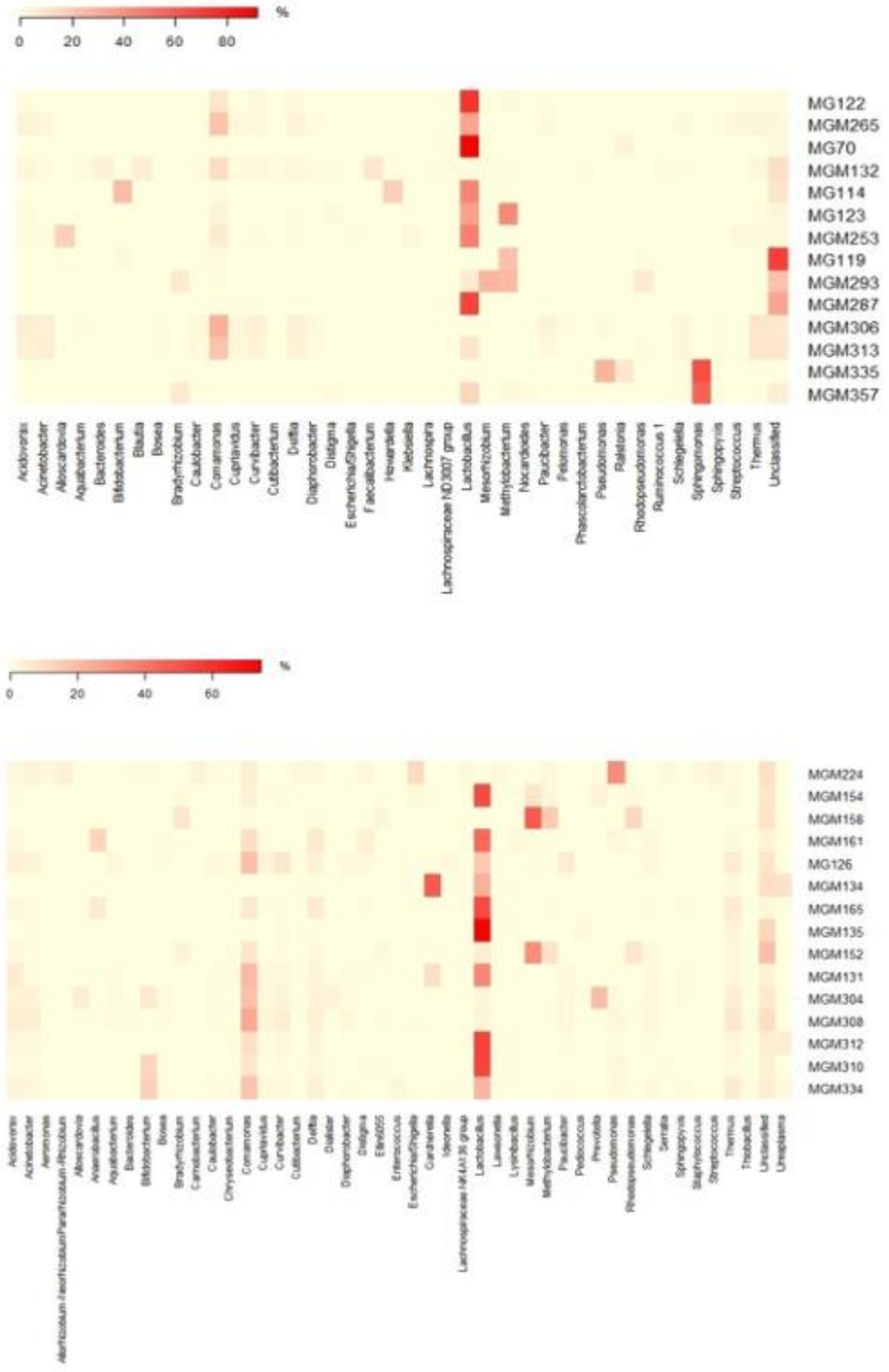
Background and Aims: Endometrial pathology as a cause of recurrent pregnancy loss (RPL) remains a problem of great interest. It is proved that even healthy endometrium is not a sterile tissue. The aim of this study was to reveal the microbes in the endometrium that can cause inflammation and chronic endometritis, thus becoming the reason of RPL.

Methods: To study endometrial microbiome Pipel biopsy of endometrium of 29 women was performed from 22 to 24 day of regular menstrual cycle. All women were divided into 2 groups: 15 healthy fertile women and 14 women with RPL. 16S rRNA sequencing was used to study endometrial microbiome and immunohistochemistry was used to reveal markers of chronic endometriris(CD138 and CXCL13).

Results: In women with RPL, the most common genus were Lactobacillus-30.3%, Comamonas-7.7%, Sphingomonas-8.6%, in the group of healthy fertile patients - Lactobacillus-29.4%, Comamonas-16.8% and Mesorhizobium-6.0%. Statistically significant differences were detected only in Brevibacillus and Corynebacterium1 abundance: in the group of healthy fertile women, Brevibacillus abundance was higher (0.19±0.24), in the group of women with RPL - 0.0014±0.0053 (p=0.008); the relative abundance of Corynebacterium1 in fertile women was less (0.013±0.052), in the group of women with RPL - 0.075±0.068 (p=0.002). Positive correlation was found between the presence of CD138 marker and high relative abundance of Pseudorhodoferax genus, and positive correlation was found between the CXCL13 marker and an increased concentration of bacteria of the genus Alistipes, Butyricimonas, Dialister, Leuconostoc, Neisseria, Parabacteroides, Phascolarctobacterium, Prevotella, Ruminococcaceae UCG-005, Sutterella, Sphingobium, Subdoligranulum iwomen with RPL.



Conclusions: Studying endometrial microbiome and markers of chronic endometritis can improve perinatal outcomes.

Topic: *AS06 PBI - Other*

ANALYSIS OF MICROBIOME PROFILE GROUPS REVEALED MORAXELLA EXPANSION DURING ASTHMA EXACERBATION IN CHINESE ASTHMATIC CHILDREN

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Background and Aims: There is paucity of data on alterations in nasopharyngeal microbiome (NPM) profile in childhood asthma exacerbation (AE). This study investigated temporal dynamics of NPM in Chinese children with AE.

Methods: Thirty-three exacerbation-prone schoolchildren with asthma were followed from September to December in 2017. Their spirometric indices and exhaled nitric oxide levels were measured at baseline, and AE occurrence were identified by asthma diary. Flocked nasopharyngeal swabs (FNPSs) were collected every 2-4 weeks for human rhinovirus detection and 16S rDNA sequencing. Twenty controls were also recruited. Microbiome communities were analyzed using QIIME2-DADA2 pipeline, and temporal dynamics evaluated by linear-mixed effect models.

Results: 121 FNPS samples from 13 stable asthmatics [AS] and 11 children with AE were studied. NPM diversity in asthmatics, at baseline and during AE, was lower than that of controls. NPM was classified into six microbiome profile groups with Moraxella, Corynebacterium 1, Dolosigranulum, Staphylococcus, Streptococcus and Anoxybacillus. Alpha diversity of NPM decreased ($P<0.001$) while microbial composition (beta diversity) remained similar over time. Moraxella ($P=0.006$) increased while Corynebacterium 1, Anoxybacillus and Pseudomonas decreased over time. However, these patterns and taxa abundances were similar between AE and AS. NPM dominated by Moraxella and Dolosigranulum exhibited temporal stability. NPM underwent Moraxella expansion during AE, which showed high microbiome resilience (recovery potential) afterwards.

Conclusions: NPM profiles in asthmatic children shift with time during autumn. Temporal pattern of NPM is not associated with childhood AE, but nasopharyngeal Moraxella expansion is linked to increased AE risk in children. (funded by Hong Kong Institute of Allergy Research Grant)

Topic: *AS06 PBI - Other*

SEMEN MICROBIOTA IN PATIENTS WITH LEUKOCYTOSPERMIA AND HEALTHY CONTROLS: CLUSTER ANALYSIS OF REAL-TIME PCR DATA

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Background and Aims: The analysis of semen microbiota is difficult due to the lack of established criteria for interpretation of microbiological tests. The aim of the study was to determine the stable clusters of semen microbiota analyzed by real-time PCR in samples with normozoospermia and leukocytospermia.

Methods: Semen samples with leukocytospermia (n=66) and normozoospermia (n=130) were included in the study. Semen microbiota was analyzed using real-time PCR kit Androflor (DNA-Technology, Russia). Cluster analysis was performed for 153 samples with the total bacterial load>10^3 GE/ml using the k-means++ algorithm, scikit-learn. The Silhouette index and the Davies–Bouldin index were used to confirm the stability of clusters.

Results: Four stable microbiota clusters were distinguished in semen samples. Cluster I was characterized by the prevalence of obligate anaerobes, Lactobacillus spp. were prevalent in Cluster II, Gram-positive facultative anaerobes were prevalent in Cluster III, Enterobacteriaceae/Enterococcus spp. were prevalent in Cluster IV. Cluster I in normozoospermia was represented by various obligate anaerobes without pronounced quantitative predominance of any bacterial group. In samples with leukocytospermia Bacteroides spp./Porphyromonas spp./Prevotella spp. group was prevalent. In leukocytospermia Cluster II was characterized by the prevalence of Lactobacillus spp., while in normozoospermia other bacterial groups were present. In normozoospermia Corynebacterium spp. and Streptococcus spp. were prevalent in Cluster III, while Staphylococcus spp. was abundant in leukocytospermia. In leukocytospermia lactobacilli were present in Cluster IV along with Enterobacteriaceae/Enterococcus, which was not typical for the samples with normozoospermia.

Conclusions: The detection rate of 4 stable semen microbiota clusters and the dominant bacterial groups varied in patients with leukocytospermia and normospermia.

Topic: *AS06 PBI - Other*

UTERINE MICROBIOME IN WOMEN WITH REPEATED IVF FAILURES AND IN HEALTHY FERTILE WOMEN

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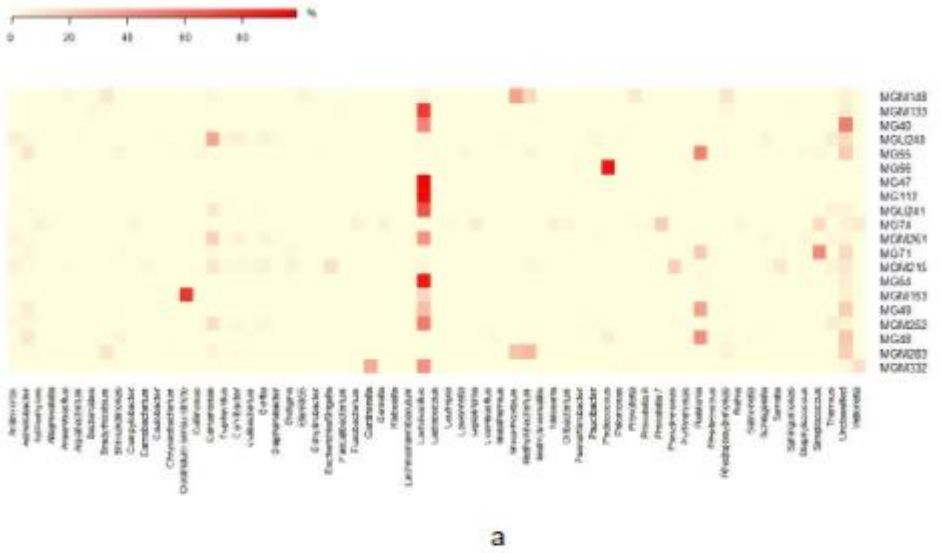
Background and Aims: The aim of the study was to reveal the features of the endometrial microbiome in healthy fertile women and in women with multiple IVF failures.

Methods: To assess the differences in the endometrial microbiota in 20 women with infertility and multiple unsuccessful attempts of IVF and in 15 fertile healthy women the endometrial microbiome was studied by NGS of 16S rRNA.

Results: Lactobacillus (29.4%), Comamonas (16.8%) and Mesorhizobium (6.0%) were the most abundant genera in the group of healthy fertile patients, and Lactobacillus (33.3%), Ralstonia (7.9%) and Pediococcus (4.8%) were mostly abundant in the group of infertile patients. The mean relative abundance of Lactobacillus did not significantly differ between groups and comprised 33.3% in the 1st group and 29.4% in the 2d group. Significantly higher mean relative abundance of bacteria of the genus Brevundimonas and Ralstonia was recorded in the 1st group. The fertile women of the 2nd group had a statistically significantly higher mean relative abundance of Acidovorax, Brevibacillus, Caulobacter, Comamonas, Delftia, Distigma, Pseudomonas, Schlegelella, Thermus.



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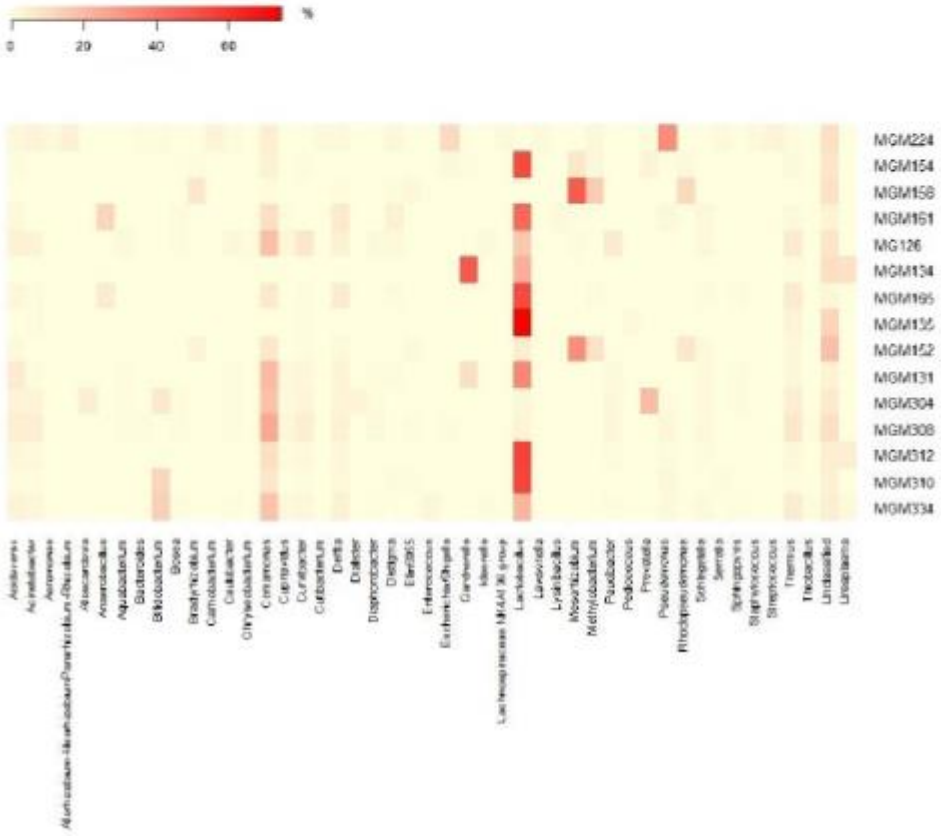

Conclusions: The presented data confirm the concept of non-sterility of the endometrium. Lactobacillus are dominant genera; however, there is no absolute dominance of Lactobacillus over 90%. The mean relative abundance of Lactobacillus in uterine microbiome in fertile patients and in patients with multiple IVF failures did not differ.

Topic: *AS06 PBI - Other*

ENDOMETRIAL MICROBIOTA IN HEALTHY WOMEN, PATIENTS WITH CHRONIC ENDOMETRITIS AND ENDOMETRIAL HYPERPLASIA: THE REAL-TIME PCR DATA

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Background and Aims: The aim of this study was to assess the endometrial microbiota composition by means of real-time PCR in reproductive-age women divided in groups depending on the morphological pattern of the endometrium.

Methods: Endometrial aspirates from 81 women (age range 22–49 years, mean age 32.0±4.9 years) were included in the study. Endometrial aspirates were collected on day 7–10 of the menstrual cycle using Endobrush Standard for Endometrial Cytology (Laboratoire C.C.D.; France). Microbiota composition was assessed using Femoflor® real-time PCR kit (DNA Technology, Russia). Depending on the results of the endometrial histopathological examination, patients were divided into 3 groups: 37 healthy women, 15 patients with chronic endometritis, 29 patients with endometrial hyperplasia.

Results: Up to 11 groups of microorganisms were detected in each sample. The total bacterial load (TBL) was the same in all three groups: $10^{3.1}$–$10^{5.8}$ (median $10^{3,9}$; interquartile range $10^{3.6}$–$10^{4.2}$) GE/ml. Lactobacillus spp. were detected in 88.9% of all samples. Opportunistic microorganisms (OM) were identified in 38.3% of all samples, including 27.1% of samples with lactobacilli and 11.1% — without lactobacilli. Based on the proportion of lactobacilli and OM, we identified 3 types of endometrial microbiota: 1) lactobacilli-dominated type (lactobacilli proportion is at least 90% from TBL); 2) mixed type (lactobacilli proportion is 10-90% from TBL); OM-dominated type (OM constitute no less than 90% of TBL).

Conclusions: Detection rates for the endometrial microbiota types varied in the three groups of patients, but there was no statistically significant difference (Pearson Chi-Square P value was 0.155).

Topic: *AS06 PBI - Other*

INOCULATING GERM-FREE MICE WITH HUMAN INFANT FECES PROVIDES A ROBUST MURINE MODEL TO STUDY MICROBIOTA SUCCESSION AND COMPOSITION

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Background and Aims: Studying the infant microbiota and the effect on the early development of the immune system can be challenging and therefore murine models are frequently used. Whether studies performed on microbiota-immune interactions in mice are relevant for humans is still a matter of debate, mainly due to species specific microbiota differences and the frequent use of single species as colonizers in models.

Methods: In order to study how human microbiota thrive in the murine intestine and their offspring, we transferred human microbiota from three distinct groups of infants (A, B and C) into mice, and studied the microbiota profile and stability over time.

Results: Each group had initially 2 dominant phyla; Bacteroidetes and Actinobacteria in group A; Proteobacteria and Actinobacteria in group B; and Bacteroidetes and Firmicutes in group C. After introduction into germ-free mice the composition switched. Major changes were seen in Proteobacteria, which were almost absent in inoculi A and C, but ended up more abundant in the mice. Actinobacteria went from very high in 2 groups to a more equal level across the 3 mice groups. Both the Firmicutes and Bacteroidetes showed minor changes in relative abundance. The alpha diversity stayed similar throughout the experiment.

Conclusions: Our data supports studies with human microbiota in murine models, but also highlights that we need to be aware of the change in composition when the microbiota switches host, especially if we are studying certain bacteria or microbiota composition patterns that are related to physical conditions.

Topic: *AS06 PBI - Other*

IDENTIFYING BACTERIAL INTERACTIONS WITHIN THE INFANT GUT AND DETERMINING THEIR IMPACTS ON SUCCESSION OF MICROBIAL COMMUNITIES

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Background and Aims: The gut microbial community, including taxonomy, species richness and diversity, is regulated by the bacterial community succession. The complex interactions of microbial populations among themselves (biotic) and with environment (abiotic) result in the formation of a functional microbial network. Primary bacterial succession in the gut starts from infancy followed by dynamic and stable changes in microbial composition over time due to various biotic and abiotic selection pressures. Although 42% of bacterial genome encodes genes related to biotic interaction, the quantitative data on how biotic microbial interactions shape microbial community assembly and succession are missing. In this study, I aim to quantify the bacterial interactions using a pairwise assemblage setting and understand the molecular mechanisms driving interactions in the infant gut.

Methods: I will utilize the probabilistic co-occurrence modeling and pairwise co-culturing method to predict the pairwise bacterial associations. Relative fitness, contact-dependent competition assay, conditioned media, and nutrient consumption competition assay will be performed to determine the type of bacterial associations. Furthermore, comparative genomics and transcriptomics profiling of bacterial mono- vs co-cultures will be done to determine the molecular mechanisms driving the bacterial interactions.

Results: Based on in silico co-occurrence analysis, I found that 1128 bacterial pairs co-occur in atleast one sample, where 31 associations were positive, 15 were negative, and remaining were random.

Conclusions: The outcome of the proposed work will, for the first time, quantify the biotic forces that contribute to driving the microbial community succession and function, which will further help in modelling the complex community interactions in the host environment.

Topic: *AS06 PBI - Other*

RESISTANCE OF SARS-COV-2 ANTIBODIES IN BREAST MILK OF INFECTED AND VACCINATED MOTHERS ALONG SIMULATED GASTROINTESTINAL DIGESTION AND ITS RELATION WITH COLONIC MICROBIOTA

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Background and Aims: Breastfeeding has been identified as a vehicle of anti-SARS-CoV-2 antibodies with a potential protective effect for lactating infants, but little is known about the resistance of these antibodies along infants' gastrointestinal digestion. The aim of this study was to assess the resistance of IgA and IgG along in vitro infants' digestion

Methods: ELISA methods were used to measure the anti-SARS-CoV-2 IgA and IgG breast milk levels after simulated gastric and intestinal digestion. Four different groups were analysed: prepandemic (n=10), naturally SARS-CoV-2 infected mothers (n=5) and mRNA-vaccinated with Pfizer (n=5) or Moderna (n=5). Digesta was also subjected to in vitro colonic fermentation during 48h using two types of infant microbial inoculum: from vaccinated and from non-vaccinated mothers. Targeted qPCR to total bacteria, bifidobacteriae, lactobacillus and enterobacteria were analysed.

Results: After simulated gastrointestinal digestion, IgA was found to be resistant whilst IgG was completely degraded in the intestinal phase, in all the study groups. After simulated colonic feremntation, total bacteria did not show significant differences among the different groups, and Enterobacteriaceae family was the dominant bacteria in both type of inoculums.

Conclusions: anti-SARS-CoV-2 IgA a IgA levels are preserved after in vitro digestion with a potential effect at intestinal level, while dramatic reduction on IgG levels were observed. Further studies are needed to stablish the impact of breast milk antibodies at oral and nasopharynx level

Topic: *AS06 PBI - Other*

PROTECTING MATERNAL AND NEWBORN EUBIOSIS TO CREATE HEALTH: NEW PERSPECTIVES FOR MIDWIVES

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Background and Aims: Recent studies on human microbiome disclose deep connections between health and birth. A descriptive observational study has been led to assess Italian midwives' knowledge on microbiome and know-how on specific midwifery care practices which can facilitate or hinder maternal and newborn eubiosis.

Methods: A sample of 502 Italian midwives, recruited by convenience sampling, was asked to fill out a tailor-made multiple-choice questionnaire shared online from 15th September to 15th October 2021.

Results: The study showed Italian midwives have a discreet knowledge about the subject: 77.7% of the respondents are aware C-section and vaginal birth differently impact on infant microbiome, but 54% is not aware of the benefits of preserving oral health during pregnancy. Several non-evidence-based practices are still widespread, like external genitalia disinfection during labour (24.9%) or incorrect executions of Group B Streptococcus antibiotic-prophylaxis (45.7%), together with positive practices like skin-to-skin contact (78.1%) or delayed first bath (47.8%) that still need to be strengthened. Moreover, the majority of the respondents (80.9%) seem to be ready to improve care of eubiosis. Analysing the 138 answers to the last open-ended question, some important advices have arisen: having balanced diets (23.1%), healthy lifestyles (6.5%), correct intimate hygiene (5%), wise use of probiotics (9.4%) and more training and information (39.1%).

Conclusions: Protecting, sowing, strengthening and guarding are the actions highlighted to describe the work of midwives to ensure optimal microbial colonization from the beginning. A renewed centrality for midwives in health processes is affirmed by the possibility of protecting the eubiosis to create health.

Topic: *AS06 PBI - Other*

MICROBIOME-MEDIATED EFFECTS OF DIET CONTROL ON GESTATIONAL DIABETES MELLITUS (GDM)

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Background and Aims: The prevalence of gestational diabetes mellitus (GDM) is rising, mainly due to the increasing obesity epidemic and older maternal age. Leading treatments include lifestyle changes, particularly diet. Dietary changes influence the composition of the microbiota almost immediately and could have direct or indirect effects on GDM progression. Here, we examine the microbiome-mediated effect of diet control on GDM.

Methods: We first examined consequences of sugar control diet on GDM in 438 pregnant women, 88 of which were diagnosed with GDM, focusing on two time points: before (2nd trimester) and after (3rd trimester) diet intervention. We then examined the direct role of the microbiome in disease progression using fecal microbiome transplantation (FMT) of stools from women with and without GDM prior to and after the dietary intervention into germ-free mice.

Results: Differences in microbiome composition, SCFAs, cytokines and hormones were minor in the longitudinal study of pregnant women with GDM. We did, however, observe significant effects of the microbiome in the germ-free mice that received FMT from women with and without GDM before and after intervention. Mice that received FMT from GDM women before intervention had impaired glucose levels, and those mice who received the GDM-post diet intervention FMT had lower glucose levels compared to mice who received control FMTs.

Conclusions: We demonstrate a definite signal of the positive effects of diet-induced microbiome modification on GDM through phenotype transfer in our murine model. Our findings suggest that diet can mediate GDM through direct effects of microbial community modification leading to a heathier metabolic profile.

Topic: *AS06 PBI - Other*

THE VAGINAL AND ENDOMETRIAL CULTUROMICS-GENERATED MICROBIOME PROFILES IN SUBFERTILITY

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Background and Aims: The microbiome of the female reproductive tract has been implicated in fertility and IVF success rates. Moreover, the presence of certain Lactobacillus species has been related to pregnancy outcome. In this pilot project, we investigated to what extent the endometrial microbiome corresponds to that of the vagina in subfertile women, applying high-throughput culturing techniques.

Methods: Paired samples of endometrial biopsies and vaginal swabs were obtained from six subfertile women. A plethora of aerobic and anaerobic culture conditions were applied to ensure exhaustive culturomics results. Agars were incubated until 30 days post-inoculation and processed applying the Copan WASPLab® system. Bacterial colonies were identified using the Brüker MALDITOF MS Biotyper® system.

Results: High inter-patient variability was observed in the total number of different isolated species (11 – 49). Also, the concordance between vaginal and endometrial microbiota differed from 4 - 48 %. Interestingly, the percentage of vagino-endometrial similarity and microbial diversity seemed to be correlated to the presence of certain Lactobacillus species. As we observed that the low-diversity microbiome profiles were dominated by eubiosis-related strains, such as L. crispatus, L. jensenii, and L. gasseri; while the more diverse microbiome profile was colonized by de dysbiosis-associated L. iners.

Conclusions: Our findings suggest a relationship between the presence of certain Lactobacillus species and microbial diversity along the female reproductive tract. With these culturomics-acquired microbiome profiles, we highlight the potential importance in fertility medicine and implications in therapy. Further research will unravel the true value of our findings and their relationship whit endometrial pathologies and IVF success rates.

Topic: *AS06 PBI - Other*

EFFECT OF INTRA-PARTUM AZITHROMYCIN ON THE DEVELOPMENT OF THE INFANT NASOPHARYNGEAL MICROBIOTA: A POST HOC ANALYSIS OF A DOUBLE-BLIND RANDOMIZED TRIAL

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Background and Aims: Sepsis is a leading cause of neonatal deaths. Intrapartum azithromycin reduced neonatal nasopharyngeal carriage of some potentially pathogenic bacteria – a prerequisite for development of infection including sepsis. As early antibiotic exposure has been associated with microbiome perturbations, we seek to understand the effect of Intrapartum azithromycin on the developing nasopharyngeal microbiota

Methods: We performed a post-hoc analysis of a double-blind, randomized placebo-controlled trial (PregnAnZI-1), to determine the impact of 2g oral intrapartum azithromycin on infant nasopharyngeal microbiota development. Using 16S-rRNA gene sequencing, we compared nasopharyngeal samples from 109 healthy children (55 from the intervention and 54 from the placebo arms) at birth, day 6, 28 and 12 months. Shannon diversity, Bray-Curtis dissimilarity, and taxa abundance were compared between the trial arms. Samples were then grouped into population types by probabilistic modelling based on community similarity.

Results: Bacterial density was lower in the azithromycin arm at birth, whereas alpha diversity was higher in the azithromycin arm at day 6 ($p = 0{\cdot}031$). Overall microbiota composition differed between arms at days 6 and 28 ($R^2 = 4.4\%$, $q = 0{\cdot}007$ and $R^2 = 2.3\%$, $q = 0{\cdot}018$ respectively). At genus level, we observed greater representation of, amongst others, Staphylococcus in the placebo arm at day 6 ($q = 0{\cdot}030$) and a slightly higher representation of Moraxella in the azithromycin arm at 12 months ($q = 0{\cdot}044$).

Conclusions: Intra-partum azithromycin caused short-term alterations in diversity and abundance of bacterial taxa in the developing nasopharyngeal microbiota but showed modest overall effect at 12 months.

Topic: *AS06 PBI - Other*

EFFECTS OF AIR POLLUTION EXPOSURE ON BACTERIAL NASAL MICROBIOME DURING PREGNANCY AS POTENTIAL MECHANISM TO EXPLAIN BIRTH OUTCOMES

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Background and Aims: The impact of exposure to respirable particulate matter (PM) during pregnancy is a growing concern, as it is associated with pregnancy and birth outcomes although the underlying molecular mechanisms are still unclarified. Bacterial nasal microbiome (bNM) is one of the first compartments hit by PM exposure and this interaction might lead to functional modifications potentially affecting the course of pregnancy.

Methods: bNM was characterized by 16S rRNA sequencing during the 11th week of gestation of 65 healthy pregnant women. The estimated levels of daily PM exposure with aerodynamic diameters ≤10μm and ≤2.5μm (PM_{10} and $PM_{2.5}$, respectively) during the whole pregnancy were attributed to each participant from the day before the enrolment back to 13 weeks before biological sampling.

Results: bNM was dominated by the Actinobacteria (58.3%), Proteobacteria (21.0%), Firmicutes (19.8%) phyla, and 61 genera were identified. After applying factor analysis, we identified the two independent factors Factor1 and Factor2 that explained about 66% of the original dataset. Factor1 had the highest contribution from Streptococcus, Granulicatella, Bergeyella, and Abiotrophia, while Factor2 from Campylobacter and Dialister. Negative associations were observed between short-term PM_{10} (β= -0.16, p-value= 0.026) and $PM_{2.5}$ (β= -0.19, p-value= 0.034) exposures and Factor1. Focusing on birth outcomes, decrements of the gestational age at birth were associated with PM concentrations throughout the gestation or during the 2nd trimester. We are testing the effect of bNM on birth outcomes after PM exposure.

Conclusions: The results obtained might suggest a possible role exerted by bNM during pregnancy in mediating the effects of PM exposure.

Topic: *AS06 PBI - Other*

HUMAN MILK OLIGOSACCHARIDES ARE PRESENT IN AMNIOTIC FLUID IN EARLY PREGNANCY AND CHANGE WITH GESTATIONAL AGE

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Background and Aims: Human milk oligosaccharides (HMOs), bioactive glycans in human milk are found in maternal circulation in early pregnancy, and their concentration and composition change during gestation. HMOs are also present in cord blood and amniotic fluid (AF) at term birth. We here aimed to assess HMO profiles in AF over the course of gestation investigating potential temporal changes.

Methods: AF was collected during diagnostic amniocentesis, fetal surgery, or C-section from 77 women with a mean gestational age of 34 ± 6.9 (14.3-40.9) weeks. Samples were analysed for lactose and HMOs using high performance liquid chromatography with fluorescence detection.

Results: HPLC analysis of AF samples revealed the presence of up to 16 HMOs, all previously reported in maternal serum in pregnancy. Overall, 3′-sialyllactose (3′SL) and 2′-fucosyllactose (2′FL) were the highest abundant HMOs in AF. We found lactose and HMOs in all AF samples investigated starting at 14 weeks of gestation. Individual and total HMO concentrations were significantly positively correlated with gestational age. HMO composition also changed from early (14-22 weeks), mid (22-34 weeks) to late pregnancy (34-41 weeks), with relative concentrations of 3'SL significantly decreasing (43.5%, 24.5% and 23.6%) and 2'FL increasing (6.7%, 12.5% and 21.0%), respectively.

Conclusions: Our study shows that HMO species, previously also found in maternal serum or urine, and cord blood, are present in AF already in the first trimester. This demonstrates extensive contact of the fetus with a broad variety of HMOs, suggesting important roles for HMOs in fetal tissue development, and potential intrauterine microbial interactions.

Topic: *AS06 PBI - Other*

THE INFANT GUT COMMENSAL BACTEROIDES DOREI PRESENTS A GENERALIZED TRANSCRIPTIONAL RESPONSE TO VARIOUS HUMAN MILK OLIGOSACCHARIDES

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Background and Aims: Human milk oligosaccharides (HMOs) are a family of glycans found in breastmilk. Despite being the third-largest solid component in breastmilk, HMOs are indigestible by infants, and they serve as food for the infant gut bacteria. Most research thus far has focused on Bifidobacterium species that harbor many glycoside hydrolases (GHs) tailored to break the carbon bonds in HMO molecules. However, there are additional microbes in the infant gut, such as Bacteroides, with increasing evidence that they, too, are able to break-down HMOs.

Methods: Here, we developed an optimized system for isolating Bacteroides strains from infant stool samples. We then examined the HMO utilization capacity of multiple Bacteroides isolates by performing growth curves on six common HMOs (2'-FL, DFL, 3'-SL, 6'-SL, LNT, LNnT). Isolates displayed similar growth characteristics on similarly-structured HMOs, like sialylated or fucosylated sugars. We identified variation in HMO utilization across multiple strains of the same species, and chose to focus here on a Bacteroides dorei isolate that was able to utilize the test HMOs. We performed RNA sequencing on B. dorei cultures, comparing the transcriptional profile in minimal media supplemented with glucose or HMOs.

Results: We showed that B. dorei employs an extensive metabolic response to HMOs. B. dorei exhibits a generalized response to HMOs, up-regulating several shared GH families across all conditions.

Conclusions: Within each GH family, B. dorei displays a consistent pattern of up-regulation of some genes with down-regulation of the others. This response pattern to HMOs has yet to be described in other commensals of the infant gut.

Topic: *AS06 PBI - Other*

METAPROTEOMIC PROFILING OF FUNGAL GUT COLONIZATION IN GNOTOBIOTIC MICE

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Background and Aims: Eukaryotic microbes modulate mammalian host health and disease states, yet the molecular contribution of gut fungi remains nascent. We previously showed that mice colonised with fungi displayed sensitivity to allergic airway inflammation and had fecal metabolite profiles similar to germ-free mice. This marginal effect on the host metabolome suggested that fungi do not primarily use metabolites to modulate the host immune system.

Methods: To describe functional changes attributed to fungal colonisation, we performed mass spectrometry-based analyses of feces and the small intestine of gnotobiotic mice colonised with defined consortia of twelve bacterial species, five fungal species, or both.

Results: We detected 6,675 proteins in the mice feces. Analysis of variance showed changes in the different gnotobiotic mouse groups; specifically, 46% of 2,860 bacterial, 15% of 580 fungal, and 76% of 405 mouse quantified proteins displayed differential levels. Fungal colonisation resulted in changes in host proteins functional in innate immunity as well as metabolism, predicting specific roles of gut fungi on host systems during early developmental stages. Many of the detected fungal proteins have been previously reported as part of extracellular vesicles and having immunomodulating properties. Using an isobaric labelling approach for profiling low abundant proteins of the jejunal tissue, we confirmed that the fungal species differentially impacted the host intestinal proteome compared to the bacterial consortium.

Conclusions: Our results suggest that an increased abundance of certain gut fungal species in early life may affect the developing intracellular attributes of epithelial and immune cells.

Topic: *AS06 PBI - Other*

THE MYCOBIOME OF BREASTFEEDING: CHANGES ACROSS LACTATION

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Background and Aims: Bacterial colonization of the infant gut from breastfeeding, vaginal delivery, and environmental sources, has been well studied. The mycobiome, or fungal microbiome, is developing simultaneously, but is often neglected in scientific research. The purpose of this study was to assess the fungal breastfeeding microbiome, i.e., all of the fungi that infants are exposed to during breastfeeding, including fungi found within human milk and any bacteria found on the breast and nipple themselves.

Methods: Fifty human milk samples were collected from 15 participants at 1-, 4-, 7-, and 10-months post-partum without sanitizing the breast prior to collection. DNA from the samples was sequenced by shotgun metagenomics for species level fungal identification.

Results: Among the participants, 10 contained fungal DNA in at least one sample with 15 positive samples total (30% of samples). Because breast antiseptics were omitted from our collection procedures, the high abundance of skin fungi was expected. Malassezia restricta was the most common overall, with 9 positive samples, and was the most abundant fungi in 6 of those samples. Other abundant fungi included, Agaricomycetes species, Puccinia arachidis, and Aspergillus fischeri. Puccinia arachidis, or peanut rust, plays a role in plant disease, but its potential impact on infant development is unknown. Candida species were not present in any samples, despite it being one of the most common fungi in the human microbiome.

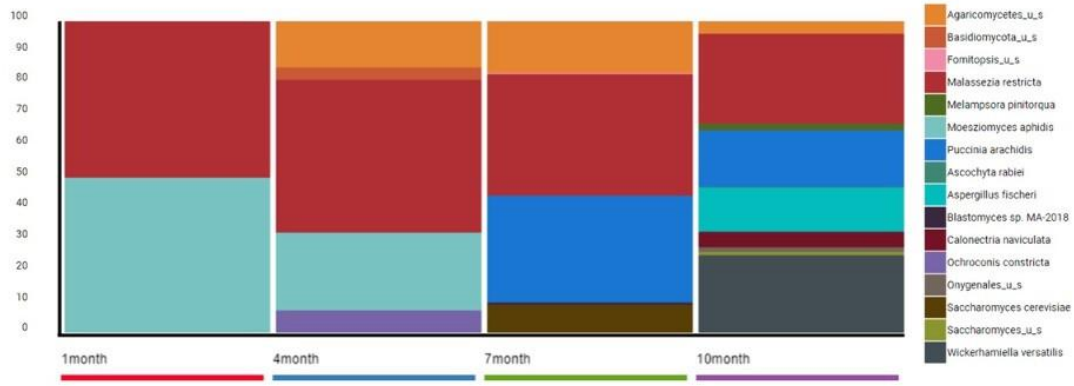

Figure 1. Longitudinal Mycobiome Diversity. Sixteen fungal species were identified in 15 of the 50 pumped human milk samples across 15 participants. Samples were collected at 1 month, 4 months, 7 months, and 10 months postpartum. The average fungal abundance at each timepoint is displayed.

Conclusions: The impact of the presence or absence of a fungal breastfeeding microbiome on infant health and development is currently being investigated, but will require larger, longitudinal investigations to confirm findings.

Topic: *AS06 PBI - Other*

VAGINAL MICROBIOTA IN TERM AND LATE TERM PREGNANCY IS AFFECTED BY PREVIOUS PREGNANCY HISTORY

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Background and Aims: Only few studies exist on vaginal microbiota in later stages of pregnancy. Our objective was to study whether vaginal microbiota differs according to previous pregnancy outcomes and whether gestational age influences the composition of vaginal microbiota at or near delivery.

Methods: We analyzed the vaginal microbiota of 324 Finnish women between 37- and 42-weeks of gestation before elective cesarean section, at the onset of spontaneous labor, and in pregnancies continuing beyond 41 weeks of gestation. Bacterial DNA was extracted using a bead beating method and subjected for quality control, index PCR and Illumina MiSeq sequencing of the V3-V4 region of the 16S rRNA gene.

Results: Vaginal microbiota composition in late pregnancy associated strongly with parity. Absence of previous deliveries was a strong predictor of Lactobacillus crispatus dominated vaginal microbiota while Lactobacillus iners associated with increasing parity. Previous pregnancies not ending in live birth, but in spontaneous or induced abortions, did not show alterations in the microbiota compared to primigravidae. Gestational age also associated significantly with microbiota variation, and the relative abundance of L. crispatus increased with advancing gestational age in women without previous deliveries.

Conclusions: Our results indicate that obstetric history influences the vaginal microbiota, and it should be accounted for in future studies on vaginal microbiota and reproductive outcomes. Further studies are needed to study whether the effect of parity can also be seen in non-pregnant women, and to address the mechanisms on how previous labor, but not pregnancy, affects the vaginal microbiota.

Topic: *AS06 PBI - Other*

FORMALIN-FIXED PARAFFIN-EMBEDDED SAMPLES ARE NOT A BENEFICIAL REPLACEMENT FOR FROZEN TISSUES IN FETAL MEMBRANE MICROBIOTA RESEARCH

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Background and Aims: Formalin-Fixed Paraffin-Embedded (FFPE) tissues are routinely collected, archived, and used for clinical diagnosis, especially for maternal and neonatal health. Application of these samples to microbiota research would be beneficial to reduce preparation, storage and costs associated with frozen sample processing and biobank limitations. This research aims to understand if routinely collected and archived fetal membrane samples are comparable to the current gold standard frozen tissue, thus could be used in microbiota analysis.

Methods: The microbiota were investigated from frozen amnion and chorion fetal membrane rolls or FFPE combined amniochorionic samples from nine matched paired patients by amplicon sequencing of the V4 16S rRNA gene region. Decontam was integrated into computational analysis to identify contaminating sequences.

Results: Over half of sequences from FFPE samples (52%), were identified as contaminants via decontam. The most abundant sequence across tissues and negative controls (Escherichia/Shigella) was detected as a genuine signal. Although, Escherichia/Shigella was the most abundant genera in both preparation methods, different bacterial communities were observed between frozen and FFPE samples. Frozen fetal membranes yielded greater quality and quantity of extracted DNA compared to highly fragmented FFPE DNA.

Conclusions: FFPE fetal membranes are not comparable to frozen samples, with reduced DNA quality and quantity, plus high levels of contamination. Although, still beneficial for histological and clinical analysis, it is not recommended to replace frozen tissues with FFPE samples for fetal membrane microbiota research.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

OBESITY AND INTESTINAL MICROBIOTA MODULATION: POSSIBLE THERAPEUTIC STRATEGY?

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Background and Aims: Obesity reached an epidemic scenario and became the subject of study in many areas. Obesity etiology is multifactorial, including hypercaloric diets, physical inactivity and intestinal dysbiosis. Changes in intestinal bacterial composition, decreasing microbial diversity and increasing pathogenic strains, could disrupt gut homeostasis causing intestinal dysbiosis. Bacterial intestinal colonization is related to host phenotype and an unbalanced ratio of Firmicutes/Bacteroidetes phyla could be associated with obesity. This review aimed to summarize scientific evidence between obesity and intestinal dysbiosis, verifying if the intestinal microbiota modulation with probiotics could assist in weight loss.

Methods: Clinical trials or observational studies that analyzed gut microbiota of human individuals classified with obesity, defined by body mass index (BMI), were eligible for this narrative review. The MeSH terms used were: “Obesity” AND “Microbiota” AND “Probiotics”, with language filter: English, Spanish and Portuguese, without publication time limitation. The search was performed in MEDLINE by PUBMED, LILACS by “Biblioteca Virtual em Saúde – BVS”, and SCIELO.

Results: Probiotics strains with the most scientific evidence about control intestinal dysbiosis were Lactobacillus spp. and Bifidobacterium spp.. Intestinal microbiota modulation with probiotics increased bacterial colonic diversity, associated with regular consumption of prebiotics. Some endogenous strains, such as Akkermansia muciniphila, use fructans and inulin as a source of energy. Individuals classified as obese usually had lower bacterial gut colonization of this strain. Intestinal microbiota modulation with Akkermansia muciniphila contributed to reverse intestinal dysbiosis, favoring weight loss in these individuals.

Conclusions: In conclusion, intestinal microbiota modulation with probiotics, associated with lifestyle changes, could improve weight loss.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

ANTIBIOTIC TREATMENT USING AMOXICILLIN-CLAVULANIC ACID IMPAIRS GUT MYCOBIOTA DEVELOPMENT THROUGH INTERACTION WITH SPECIFIC BACTERIA

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Background and Aims: Antibiotics effects on gut bacteria have been widely studied, but very little is known on the consequences of such treatments on the fungal part of the microbiota. It is commonly believed that the fungal load increases in the gastrointestinal tract following an antibiotic treatment.

Methods: We used conventional and humanized mice to study the consequences of an antibiotic treatment (Amoxicillin-clavulanic acid) on the intestinal microbiota. Bacterial and fungal community were followed by qPCR or 16S and ITS2 amplicon-based sequencing for microbiota analysis. In vitro and in vivo assays were performed for further bacterial – fungal interaction characterization, with mixed culture between specific bacteria and fungi.

Results: Amoxicillin-clavulanic acid treatment triggered a decrease of the total fungal population while other antibiotics had opposite effects on fungal load. In the presence of Amoxicillin-Clavulanate, fungal and bacterial microbiota analysis showed a remodeling of the bacterial microbiota with an increase of specific bacteria such as the Enterobacteriaceae family. Using in vitro assays, we isolated different Enterobacteriaceae and explored their effect on several fungal strains. We showed that Enterobacter hormaechei was able to reduce the fungal population in vitro and in vivo through yet unknown mechanism.

Conclusions: Bacteria and fungi have strong interactions within the microbiota, hence the perturbations initiated by an antibiotic treatment targeting bacterial community have complex consequences and can indirectly induce alterations of the mycobiota. Interestingly, Amoxicillin-Clavulanate treatment has deleterious effect on the fungal community and this may be partially due to the overgrowth of specific bacterial strains with inhibiting or competing effects on fungi.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

IMPACT OF INTRA-BLADDER INSTILLATIONS WITH PROBIOTICS IN PATIENTS WITH RECURRENT URINARY TRACT INFECTIONS. THIS TREATMENT IS A NOVEL APPROACH TO AVOID ANTIBIOTIC RESISTANCE AND COSTS.

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Background and Aims: The purpose of this study is to evaluate an alternative treatment to avoid antibiotic therapy (AT) in patients with Recurrent Urinary Tract Infections (RUTI), which may lead to bacterial resistance, reduce costs of medications and hospital stays. Probiotics stimulate the immunological system producing cytokines mature B lymphocytes and lgA, which strengthen the intestinal barrier.

Methods: This research was done on 8 women between the ages of 27-73 with multiple Urinary Tract Infections (UTI). Probiotics were performed with Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus helveticus and Bifidobacterium animalis lactis ssp lafti B94 with a live cell colony count (>109 CFU/mL). Results: Improvement in 6 of the 8 cases with a reduction of bacteria colony culture counts and leucocytes in the urinalysis. This probiotic instillation showed a frank reduction of countless leucocytes to 8-10 HPF, plus decreases in bacteria colony counts: initial (>105 CFU/ mL) to 8.000 CFU/ml.

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Conclusions: This alternative treatment avoids side-effects of AT and costs related to it. It also reduces amounts of money and time spent on laboratory tests, hospital admissions, health complications, work absenteeism, and medications. In conclusion, it tends to improve the quality of life of patients with RUTI or UTI, and it avoids harmful effects caused by the chronic use of AT.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

EFFECTS OF THE ADMINISTRATION OF SACCHAROMYCES BOULARDII CNCM I-745 DURING AND AFTER ANTIBIOTHERAPY ON THE BALANCE OF THE FUNGAL AND BACTERIAL MICROBIOTA

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Background and Aims: Antibiotics effects on gut bacteria have been widely studied, but very little is known on the consequences of such treatments on the fungal part of the microbiota. Here we focused on the role of antibiotics on the bacterial and the fungal microbiotas, and how administration of Saccharomyces boulardii CNCM I-745 can influence both microbiotas. While several studies have described its effect on bacterial microbiota, nothing is known on its effect on fungal microbiota and how the length of the administration influences both bacterial and fungal microbiotas.

Methods: We used humanized mice with fecal microbiota transfer and treatment with S. boulardii during and after an antibiotic treatment. Bacterial and fungal community were followed by CFU, qPCR or 16S and ITS2 amplicon-based sequencing for microbiota analysis.

Results: We showed that administration of S. boulardii during the antibiotic treatment allowed a better implantation of the probiotic yeast and limitation of the population of other fungi, and in particular a decrease in Debaryomyces genus, that was recently associated with inflammatory bowel diseases. Concerning bacterial microbiota, S. boulardii administration permitted a better recovery of the bacterial populations after the end of the antibiotic treatment. Additionally, 16S and ITS2 sequences analysis revealed that to continue the administration of S. boulardii for at least 7 days (17 days in total) was globally better for a quicker return to the initial bacterial equilibrium.

Conclusions: In this study, we provide a comprehensive analysis of how yeast probiotic administration can influence the fungal and bacterial microbiota in a model of broad-spectrum antibiotherapy.

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INFLUENCE OF COCOA AND ORANGE POLYPHENOLS ON CAECAL MICROBIOTA COMPOSITION OF RATS FOLLOWING AN INTENSIVE TRAINING AND EXHAUSTING EXERCISE

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Background and Aims: Dietary and non-dietary lifestyle factors influence the gut microbiota composition. We aimed to assess the influence of diets containing cocoa and orange polyphenols on caecal microbiota composition after intensive training and exhausting exercise in rats.

Methods: For this purpose, rats were fed either a standard diet, a diet containing 10% cocoa (C10) or a diet containing 10% cocoa plus 0.5% hesperidin (CH) for 6 weeks. In this period, animals were submitted to an intensive running training on a treadmill or remained as a sedentary control group. At the end, caecal content samples were obtained 24h after performing a regular training (trained groups) and immediately after carrying out a final exhaustion test (exhausted groups). The composition of the caecal microbiota was assessed through 16S rRNA sequencing technique.

Results: Both exercise and the experimental diets were associated with changes in the microbial richness of the samples. Moreover, the C10 and CH diets modified the caecal microbial composition as assessed by PCoA plots and PERMANOVA analysis. Whereas exercise “per se” did not induce any substantial change in the relative abundance of the studied bacterial groups, it must be highlighted that the intake of the C10 and CH diets induced an increase in the proportion of the genus Lactobacillus in the sedentary control animals. In trained animals, the Monoglobaceae family proportion was also increased due to the experimental diets.

Conclusions: Overall, our exercise model barely modified the caecal microbiota composition, however, both polyphenol-enriched diets exerted positive effects, encouraging further studies to ascertain their potential role as prebiotics.

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THE ECOLOGY OF THE MICROBIOTA IN CHILDREN WITH OBESITY IS ASSOCIATED TO INSULIN RESISTANCE AND DIET COMPOSITION

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Background and Aims: In the pediatric population, the progression of obesity-related diseases can be delayed or prevented through lifestyle changes, including the promotion of a Mediterranean-like dietary (MD) pattern. We aimed to evaluate the gut microbiome ecology in relation to dietary and clinical parameters in the pediatric subjects with obesity recruited at baseline in a protocol on an educational training to MD.

Methods: A total of 55 subjects (6 and 18 years) with obesity, diet naïve or with failure to a previous weight loss program were recruited. We collected auxological, metabolic, nutritional parameters (KIDMED score; IDEFICS food frequency questionnaire), and stool samples. DNA was extracted directly from 0.25 g of stool using the Power SoilKit. DNA was amplified with primers for the V3 and V6 regions of 16S rDNA tagged with Multiplex Identifier sequences using Microbiota Solution B Kit optimized for Illumina Miseq sequencing. FastQ sequences were analyzed using MicrobAT Software. Statistical analyses were performed using R software.

Results: All the 55 subjects showed a Bacteroides enterotype: 38% Bacteroidetes, 34% Firmicutes, 22% Unclassified Bacteria, 4% Actinobacteria, 1% Proteobacteria. At baseline, clinical and metabolic characteristics were homogeneous among children while microbial communities associated with the different subjects showed statistically significant differences according to: Tanner stage considering sex, fasting insulin levels, fasting insulin resistance, percentage of carbohydrates.

Conclusions: These preliminary results highlight as diet, insulin sensitivity and microbiome are strictly related in children with obesity. We identified several bacterial groups not previously described in obesity. These findings are of importance for clustering patients and studying tailored dietary programs.

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EFFECT OF FOOD SUPPLEMENTS ON GUT MICROBIOTA IN OBESE PATIENTS: PRELIMINARY RESULTS OF ZIMBA TRIAL

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Background and Aims: Human bodies harbor a diverse community of microbes that together compose the human microbiota. Non-digestible carbohydrates can highly modify the composition and function of gut microbiota. Prebiotics are a group of nutrients that are degraded by gut microbiota and their relationship with human health has been an area of increasing interest in recent years. Zimba project is a placebo-controlled clinical trial that study the efficacy of the association of Zinc, Myoinositol and GOS in respect to the administration only GOS (placebo) in pediatric obesity.

Methods: DNA was extracted from stool using the DNeasy® PowerSoil® kit. The bacterial 16S DNA libraries were prepared using the Microbiota solution B kits, Arrow Diagnostics srl.. The amplicon pool was processed using the Nano Kit v2 kit, Illumina. Bioinformatic analysis workflow was according to Bona et al. Raw sequences were processed by MicrobAT software. Statistical analysis was performed using both MicrobiomeAnalyst and R softwares.

Results: The preliminary results on microbiota characterization of 27 enrolled patients showed a decrease in biodiversity in active treated patients in respect to placebo ones, probably due to Zn. Moreover, the prebiotic intake induced a significant increase in Firmicutes, essential for a healthy microbiota homeostasis, such as Dorea longicatena, Clostridium colstridioforme and Faecalibacterium sp. and the decrease in Bacteroidetes.

Conclusions: Concluding, further analyses linked to the clinical and metabolic responses will be necessary to validate the proposed mechanisms at the base of the microbiota modulation.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

BUTYRATE PROTECTS PANCREATIC BETA CELLS FROM CYTOKINE-INDUCED DYSFUNCTION

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Background and Aims: The gut microbiota has emerged as an important regulator of glucose metabolism, possibly through the production of short chain fatty acids. Type 2 diabetes (T2D) individuals have less butyrate-producing bacteria and reduced functional beta cell mass. Identification of factors that can prevent or reverse beta cell dysfunction is therefore of great interest in diabetes research. The aim of this study was to investigate the effect of butyrate on cytokine-induced beta cell dysfunction.

Methods: Mouse islets were exposed to non-cytotoxic concentrations of IL-1β with or without butyrate for 10 days, to resemble the slow onset of inflammation in T2D. Beta cell function was assessed by glucose stimulated insulin secretion (GSIS), the transcriptome by RNA-seq and the mechanisms of actions were investigated in insulin secreting INS-1E cells by western blotting and chromatin immunoprecipitation (ChIP).

Results: Butyrate protected beta cells from IL-1β-induced impairment of GSIS and insulin content. This was associated with downregulation of genes involved in inflammation such as nitric oxide synthase 2 (Nos2), chemokines, interleukins and immunoproteasome subunits. IL-1β-induced nuclear translocation of NF-κB p65 was unaffected by butyrate, but butyrate inhibited the recruitment of p65 and RNA polymerase II to inflammatory gene promoters.

Conclusions: Our results suggest that butyrate prevents IL-1β-induced beta cell dysfunction by suppression of NF-κB activation and inflammatory gene expression.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

PROBIOTIC INTERVENTION OF THE MICROBIOME TO FIGHT TYPE 2 DIABETES MELLITUS AND OBESITY

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Background and Aims: Diabetes mellitus is a metabolic disease characterized by high glucose levels in blood. Currently, approximately 350 million people have diabetes mellitus worldwide, and this number is believed to reach almost 500 million people by 2030. In particular, type 2 diabetes mellitus accounts for more than 90% of total diabetes worldwide. Worsening the situation, a growing number of type 2 diabetic patients have arisen after suffered COVID-19. Therefore, there is an urgent need for the exploration and development of novel strategies against the onset and progression of diabetes mellitus. The gut microbiota influences the efficiency of energy extraction from ingested foods, time of food intestinal residence, mucosal immunity, intestinal permeability, and systemic inflammation, all factors involved in the triggering and progression of type 2 diabetes. Our working hypothesis is that the administration of probiotic bacteria (live microorganisms conferring health benefits to the host when consumed in adequate amounts) could be a promising approach to modulate the gut flora.

Methods: Here, we report how the administration of the probiotic bacterium Bacillus subtilis DG101 to: (1) non-COVID-19-patients refractory to anti-diabetic medication (i.e., metformin) and (2) post-COVID-19-patients; successfully responded to probiotic consumption.

Results: After six months of treatment, B. subtilis DG101 restores the levels of glycemia, insulinemia and HbA1c of both groups to physiological values. In addition, probiotic B. subtilis DG101, combined with a hypocaloric diet, significantly contributes to weight management (i.e., descences of weight, body mass index and fat content).

Conclusions: Overall, probiotic B. subtilis intervention represents a novel and safe tool against type 2 diabetes mellitus and obesity.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

ANALYSIS OF BRONCHOALVEOLAR LAVAGE FLUID METATRANSCRIPTOMES AMONG PATIENTS WITH COVID-19 DISEASE

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Background and Aims: In order to better understand how COVID-19 impacts the microbial community dynamics / functional profile from a hologenome standpoint, we conducted a multivariate comparison of publicly available human bronchoalveolar lavage fluid (BALF) metatranscriptomes samples amongst COVID-19 (n=48), community acquired pneumonia(n=25), and uninfected patients(n=32), with the objective to compare the BALF metatranscriptome amongst and between each of the three disease cohort classifiers, and identify significantly associated taxonomic changes in microbial derived community dynamics / and functional changes derived from gene ontologies. Our overarching testable hypothesis was that there is a potential informative and discernably significant relationship between the BALF microbiome and COVID-19 disease severity, including death.

Methods: After read filtering and batch effect sample removal, the remaining SARS-CoV-2 viral and microbial reads taxonomically classified (Kraken2), functionally characterized (SeqScreen), and analyzed for multivariable associations with linear models (MaAsLin2) associated with disease case and outcome (deceased n=20, survived n=19), while controlling for differences in publication and study design.

Results: demonstrated unique taxonomic and functional changes to the hologenome associated with COVID-19 disease and outcome associated the COVID-19 cohort, with notable functional profiles with notable functional profiles associated phosphate / phosphorylation , metal ion binding , RNA binding, and lytic activity (hydrolase, endopeptidase, oxidoreductase, etc.) with which analysis of predicted proteins and gene ontology terms could be used to predict disease and outcome severity.

Conclusions: These reults improve undertanding about how the relationship between the human microbiome and the host susceptibility to and severity of COVID-19, and help to generate important hypotheses that warrant further investigation.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

TARGETING THE PERINATAL DIET TO ALTER THE INFANT GUT MICROBIOME: PRELIMINARY METAGENOMIC RESULTS FROM A RANDOMISED CONTROLLED TRIAL

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Background and Aims: It is unclear whether the perinatal diet can influence the infant gut microbiota. This study aimed to assess whether a perinatal dietary program focused on the gut microbiota modulates infant gut microbiota.

Methods: Participants were 45 infants born to mothers (n=44) that participated in a dietary RCT (ACTRN12616000936426). The intervention increased prenatal diet quality, variety and intakes of prebiotic and probiotic foods compared to the control group receiving dietary advice as part of standard pregnancy care. Metagenomic data were generated from infant stool samples collected at four weeks of age. A between-group difference in Shannon diversity was estimated using a parametric T-test and Cohen’s D effect size. Differential CLR-transformed species abundance was investigated using Welch's T-test with a Bonferroni correction.

Results: Infants in the intervention group (n=22) had a mean Shannon index -0.28 units lower than the control group (n=23) (95%CI: -0.003, 0.56). This difference represented a moderate effect size of 0.6 (95%CI: 0.03, 1.23). Compared to controls, the intervention group had significantly lower abundances of Escherichia flexneri, Streptococcus parasanguinis_B, Bacteroides faecis and a higher abundance of Bifidobacterium MIC6680, however these differences were attenuated after adjustment for multiple testing.

Conclusions: There was some evidence, although not conclusive, that infants of women receiving a dietary intervention focused on the gut had lower alpha diversity compared to those whose mothers received standard care. Given the modest sample size, these preliminary findings should be interpreted with caution, however we anticipate they will support larger studies aiming to guide the assembly of the early life gut microbiota.

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MITOCHONDRIAL HYPERATIVATION DETERMINES A SPECIFIC MICROBIOTA PROFILE CONFERRING A TRANSFERABLE PROTECTIVE EFFECT ON NON-ALCOHOLIC FATTY LIVER DISEASE PROGRESSION

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Background and Aims: Mitochondrial dysfunction is frequent in non-alcoholic fatty liver disease (NAFLD) pathogenesis. Deletion of MCJ protein, a negative regulator of mitochondrial complex I, enhances mitochondrial activity and diminishes diet or hepatotoxic drug-induced lipid accumulation and hepatic damage. Our aim was to determine gut microbiota involvement in the protective effect of MCJ deficiency in NAFLD development.

Methods: Wild-type (WT) and MCJ knock-out (MCJ-KO) mice were fed with control or choline-deficient, L-amino acid-defined, high-fat diet (CDA-HFD) for 6 weeks. Concerning NAFLD-related parameters, a donor mouse from each group was selected. Fecal microbiota transplantation was performed to germ-free mice (GFm), being fed under the same conditions for 3 weeks. Liver disease progression, mitochondrial status and gut microbiota composition were measured.

Results: An inflammatory and fibrotic status was induced by CDA-HFD. MCJ-KO mice revealed a reduced expression of inflammatory and liver fibrosis markers. Similar results were observed in CDA-HFD-fed GFm and colonized with microbiota from MCJ-KO genotype donors. Moreover, niconinamide-adenine dinucleotide (NAD) intestinal production and its related synthesis enzymes were incremented in MCJ-KO mice, which augmented their fatty acids oxidation potential comparing to WT mice. Moreover, metagenomic analysis indicated a specific gut microbiota profile associated to MCJ-KO, increasing Dorea and Oscillospira and decreasing AF12, Allobaculum and Ruminococcus. This pattern was transferred to GFm colonized with MCJ-KO microbiota, being associated to an incremented intestinal and hepatic NAD synthesis.

Conclusions: The protective effect of MCJ deficiency in NAFLD involves a mitochondrial hyperactivity mechanism that is transferable through gut microbiota.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

PROTECTIVE EFFECT ON OBESITY AND NON-ALCOHOLIC FATTY LIVER DISEASE OF QUERCETIN AND A. MUCINIPHILA COMBINATION MODIFYING INFLAMMATORY STATUS, LIPID METABOLISM AND GUT MICROBIOTA PROFILE

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Background and Aims: Alteration of the gut microbiota composition is a hallmark of the development of obesity and non-alcoholic fatty liver disease (NAFLD). Targeting the gut microbiota with probiotics, prebiotics or their combination may be a potential therapeutic strategy. Our aim was to determine the benefits of the combination of Akkermansia muciniphila and quercetin with a nutritional intervention in an in vivo model of early obesity and NAFLD.

Methods: 21-days-old Wistar rats were fed with control diet (CD) or high fat diet (HFD) for 6 weeks. Then, every rat was given CD supplemented or not with the symbiotic during 3 weeks. Plasmatic and hepatic parameters were measured. Faecal samples collected the 6th and 9th week were sequenced using Illumina Myseq system.

Results: HFD rats displayed alterations compatible with obesity and NAFLD development, and an associated intestinal dysbiosis. Steatosis was enhanced, triglycerides were reduced and a lipid metabolism modulation was observed (lower hepatic expression of CEBP/a, DGAT2 and SREBP) after 3 weeks under symbiotic administration. Liver inflammatory status was improved by the symbiotic, showing a reduced expression of proinflammatory cytokines. Additionally, the HOMA-IR index, plasmatic leptin and triglycerides concentration were enhanced with the symbiotic. Metagenomic analysis revealed that the combination reversed dysbiosis showing a specific profile characterized and incremented on Cyanobacteria and Oscillospira taxa, and a reduction of Actinobacteria, Lactococcus, Lactobacillus and Roseburia.

Conclusions: The symbiotic could counteract obesity and NAFLD development in an in vivo model, modulating intestinal dysbiosis and reversing inflammatory and metabolic alterations.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

PROBIOTICS AS A VIABLE THERAPEUTIC OPTION: THE CLINICAL GUIDE TO PROBIOTIC PRODUCTS AVAILABLE IN CANADA AND IN THE US, TRANSLATING SCIENTIFIC EVIDENCE TO CLINICAL PRACTICE

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Background and Aims: The probiotic clinical reference tool is independently designed to translate scientific evidence available up to date for commercially available probiotic products into practical, clinically relevant information, enabling clinicians to easily select the appropriate product, dose, and format for a specific indication.

Methods: Published studies with defined clinical outcomes for probiotic strain(s) were searched using defined inclusion criteria. Commercially available products containing said strain(s) were identified, and the Levels of Recommendation were used to rate the strength of the evidence. This information was compiled into a chart form, assessed by independent expert reviewers. This guide is a clinical decision-making tool to assist health care professionals in providing evidence-based recommendations for their patients. In the case of probiotics, the clinical evidence supports only certain formulations/brand names of the probiotics. The authors made every attempt to include the published clinical data for the available probiotic formulations.

Results: In the Clinical Guide, the available strains were organized based on probiotic strain(s), doses, and evaluated evidence levels based on our pre-defined criteria. This document is easily accessible in print and digital formats.

Conclusions: There is evidence to support the use of probiotic products for various aspects of human health, however, applications and results are strain-specific and disease or symptom-specific. Due to frequent changes in commercial availability of probiotic strains, newly published evidence, and growing research, an annual review and updates of this Clinical Guide have been conducted since 2008. Lack of adverse effects supports the widespread use of these products, and further investigation is recommended.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

INTESTINAL MICROBIOTA MODULATES DENDRITIC CELL FUNCTION IN THE BONE MARROW AND IMPACTS DISEASE PROGRESSION IN MICE AFFECTED BY MULTIPLE MYELOMA

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Background and Aims: The host microbiota impacts human health beyond skin and mucosae. We showed that Prevotella heparinolytica, a commensal of the human microbiota, induces a pro-tumoral Th17 response that favors the progression of multiple myeloma (MM), a treatable but incurable neoplasia of plasma cells accumulating in the bone marrow (BM). Microbiota-propelled Th17 cells act on IL-17 receptor expressed by neoplastic plasma cells, supporting their survival and proliferation. At odds, P.melaninogenica restrains MM progression by limiting expansion of Th17 cells. The mechanism by which the two Prevotellae differently affect the immune response remains largely unknown.

Methods: We modulated the gut microbiota of MM-bearing mice by administrating P.heparinolityca or P.melaninogenica, or by butyrate supplementation. Human and mouse dendritic cells (DCs) were differentiated in vitro from BM precursors and stimulated with Prevotellae or their conditioned medium, and co-cultured with naïve T cells.

Results: Our data show that P.melaninogenica administration increased the therapeutic potential of immune checkpoint inhibitors in MM-bearing mice. Dietary intervention with butyrate, a microbiota-derived metabolite, delayed MM appearance and reduced the proportion of pathogenic Th17 cells. Mechanistically, DCs in the BM of MM-bearing mice receiving P.heparinolytica produced more IL-6 and IL-1β, cytokines involved in Th17 polarization, than P.melaninogenica-conditioned mice. in vitro stimulation of DCs with P.heparinolytica or its conditioned medium resulted in stronger Th17 polarization of naïve T cells when compared to P.melaninogenica.

Conclusions: Alteration of the gut microbiota impacts immune response beyond the intestinal tract, opening the path for the development of microbiota-based immunotherapies to prevent the progression from asymptomatic to fully blown MM.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

DIETARY CHOLINE CONTENT AND HDL LEVELS MODULATE GUT MICROBIOTA COMPOSITION AND CONTRIBUTE TO A PRO-ATHEROGENIC PLASMA METABOLOMIC PROFILE IN MICE

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Background and Aims: Gut microbiota can influence atherosclerosis development by metabolizing dietary choline: experimental and observational studies have highlighted a positive correlation between increased plasma choline-derived TMAO concentrations and adverse cardiovascular events. This study was aimed at investigating how the plasma metabolome of mice prone to atherosclerosis development was modulated by HDL levels and the dietary intake of choline.

Methods: Low-fat, no cholesterol diets with different choline content (0.09% or 1.2%) were administered for 16 weeks to two groups of atherosclerosis-prone female mice: 1) extremely low-HDL mice, deficient for both murine apoA-I and apoE (DKO); 2) high-HDL mice, deficient for both apoA-I/apoE, but overexpressing human apoA-I (DKO/hA-I). At sacrifice, atherosclerosis was evaluated, and a targeted metabolomics of plasma was performed.

Results: The high-choline diet increased the proportion of Proteobacteria (mainly Burkholderiaceae). and decreased that of Rikenellaceae only in DKO mice. Surprisingly, although the high-choline diet resulted into elevated plasma TMAO levels in both genotypes, choline supplementation significantly worsened plaque development only in DKO/hA-I mice. Noteworthy, only in DKO/hA-I mice, high-choline diet led to an increased concentration of plasma lipids (triglycerides, hexosylceramides, ceramides and sphingomyelins), as well as several markers of increased cardiovascular disease risk and compromised renal function such as asymmetric dimethylarginine, symmetric dimethylarginine, indoxyl sulfate, creatinine and the microbiota-derived metabolite phenylacetylglutamine.

Conclusions: In conclusion, dietary choline supplementation modifies gut microbiota composition and atherosclerosis development. Plasma metabolomics clearly indicated that choline supplementation, only in the presence of HDL, increases the concentration of several metabolites indicative of augmented cardiovascular risk and impaired kidney function.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

IMPACT OF BLUEBERRY JUICE ON KIDNEY AND PERIRENAL ADIPOSE TISSUE IN A RAT MODEL OF PREDIABETIC NEPHROPATHY – GUT MICROBIOTA AND BEYOND

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Background and Aims: Prebiotic properties of blueberries (BB) have been described as promising against metabolic diseases, but its effects on Prediabetic Nephropathy (PreDN) remain unclear. This study aimed to evaluate the effects of blueberry juice (BJ) on PreDN, focus on gut microbiota modulation and on kidney and perirenal adipose tissue (PAT).

Methods: Polyphenol (PP) content in BJ was assessed by HPLC/PDA/ESI-MSn. Twenty-four male Wistar rats were divided in 3 groups (n=8) for 24 weeks: Standard chow (Sd); PreDN: 45% High-Fat diet (HFD)-induced group and HFD+BJ: 25g/kgBW/day from week 16 onwards. Glycemic, insulinemic and lipid profiles were evaluated, as well as renal function and kidney and PAT histomorphology. Gene expression was evaluated in kidney (KIM-1, NLRP3) and PAT (PRDM16, CD36, UCP1, CPT1) and gut microbiota and SCFAs composition were evaluated in faeces.

Results: HPLC/PDA/ESI-MSn analysis revealed that the main PP's present in BJ are anthocyanins (e.g. malvidin derivative), flavonoids (e.g. quercetin-O-hexoside) and phenolic acids (e.g. caffeic acid). Apart from ameliorating prediabetic glucose intolerance, BJ supplementation elicited hepatic lipid accumulation, inhibition of PAT browning mechanisms and fatty acid β-oxidation, increased renal/PAT inflammasome and kidney injury markers without major improvements on renal function and/or histopathology. In addition, BJ treatment was unable to correct the HFD-induced reduction of Lactobacillus spp and Prevotella spp, and aggravated the reduction of SCFAs.

Conclusions: In this animal model of PreDN, BJ was able to ameliorate prediabetic glucose intolerance but promoted harmful effects in the kidney/PAT duo, which was accompanied by aggravation of SCFAs' reduction. Support: SFRH/BD/109017/2015, PTDC/SAU-NUT/31712/2017 and POCI-01-0145-FEDER-031712.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

PREBIOTIC BLUEBERRY INTERVENTION IMPROVES METABOLIC DYSFUNCTION IN A HYPERCALORIC DIET-INDUCED MICROBIOTA DYSBIOSIS SCENARIO

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Background and Aims: This study aimed to assess the effects of blueberry juice (BJ) on metabolic profile, gut microbiota (GM) composition, intestinal barrier integrity, liver metabolism and hepatic mitochondrial-related parameters in prediabetes.

Methods: A prediabetic rat model [Male Wistar rats] was established by ingestion of a high-sucrose (HSu, 35%) diet for 9 weeks (W9), further supplemented with a high-fat diet (HF, 60%) for 14 weeks (HSuHF, W23), vs control. Half of the HSuHF animals ingested BJ (25g/Kg BW, daily) between W9 and W23 (HSuHF+BJ). Along with metabolic characterization, GM and SCFAS composition as well as intestinal permeability were analyzed by standard techniques. Morphological/functional markers of liver damage were assessed by ultrasonography, histological techniques, and mitochondrial bioenergetics assays. Hepatic expression profile of genes involved in chief-metabolic pathways was assessed by RT-qPCR.

Results: HSuHF+BJ rats displayed improved the metabolic features of prediabetes along with amelioration of hepatic steatosis and mitochondrial function. Alongside restoring hepatic antioxidant metabolites, BJ positively affected gene expression of key targets of fatty acid oxidation, insulin signaling, inflammation, as well as mitochondrial respiratory chain-related genes, which were all downregulated in HSuHF animals' livers. Subtle GM modulation was also observed upon BJ treatment, including a suppression of Bifidobacterium and Prevotella abundance and lower fecal succinate levels compared to the GM of prediabetic animals, despite of unchanged SCFAs contents.

Conclusions: BJ can be effective to counteract prediabetes progression induced by hypercaloric diet due to beneficial effects against hepatic steatosis and mitochondria dysfunction, with subtle changes on GM profile as early as prediabetes. Support: SFRH/BD/109017/2015; PTDC/SAU-NUT/31712/2017; POCI-01-0145-FEDER-031712.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

GUT MICROBIOTA AND NAFLD: ASSOCIATION OR CONFLICT?

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Background and Aims: Nonalcoholic fatty liver disease is a liver disease that affects about 25-30% of the population. Gut microbiota may affect all risk factors for the NAFLD development by disturbing metabolic homeostasis, enhancing IR, increasing oxidative stress and developing liver inflammation. The aim of this study was to explore the influence of gut microbiota on liver fat increasing.

Methods: The study included 54 patients with NAFLD based on elastography (34 men, 20 women) (average age 46.64±2.52). The controls involved 32 paients (14 men, 18 women) (average age 32±1.54) without NAFLD. Microbiom quantification of different taxa by qPCR using primers targeting the 16S rRNA gene, specific for Firmicutes, Actinobacteria and Bacteroidetes was performed.

Results: The Bacteroidetes level was significantly higher in controls (45,54±5,49 vs.21,10±3,39). Actinobacteria (22,13±2,47 vs 14,53±2,75), F/B index (4,02±1,00 vs 1,82±0,45) in patients with NAFLD was above than in controls. F/B index growth was leading to the triglycerides (r=0.53), ALT (r=0.61) and VLDL (r=0.4) increasing. Microbiota of control group played a protective role by reducing aggressive factors - the Actinobacteria growth led to the decreasing of GGTP (r=-0.42), direct bilirubin (r=-0.34) and CRP (r=-0.36). F/B index growth caused the decreasing of GGTP (r=-0.36) and TNF-a (r=-0.29)

Conclusions: The microbiota regulation could be effective in the direction of reducing harmful bacteria (F/B index) and increasing the beneficial ones (Bacteroidetes) only in patients with NAFLD. The same bacteria intake in patients without NAFLD will not prevent the disease occurence and could provoke the unespecial effects, thus the question remains whether it is necessary to take probiotics profilactically.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

GUT MICROBIOTA- DO WE HAVE THE CHANCE TO CHANGE THE MIND ABOUT NAFLD?

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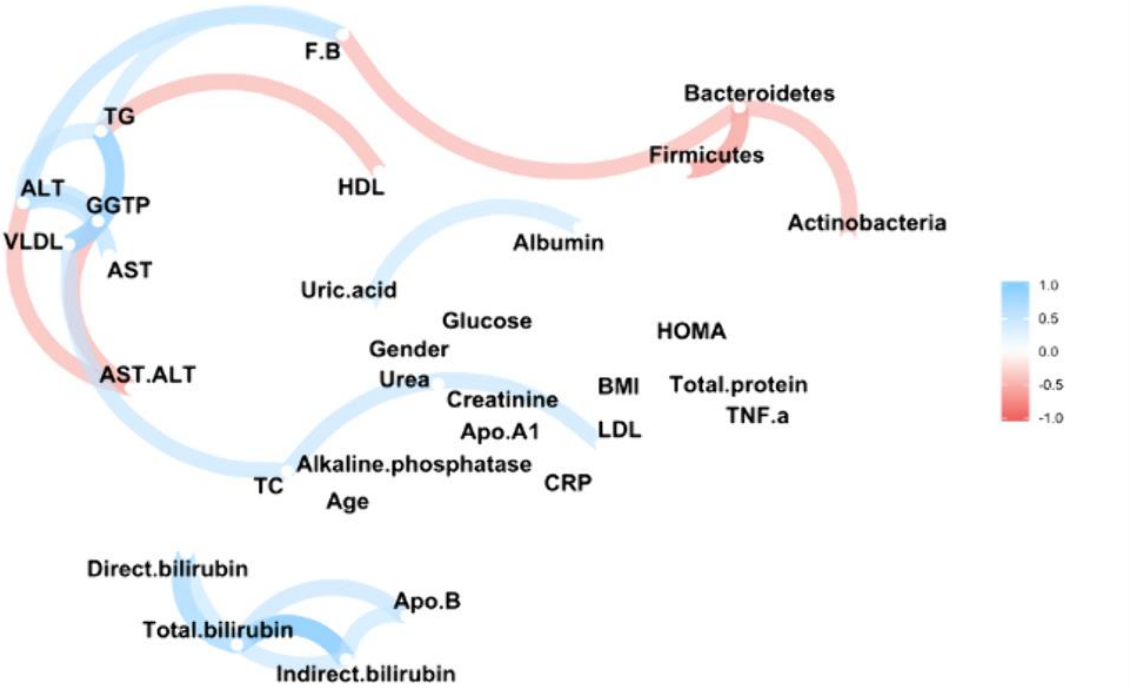
Background and Aims: Nonalcoholic fatty liver disease affects about 25-30% of the population. All evidence points to a close relationship between the composition of microbiota and the development of NAFLD with complications. The gut microbiota is now considered as a major metabolic internal organ. The gut microbiota may affect all risk factors for the NAFLD occurence by increasing metabolic disorders, IR, oxidative stress and inflammation. The aim of this study was to explore the impact of gut microbiota on fatty liver infiltration and its progression.

Methods: The study included 54 patients with NAFLD based on elastography (34 men, 20 women) (average age 46.64±2.52). The control group involved 32 patients (14 men, 18 women) (average age 32±1.54) without NAFLD. Both groups underwent biochemical tests. Microbiom quantification by qPCR using primers of 16S rRNA gene, specific for Firmicutes, Actinobacteria and Bacteroidetes was performed.

Results:



The negative correlational relationship was marked in NAFLD group between Bacteroidetes and Firmicutes (r=-0.68), Bacteroidetes and Actinobacteria (r=-0.56), Bacteroidetes and F/B index (r=-0.56). Actinobacteria in NAFLD group correlated with TNF-a (r=0.41). F/B index was in strong relationship with ALT (r=0.61), TG (r=0.53), VLDL (r=0.4). In controls, Bacteroidetes strongly correlated with Firmicutes (r=-0.93), Actinobacteria (r=-0.84), F/B index (r=-0.88). Actinobacteria was in correlation with Apo-A1 (r=0.74), Apo-B (r=-0.74), GGTP (r=-0.42). Bacteroidetes and F/B index correlated with Apo-A1 (r=-0.43) (r=0.6), Apo-B (r=0.44) (r=-0.61). Firmicutes with ALT (r=-0.37)

Conclusions: Microbiom plays one of the particular roles in NAFLD development. In NAFLD absence – Actinobacteria and F/B index will play a protective role, in case of NAFLD - aggressive.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

HUMAN MILK OLIGOSACCHARIDE SUPPLEMENTATION DURING GESTATION INDUCES A TOLEROGENIC ENVIRONMENT IN OFFSPRING PROTECTING FROM FOOD ALLERGY

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Background and Aims: Food allergy (FA) severity is constantly increasing and there is no effective preventive strategy. Allergy is associated with the dysfunction of the microbiota and the immune system leading to the failure of tolerance. These actors are set up in utero, making pregnancy an optimal window of intervention. Human milk oligosaccharides (HMO) are able to modulate the microbiota and the immune system. We hypothesized that supplementation with an HMO (2'-FL) during pregnancy would allow the establishment in the fetus/newborn of a tolerogenic immune system and a benefic microbiota, protecting from FA occurrence.

Methods: Mice were fed a standard diet or a diet enriched in 2'-FL during gestation only. Wheat FA was induced in female pups born from mothers that received either a control diet or 2'-FL diet. Not sensitized pups were used as controls. Symptoms (drop in temperature) and immune biomarkers of FA, were evaluated.

Results: In mothers, HMO supplementation increases the frequency of IL-10-secreting B cells and Th1 cells. They are found in pups issued from HMO-supplemented mothers associated with the increase of regulatory B cell expressing CD25. No effect on the frequency of regulatory T cell was observed in dams and offspring. In FA mice born from HMO-supplemented mothers, symptoms of allergy were abrogated, IgE concentration was reduced and the frequency of regulatory B cell expressing CD9 was increased.

Conclusions: In conclusion, HMO supplementation during pregnancy increases the frequency of regulatory B cells in the mother and the pups, reflecting the mother-to-child transfer of a tolerogenic immune environment protecting from FA occurrence

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

ENGINEERING A NOVEL BACTERIAL POPULATION CONTROL SYSTEM

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Background and Aims: In situations where specific microbes play antagonistic or causative roles in human disease, an engineered live bacterial therapeutic (LBT) could be used to target these specific pathogenic microbes. However, the competition pressures faced by LBT are person, strain, and region specific. Therefore, we need systems that are controllable, targeted, and work uniformly across individuals. We developed the SPoCK system to address this challenge.

Methods: Our SPoCK system is a population control system with highly controlled colonisation and selective killing. The The SPoCK system controls other microbes by producing toxins, called bacteriocins, that kill competitors. The system works as follows: when SPoCK enters a community it produces bacteriocins, these kill competitors and SPoCK wins. As the SPoCK population increases, negative feedback via quorum sensing molecules turns off production of the bacteriocin, allowing the competitor populations to re-grow, now SPoCK loses. Once the SPoCK population is low the cycle repeats again, SPoCK wins once more. In this way, the SPoCK system creates a dynamic win-lose cycle enabling its co-existence within a microbial community. This work focuses on the construction of an upgraded SPoCK, SPoCK2, system where the bacteriocin (microcin V) and corresponding immunity (Cvi) are both under the control of an inducible promoter.

Results: Inhibition assays and qPCR were used to determine successful construction and behaviours of the SPoCK2 plasmid and system. SPoCK2 is successfully able to kill bacteriocin sensitive strains and respond to repressors.

Conclusions: Future work would focus on identifying bacteriocins to kill cancer-associated bacteria suitable for use in humans

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

ENGINEERED ACETOACETATE-INDUCIBLE WHOLE-CELL BIOSENSORS BASED ON THE ATOSC TWO-COMPONENT SYSTEM

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Background and Aims: Whole-cell biosensors (WCBs), engineered bacteria designed to sense specific inputs, hold the potential to increase our understanding of the microbiota and host-microbe interactions. WCBs can be engineered to detect environmental factors (e.g., pH or metabolite concentrations) and subsequently report on these levels via a measurable output (e.g., fluorescent proteins), or influence the community through targeted expression of a therapeutic molecule. These WCBs offer several advantages over more traditional sensing technologies, such as portability and self-replication. However, WCB input-output responses are often not suitable for the desired application and methods of reliably tuning their behaviour are needed if they are to become more widely used.

Methods: Here we report the construction of WCBs sensitive to acetoacetate. Acetoacetate is a metabolite found systemically within the blood and is linked to mammalian metabolism and energy regulation. More specifically, elevated levels of acetoacetate can be indicative of diabetic or alcoholic ketoacidosis. If left untreated, these conditions lead to severe complications.

Results: Initially, we engineered a WCB that produces a GFP signal when exposed to increasing acetoacetate levels, via the AtoSC two-component system. Subsequently, we developed an ODE model of this circuit and used sensitivity analysis to inform design strategies that were used to modify the response of our acetoacetate-WCBs.

Conclusions: The final range of biosensors produced display a range of switching behaviours. It is hoped these biosensors may be used in future for complex in vivo monitoring applications and that the design strategy developed here could be used to tune other two-component based biosensors.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

GUT MICROBIOME COMPOSITION AND SERUM METABOLOME PROFILE AMONG INDIVIDUALS WITH SPINAL CORD INJURY AND NORMAL GLUCOSE TOLERANCE OR PREDIABETES/TYPE 2 DIABETES

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Background and Aims: Objective: To compare the gut microbiome composition and serum metabolome profile among individuals with spinal cord injury (SCI) and normal glucose tolerance (NGT) or prediabetes/type 2 diabetes (preDM/T2D). Design: Cross-sectional design. Setting: Research university.

Methods: Participants: A total of 25 adults (N=25) with SCI were included in the analysis and categorized as NGT (n=16) or preDM/T2D (n=9) based on their glucose concentration at minute 120 during a 75-g oral glucose tolerance test. The American Diabetes Association diagnosis guideline was used for grouping participants. Main outcome measures: A stool sample was collected and used to assess the gut microbiome composition (alpha and beta diversity, microbial abundance) via the 16s ribosomal RNA sequencing technique. A fasting serum sample was used for liquid chromatography-mass spectrometry-based untargeted metabolomics analysis, the results from which reflect the relative quantity of metabolites detected and identified. Gut microbiome and metabolomics data were analyzed by the Quantitative Insights into Microbial Ecology 2 and Metaboanalyst platforms, respectively.

Results: Gut microbiome alpha diversity (Pielou's evenness index, Shannon's index) and beta diversity (weighted UniFrac distances) differed between groups. Compared with participants with NGT, participants with preDM/T2D had less evenness in microbial communities. In particular, those with preDM/T2D had a lower abundance of the Clostridiales order and higher abundance of the Akkermansia genus, as well as higher serum levels of gut-derived metabolites, including indoxyl sulfate and phenylacetylglutamine ($P < .05$ for all).

Conclusions: Our results provide evidence for altered gut microbiome composition and dysregulation of gut-derived metabolites in participants with SCI and preDM/T2D.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

ORAL SUPERABSORBENT HYDROGEL EXPANDS AKKERMANSIA AND DRIVES CHANGES TO THE GUT MICROBIOTA ASSOCIATED WITH METABOLIC BENEFITS IN A MOUSE MODEL OF DIET INDUCED OBESITY

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Background and Aims: Gelesis oral superabsorbent hydrogels (OSH) are cellulose-based devices that mimic 3D mechanical properties of masticated vegetables during digestive system transit. We previously developed a murine diet-induced obesity (DIO) model and observed improvement in several metabolic parameters after treatment with the OSH Gel-B. This study aimed to define the gut microbiota associated with these improvements and uncover how Gel-B may drive compositional changes to these communities.

Methods: Male C57BL6/J mice with DIO were treated with high fat diet (HFD), HFD+Gel-B, or chow for 12 weeks. Whole-genome shotgun sequencing was performed on fecal samples. Count tables were generated using the CosmosID Metagenomics Cloud. Statistical modeling was performed in R.

Results: On PCoA analysis, gut microbiota of the groups showed significant separation ($p< 0.01$; ADONIS permutation). At 12 weeks, relative to HFD, treatment of Gel-B 4% increased relative abundance of Bacteroidetes ($p < 0.02$) and Verrucomicrobia (exclusively Akkermansia muciniphila; $p =0.05$), and decreased Firmicutes ($p <0.01$) and Actinobacteria ($p<0.01$). The impact of Gel-B on A. muciniphila (AM) growth was studied in vitro, and the addition of Gel-B to growth media increased AM growth over that in growth media alone ($p<0.01$). Non-crosslinked Gel-B backbone did not increase AM growth, indicating that Gel-B 3D structure is required for this phenomenon ($p<0.01$).

Conclusions: Gel-B treatment induced dramatic changes to the gut microbiota in DIO mice associated with improved metabolic outcomes, including an increase in A. muciniphila relative abundance. In vitro experiments found that Gel-B enhances A. muciniphila growth, providing a unique mechanism to explain observations from the animal study.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

ANALYSIS OF MICROBIOME DATA FROM THE CAN-BIND12: EFFECTS OF PROBIOTICS ON SYMPTOMS OF DEPRESSION (EPSD) CLINICAL TRIAL

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Background and Aims: A double-blind randomized placebo-controlled trial recently took place investigating the effects of probiotic administration on symptoms of depression. The primary objective of this analysis is to investigate changes in the gut microbiome of individuals with depression before, during, and after probiotic supplementation.

Methods: Stool samples were collected from participants (n=13) at 4 time points, baseline, week 2, week 8, and week 16. Stool samples (n=51) were analysed using 16s rRNA metabarcoding to assess for measures of alpha diversity and taxonomic information. Subgroups were analysed to determine the relationship between measures of microbiome diversity and sex, treatment group, responder status, and dosage.

Results: Subgroup analyses for measures of alpha diversity found significantly lower scores on most measures of alpha diversity in placebo groups compared to low or high-dose probiotic groups. Males were found to be significantly lower than females in most measures of alpha diversity. Responder status-based subgroup analysis found responders to be significantly lower than non-responders in one alpha diversity measure at week 8.

Conclusions: These findings provide evidence to support the use of probiotic supplementation as a tool to increase the microbial diversity of one's gut, however, increased microbial diversity was not positively associated with responder status or improvement in symptoms of depression. Significant sex-based differences in microbial diversity were consistently observed, which has implications for sex-specific probiotic development and dosing. This study is heavily limited by sample size suggesting the need for larger-scale randomized controlled trials, as well as the addition of microbiome analysis to ongoing and future clinical trials.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

COMPARISON OF JUVENILE GROWTH OF SPF AND GNOTOBIOTIC GM15 AND OMM12 COLONISED MICE ON BREEDING DIET AND DURING CHRONIC UNDERNUTRITION

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Background and Aims: Biomedical research has benefited from genetic and breeding conditions standardization of Mus musculus. Despite these efforts, differences in microbiome among facilities remain a limitation. In an attempt to standardize the gut microbiome, mouse minimal microbiota were established. We compared growth dynamics of mice colonized with GM15 and oMM12 minimal microbiota to SPF mice.

Methods: SPF, GM15 and oMM12 colonized mice were weaned at day 21 after birth either on breeding diet (BD)(males and females) or depleted diet (DD)(males). DD is low in proteins and fat, but calorically equivalent. The body weight and lenght of the mice were monitored for five weeks. Serum levels of IGF-1 and IGFBP3 were measured by ELISA.

Results: Females and males kept on BD grew comparably regardless of microbiota status. We detected no difference in the circulating levels of IGF-1 and IGFBP3 neither for females nor for males on BD. Weaning on DD caused stunting, manifested by lower weight and length gains. GM15 and oMM12 grew better (both weight and length growth rates) compared to the SPF mice. The improved growth of GM15 and oMM12 mice was accompanied by higher levels of serum IGF-1 and IGFBP3 compared to SPF mice.

Conclusions: GM15 and oMM12 colonized mice grow comparably to SPF mice on breeding diet. Both minimal microbiota improved growth during juvenile chronic undernutrition. In the future, modular aspect of minimal microbiota will allow us to manipulate the bacterial composition and further dissect the impact of bacterial strains and defined communities on host physiology and growth.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

ANTI-INFLAMMATORY EFFECT OF BACTEROIDALES SPECIES ON HT-29 CELLS AND ASSOCIATED METABOLOMIC CHANGES

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Background and Aims: In the intimate relationship between the host and the gastrointestinal microbiota, the latter has adopted key functions for the host including nutrients digestion, fermentation of fibers, energy generation, synthesis of vitamins, pathogen protection and immune system modulation. Some strains of the phylum Bacteroidetes, the most abundant gram-negative phylum in the human gut, are currently being investigated as next generation probiotics (NGPs). The health-promoting benefits of some members has been associated to the production of molecules with anti-inflammatory properties, such as polysaccharide A (PSA), sphingolipids, or short-chain fatty acids (SCFAs). In this study we aim to find potentially new beneficial Bacteroidetes strains and to unravel the mechanisms underlying their anti-inflammatory properties.

Methods: A panel of Bacteroidales strains have been tested for their ability to modulate IL-8 production on the gut mimicking HT-29 in vitro cell system. Subsequently, several strains have been selected for their anti-inflammatory effect and untargeted liquid chromatography-mass spectrometry (LC-MS)-based metabolomic analysis has been performed.

Results: From the panel of Bacteroidales, the strain B6 has been selected for its ability to reduce IL-8 levels in challenged HT-29 cells with TNF-α. Metabolites belonging to amino acids and derivatives in the treatment group were significantly different from the controls. Other classes of significantly different metabolites include fatty acyls and imidazopyridines.

Conclusions: To conclude, the anti-inflammatory properties of Bacteroidales order are strain dependent, with some strains having the opposite effect. Specifically, the strain B6 is able to reduce the levels of proinflammatory cytokine IL-8 and to affect the metabolic environment of HT-29 cells.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

LACTOBACILLUS PARACASEI CNCM I-5220 POSTBIOTIC EFFECTIVELY PRESERVE EPITHELIAL BARRIER INTEGRITY

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Background and Aims: Postbiotica is a new innovative biopharmaceutical company engaged in the discovery and development of postbiotics. Postbiotics are novel classes of natural molecules released during human microbiome's metabolic activities. These metabolites can regulate hostmicrobe interaction, epithelial homeostasis and are beneficial for our health. Due to their intrinsic toxicity and immunomodulatory properties, they represent a novel solution for a number of medical applications.

Methods: In particular, Postbiotica has developed a novel postbiotic from Lactobacillus paracasei CNCM I-5220 strain. This postbiotic has found to have a role in protecting the epithelial integrity barrier, both intestinal (IEB) and skin epithelial barrier. We showed that Lactobacillus paracasei CNCM I-5220 postbiotics are able to repair the damaged monolayer of Caco2 epithelial cells in vitro, where damage is induced by invasive pathogens, such as Salmonella typhimirium. This protective effect of postbiotics has been confirmed in vivo as well, where mice pre-treated with postbiotics were able to preserve IEB upon Salmonella typhimirium exposure. At the level of skin, this postbiotic was able to induce the dosedependent release of hyaluronic acid and pro-collage by human keratinocytes and fibroblast, respectively and exhibit wound healing properties and reduce skin roughness. Thus, suggesting that postbiotic is able to improve overall vitality of the skin and its barrier properties.

Results: Overall, this novel Lactobacillus paracasei CNCM I-5220 postbiotic has barrierprotective characteristics that can be implemented in treatment of different pathologies, from intestinal-specific to skin-specific diseases.

Conclusions:

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

VITAMIN A METABOLIC POTENTIAL OF THE GUT MICROBIOTA

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Background and Aims: Vitamin A (VA) and its active metabolite retinoic acid (RA) are major regulators of gene expression in mammals. RA plays a major role in maturation of immune cells and maintaining the integrity of the gut barrier. Previous work in the laboratory demonstrated gut bacteria regulate VA metabolic machinery and RA production in the intestinal epithelium however, whether gut bacteria themselves can metabolize dietary VA is not known.

Methods: We used various methodologies including assays to measure enzymatic activity, in-vitro cultures and 16S sequencing.

Results: I observed that conventional mice harboring a commensal bacteria have significantly higher RA in the gut lumen compared to germ-free mice. Furthermore, cecal contents from conventional mice showed significantly higher activity of enzymes involved in VA metabolism compared to germ-free mice where VA metabolism enzyme activity was undetectable. Oral antibiotics treatment significantly diminished VA metabolic activity in the cecal contents of conventional mice suggesting that gut bacteria have the metabolic potential to metabolize VA into RA that could influence RA-signaling in intestinal mucosa. Finally, bacteria isolated from mouse gut showed a strong VA metabolic activity in vitro.

Conclusions: I propose that gut microbiome is a novel and potent source of VA metabolic activity that plays a crucial role in regulating RA dependent epithelial barrier and immune function in the intestine. As VA deficiency is one of the most common dietary deficiencies worldwide our data potentially suggests that gut microbiome can be used as a key therapeutic factor in addressing this public health issue.

Topic: *AS07 DMH - Manipulation of the Microbiome for treating diseases (Phage therapy, diet, nutrition, new approaches, etc.)*

PROTECTIVE EFFECT OF APOLIPOPROTEIN A-I/HDL LEVELS IN CHOLINE-INDUCED ATHEROSCLEROSIS : A ROLE FOR THE GUT MICROBIOTA ?

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Background and Aims: Apolipoprotein A-I/ high-density lipoprotein (apoA-I/ HDL) has been recognized to exert a beneficial anti-atherogenic effect. The gut microbiota exert a strong association with HDL levels and a direct pro-atherogenic effect through the metabolism of dietary choline. Hence, the aim of this study is to investigate the role of microbiota in choline-induced atherosclerosis through the modulation of HDL levels.

Methods: Three genetically modified, conventionally raised, atherosclerosis-prone mouse models were fed with either low or high choline diet for 16 weeks: i) extremely low-HDL, apoA-I and apoE double knock out (DKO) mice; ii) low-HDL mice, deficient for murine apoE (EKO); iii) high-HDL mice, apoA-I and apoE double KO but overexpressing human apoA-I (DKO/hA-I). Development of atherosclerosis and the microbiota composition have been assessed. Then, fecal samples from the different mice groups were transplanted in EKO mice to decipher whether differences in atherosclerosis development could be transmitted through the gut microbiota.

Results: High HDL DKO/hA-I mice showed less plaque area than the other two models and high choline diet was associated with more severe atherosclerotic plaque than low choline diet. EKO mice harboured a microbiota composition different from the other two models at both T0 and TF. Interestingly, 16 weeks of choline feeding strikingly shifted the microbiota composition.

Conclusions: In conclusion, high HDL levels exert protective effects on atherosclerosis under choline diet that might related to microbiota modifications. Ongoing analyses of the gut microbiota transplant experiment will reveal if these microbiota changes contribute to the protective properties.

Topic: *AS08 DMH - Metabolomics (designing interventions based on metabolomics, with a focus on those of importance to establishing gut integrity and mucosal immunity)*

MATERNAL HIGH FAT DIET EXPOSURE AUGMENTS DEVIATIONS IN THE NEUROACTIVE METABOLOME OBSERVED IN NON-HUMAN PRIMATE OFFSPRING FED A HIGH FAT DIET

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Background and Aims: In a non-human primate (NHP) model, offspring exposed to maternal high fat diet (mHFD) display physiological changes compared to offspring exposed to maternal control diet (mCTR). To investigate the relationship between maternal diet and offspring post-weaning diet, we hypothesized that mHFD exposure plus a post-weaning high fat diet (HFD) will produce a persistently altered gut metabolome profile in vivo compared to mCTR exposure.

Methods: Japanese macaques were fed mHFD, mCTR, or a mHFD-to-mCTR reversal diet during gestation/lactation then weaned onto HFD. Serum and fresh stool were collected longitudinally. Collection of intestinal tissue was performed at necropsy (early third trimester or 15 months). Serum and polar metabolite extractions of stool and intestine were analyzed by quantitative liquid chromatography-mass spectrometry targeting 34 metabolites spanning neuroactive pathways (serotonin, GABA, dopamine) and short chain fatty acids.

Results: We have data on 34 neuroactive metabolite levels in n=191 serum, n=129 stool, and n=250 intestinal samples from n=79 fetal and n=61 juvenile (15 months old) NHP offspring. In stool from mHFD compared to mCTR exposure, we observe decreased serotonin (p=0.009), tryptophan (p=0.051), 5-hydroxyindoleacetic acid (p=0.052), GABA (p=0.001), and glutamate (p=0.009) in association with mHFD exposure.

Conclusions: Our findings indicate that gut neurometabolites in NHP offspring are sensitive to maternal diet/post-weaning diet synergism. Exposure to mHFD prior to HFD-weaning is associated with changes in multiple neuroactive metabolite levels compared to mCTR animals also weaned onto HFD. These data suggest a programmable feedback mechanism in the early life gut that may be critical to development of the gut-brain axis in primates.

Topic: *AS08 DMH - Metabolomics (designing interventions based on metabolomics, with a focus on those of importance to establishing gut integrity and mucosal immunity)*

THE NON-HUMAN PRIMATE NEUROMETABOLOME IS ALTERED BY MATERNAL HIGH FAT DIET EXPOSURE IN NON-HUMAN PRIMATE OFFSPRING INDEPENDENT OF POST-WEANING DIET

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Background and Aims: In a non-human primate model, we have shown that offspring exposed to maternal high fat diet (mHFD) display persistent anxiety and a persistently altered gut microbiome, even when weaned onto a control diet for 2.5 years. Since the gut and its microbes influence behavior/neuroactivity, we hypothesized that mHFD exposure influences offspring behavior years after birth via a persistently altered gut metabolome in vivo.

Methods: Japanese macaques were fed mHFD or control diet (mCTR) during gestation/lactation then weaned onto CTR. Serum and fresh stool were collected longitudinally. Collection of intestinal tissue was performed at necropsy (early third trimester or 3 years). Serum and polar metabolite extractions of stool and intestine were analyzed by quantitative liquid chromatography-mass spectrometry targeting 34 metabolites spanning neuroactive pathways (serotonin, GABA, dopamine) and short chain fatty acids.

Results: We quantified 34 neuroactive metabolite levels in n=216 serum, n=261 stool, and n=339 intestine from n=72 fetal and n=73 juvenile offspring. We observed significant alterations of the GABA neuroactive pathway in mHFD exposed offspring, beginning in the fetus (serum GABA: mCTR 20.5 ng/mL v. mHFD 23.9 ng/mL, p=0.030) and persistent through 3 years old (stool Glutamate: mCTR 107.6 ng/mg wet feces v. mHFD 67.0 ng/mg wet feces, p=0.038).

Conclusions: In our large experimental cohort of primates, metabolomic profiles of offspring were persistently altered by mHFD exposure, even after 2.5 years of post-weaning control diet feeding. These data suggest a programmable feedback mechanism in the early life gut that may be critical to development of the gut-brain axis in primates.

Topic: *AS08 DMH - Metabolomics (designing interventions based on metabolomics, with a focus on those of importance to establishing gut integrity and mucosal immunity)*

EXERCISE EFFECTS ON NAFLD USING OMICS APPROACH IN ADIPOSE TISSUE, PLASMA, URINE, AND STOOL

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Background and Aims: Exercise such as high interval intensity training (HIIT) is part of the recommended standard treatment against NAFLD, but poorly understood which metabolites, specific pathways, and if gut microbiota (GM) contributes to these improvements.

Methods: A 12-week randomized controlled exercise intervention in NAFLD patients (n=46) without diet change and weight loss has been conducted. While the control group (n=25) kept their lifestyle unchanged, the intervention group (n=21) performed HIIT. At baseline and endpoint, plasma, urine, adipose tissue (AT), and stool samples were collected, ergospirometry tests and MRI scans were conducted. Metagenomics, lipidomics, and non-targeted metabolomics will be performed to identify metabolic changes and pathways associated with NAFLD in humans upon exercise intervention.

Results: HIIT significantly decreased fasting plasma glucose concentration and waist circumference, and increased maximum oxygen consumption rate and maximum achieved workload. Metabolic changes ensued in all sample types after HIIT. Increased accumulations of amino acids (AA) and its derivates in AT and plasma and decreased urinary and fecal excretion were observed. Furthermore, very-long-chain sphingomyelins increased in plasma, whereas glycerophospholipids decreased in stool and certain oxylipids decreased in plasma and urine. Glycoursodeoxycholic acid and glycodeoxycholic acid produced by GM decreased in AT and urine. Gut microbial metabolite indole lactic acid and 3-(4-hydroxyphenyl)lactate) increased in plasma.

Conclusions: Overall, altered AA and lipid metabolism and changes in GM might contribute to the mechanisms underlying the beneficial effects of exercise in NAFLD patients. ClinicalTrials.gov (NCT03995056), Marie Skłodowska-Curie grant agreement No 813781

Topic: *AS08 DMH - Metabolomics (designing interventions based on metabolomics, with a focus on those of importance to establishing gut integrity and mucosal immunity)*

UPCYCLING BERRIES' AGROWASTE INTO SUSTAINABLE PREBIOTICS FAVORING GUT MUCOSAL IMMUNITY: THE CASE OF SENESCENT BLUBERRY LEAF

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Background and Aims: Upcycling strategies for senescent blueberry leaves (BL) agro-waste are promising to impart novel plant food sources of prebiotics. Herein, we aim to characterize the gut-health benefits and in vivo prebiotic surrogates of an innovative senescent BL biomass (BB) obtained from biotechnologic processing.

Methods: BB biomass was assayed for total phenolic content (TPC)/antioxidant capacity (TAC), total dietary fiber (TDF), total digestible starch (TDS) and resistant starch (RS). Male C57BL/6J mice were allocated in two experimental groups (n=8/group): control (Ctl) and orally given BB in an established safe dose (500mg/Kg) for 28 days. Gut health was evaluated by intestinal histomorphology, gut microbiota (GM) composition/fecal SCFA's contents (GC-MS), relative tight junctions gene expression (Occludin, ZO-1; qRT-PCR) and Treg(CD45+CD3+CD4+FoxP3+CD25+)/Th17(CD45+CD3+CD4+IL-17+RORγt+) balance in gut-associated lymphoid tissue (GALT, flow cytometry).

Results: BB biomass displays an enriched polyphenolic content and antioxidant capacity (220/38 fold increase compared to the fruit) along with 35.30%±0.07 TDF/g BB, 3.35±0.07 g/100g BB total digestible starch and 0.67±0.03 g/100g BB resistant starch. BB biomass treated animals show increased fecal SCFA's contents (% of control) – propionic (C3, 35%), butyric (C4, 239%) and valeric (C5, 65%) acids - along with normal crypt/villi histomorphology and mucin/occludin/ZO-1 relative gene expression. Notably, GALT presented a more favorable Treg/Th17 balance due to a repression of Th17 cells (29% compared to Ctl, $p<0.01$), a feasable consequence of lower Prevotella abundance (24.2%, $p<0.05$ of control).

Conclusions: This work paves the way for novel BB-derived prebiotics with gut-immunomodulatory effects to tackle unhealthy paradigms, adding in the transition to more sustainable food systems.
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Topic: *AS09 DMH - Post-operative care with microbiome manipulation*

SURGICAL BOWEL PREPARATION CAUSES PERSISTENT DISRUPTION OF INTESTINAL MICROBIOTA FOLLOWING COLORECTAL SURGERY

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Background and Aims: Despite the intestinal microbiota being implicated in complications following colorectal surgery, perioperative changes in the gut microbiota are poorly understood. In particular, the contribution of surgical bowel preparation (SBP) to changes in gut microbiota and its effect on surgical recovery are not well described. The aim of this study was to characterize the disruption and recovery of the microbiota following SBP and surgery.

Methods: The intestinal microbiota of patients undergoing colonoscopy (n=19) was compared with that of patients undergoing SBP and subsequent colorectal surgery (n=25). Microbiota composition was analyzed from fecal samples collected prior to and up to six months following colonoscopy or surgery by amplicon sequencing of the 16S rRNA gene.

Results: A total of 44 patients were recruited from 2019 through 2021. Colonoscopy and surgery cohorts had similar bacterial compositions at baseline. The bacterial community composition of colonoscopy patients changed intra-procedure compared to pre-procedure (ANOSIM, $R=0.076$, $P=0.003$), and rebounded quickly (median 2.5 [1-5] days). In contrast, bacterial communities from the surgery group demonstrated directional shifts with greater abundances of Streptococcus and Lactobacillus in the intraoperative and early postoperative samples. Community composition returned to baseline at a median of 30 (26-41) days postoperative (ANOSIM, $R=0.20$, $P<0.001$), and co-occurrence networks demonstrated significant decreases in measures of network centrality ($P\leq0.04$).

Conclusions: SBP significantly changes gut microbiota composition in the immediate postoperative period, with durable disruption in community composition and structure. Streptococcus is significantly increased in the SBP cohort in the immediate and early postoperative periods and may be associated with surgical complications.

Topic: *AS10 DMH - Impact of environmental chemicals on the establishing gut, oral and airways immunity*

STIMULATION OF MUCOSAL-ASSOCIATED INVARIANT T (MAIT) CELLS BY A BACTERIAL CONSORTIUM TREATED WITH MIXTURES OF ENDOCRINE DISRUPTING CHEMICALS

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Background and Aims: Bisphenols and PFAS (per- and polyfluoroalkyl substances) are widely distributed endocrine disrupting chemicals (EDCs) with potential to influence the intestinal microbiota. Chemical-induced alterations of the bacterial community or metabolism might in turn impact on activation of immune cells such as mucosal-associated invariant T (MAIT) cells. Most of the previous research on EDCs, however, has focused on exposure to single substances. Consequently, there is a big gap of knowledge about the mutual influence of different chemicals.

Methods: A simplified human intestinal microbiota (SIHUMIx) consisting of eight bacterial species was cultivated in vitro in a bioreactor that enables mimicking of intestinal conditions in a high-throughput dimension. The bacteria were constantly exposed to bisphenols, PFAS and their combinations and effects on the microbial community were investigated using proteomics and metabolomics. Activation markers of MAIT cells were measured after stimulation of human PBMCs with EDC-treated SIHUMIx via flow cytometry.

Results: Our data reveal that the production of IFN-γ and TNF-α as well as the expression of CD69, CD154 and CD107 by MAIT cells was increased by stimulation with bacteria previously treated with EDCs for 3 days. These effects were even more prominent if cells were treated with bacteria that were exposed to the mixture of bisphenols and PFAS in comparison to the exposure to a single class of EDCs.

Conclusions: Overall, the present study provides insights, how EDCs can indirectly impact on immune cells via microbes. Moreover, it contributes to the urgently needed knowledge about cumulative exposure scenarios.

Topic: *AS11 DMH - Innovation in microbiome clinical trials design*

CHALLENGES AND EXPERIENCES OF CLINICAL IMPLEMENTATION OF A VAGINAL MICROBIOME TEST PREDICTIVE FOR PREGNANCY CHANGE

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Background and Aims: Background Previous studies have shown that the bacterial composition of the vaginal microbiome using the ReceptIVFity-test can predict the chance to conceive with IVF or IVF/ICSI in a specific population. The ReceptIVFity-test allows for stratification of the vaginal microbiome in several profiles, which correspond with the chance to conceive with IVF or IVF/ICSI treatment within two months. Aims 1. To share and discuss the resulting ReceptIVFity profile and associated chance to conceive with the couple by their physician using a shared decision-making (SDM) protocol before initiating ovarian stimulation. 2a. Analyse the predictive accuracy of the ReceptIVFity-test in a general population of couples undergoing IVF or IVF/ICSI treatment trying to conceive. 2b. Determine and investigate factors influencing predictive accuracy of the ReceptIVFity profiles in a general population couples undergoing IVF or IVF/ICSI treatment trying to conceive.

Methods: In a prospective cohort study, > 800 women undergoing IVF or IVF/ICSI treatment were included between October 2018 and Augustus 2021 in two large hospitals in the Netherlands. All women were asked to complete the Shared Decision-Making Questionnaire (SDM-Q-9) after the test results were discussed and a decision regarding treatment was made. Differences in ReceptIVFity profiles between self swab versus physician performed swabs were analysed.

Results: 1. After SDM, ~ 50% of these couples will decided to temporarily refrain from initiating further treatment depending on the profile. 2. Significant difference between profiles can occur between self- and physician sampling of vaginal microbiome.

Conclusions: SDM is an approach is highly apprectiated by patients. Adequate instruction about vaginal sampling techniques is essential.

Topic: *AS11 DMH - Innovation in microbiome clinical trials design*

IDENTIFICATION AND QUANTIFICATION OF THE MICROBIOME IN COLORECTAL CANCER METASTASES

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Background and Aims: Bacterial genomes and viable bacteria have been identified in human tumor tissues. Also, bacterial strains from primary colorectal tumors (PT) have been identified in liver metastases. Here, we aimed to identify and quantify bacterial genomes in PT and associated metastases (CRCM), with normal adjacent tissue (NAT) as control, to enlighten to which extent the microbiota from the PT is transferred to metastases and how it could influence their behavior.

Methods: We collected 391 frozen samples and clinical data from 125 patients including PT, CRCM, and NAT, with hepatocellular carcinoma (HCC) and cholangiocarcinoma (CGC) as negative controls for liver metastases. We sequenced the V4 region of the bacterial 16S rRNA gene in all samples. Amplicon sequence variants were quantified using the DADA2 pipeline and potential contaminants were removed bioinformatically.

Results: We identified three main clusters based on the sample type with significant differences in their microbiota composition ((i)PT-NAT; (ii)CRCM-NAT; (iii)HCC-CGC). We also observed a significant transfer of well-known prevalent taxa in the colorectal cancer literature from PT to CRCM.

Conclusions: This first and major step allows us to move forward and deepen our analyses on the CRCM microbiome and its associations with clinical data, local immune response and genomic features in the metastases.

Topic: *AS12 DMH - Other*

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF BACTERIA REPRESENTING THE MICROBIOTA OF A COLOMBIAN CHEESE

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Background and Aims: Antibiotics have been the mechanism used to control the growth of microorganisms; however, their excessive and inappropriate use has generated resistant strains. According to the World Health Organization, antibiotic resistance is the ability of a microorganism to prevent the action of an antibiotic to which it was previously sensitive. Therefore, the use of alternatives such as antimicrobial substances produced by Lactic Acid Bacteria (LAB) is a promising solution. The aim of this work was to evaluate the antimicrobial activity of LAB representing the microbiota of Double Cream Cheese (DCC) against pathogenic microorganisms.

Methods: Cell-Free Culture Supernatant (CFCS) were obtained from bacterial isolates of a DCC and evaluated the antimicrobial activity against Escherichia coli ATTC25922, Salmonella Typhimurium ATCC14028, Staphylococcus aureus ATCC25923 and Listeria monocytogenes ATCC19111 using agar-well diffusion method, and microdilution broth method. CFCS were subjected to different pH and the antimicrobial activity was evaluated to determine the presence of antimicrobial peptides.

Results: CFCS of the 15 strains LAB showed growth inhibition of all pathogens tested. 8 strains of LAB were selected for pH treatments and growth inhibition was evidenced for the pathogens at pH 4, while at pH 7 the CFCS of Leuconostoc mesenteroides and Lactobacillus casei showed inhibition against S. aureus y L. monocytogenes. Finally, at pH 10.0 there wasn’t inhibition.

Conclusions: CFCS of Leuconostoc mesenteroides and Lactobacillus casei showed greater antimicrobial activity against pathogens tested, this inhibition could be due to the presence of organic acids or antimicrobial peptides, which classifies them as a possible alternative to the use of antibiotics.

Topic: *AS12 DMH - Other*

IN VITRO ASSESSMENT OF THE PROBIOTIC PROPERTIES OF THE NATIVE MICROBIOTA ISOLATED FROM COLOMBIAN DOUBLE CREAM CHEESE

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Background and Aims: Countless health benefits are provided by the ingestion of foods containing probiotic cultures. Double Cream Cheese is a type of artisanal Colombian cheese, manufactured in the regions of Ubaté and Chiquinquirá Valleys. This cheese contains a native microbiota that gives it its sensory organoleptic and physicochemical characteristics, mainly from the group of Lactic Acid Bacteria (LAB). However, the probiotic capacity of this native microbiota is unknown. The aim of this study was to identify and characterize the probiotic properties of some bacteria present in natural microbiota of Double Cream Cheese.

Methods: Three LAB strains (21-Leuconostoc mesenteroides subsp. mesenteroides, 29-Pediococcus acidilactici, 32- Limosilactobacillus fermentum) were evaluated for probiotic propertis such as: morphological characterization, catalase, tolerance to pH (3, 5.5, 6.5 and 8) and bile salts (0.3, 0.6 and 1% w/v) conditions, auto aggregation, coaggregation with pathogens, bacterial affinity to solvent (BATS) assay and safety to the host.

Results: All bacterial strains were not hemolytic and catalase negative. The strains evaluated were able to survive under acidic pH and bile salts conditions. Atypical antibiotic resistances were not observed, except for vancomycin resistance in 29 strain. They also exhibited coaggregation with L. monocytogenes, E. coli and S. aureus. The affinity to solvents indicated that only strain 32 was able to adhere to xylene indicating good cell surface hydrophobicity.

Conclusions: These autochthonous LAB presented probiotic properties that could provide benefits to the hosts that consume dairy products made with these bacteria.

Topic: *AS12 DMH - Other*

DELETION OF BOTH DECTIN-1 AND -2 IN MICE AFFECTS BACTERIAL BUT NOT FUNGAL GUT MICROBIOTA AND SUSCEPTIBILITY TO COLITIS IN MICE

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Background and Aims: Innate immunity genes have been reported to affect susceptibility to inflammatory bowel diseases and colitis in mice. Dectin-1, a receptor for fungal wall β-glucans, has been clearly implicated in gut microbiota modulation and modification of the susceptibility to gut inflammation. Here we explored the role of Dectin-2, and both Dectin-1 and -2 deficiency in intestinal inflammation.

Methods: Susceptibility to DSS-induced colitis was assessed in wild-type, Dectin-1KO, Dectin-2KO or double Dectin-1 and -2KO (D-1/2KO) mice. Inflammation severity, as well as bacterial and fungal microbiota compositions, were monitored.

Results: While deletion of Dectin-1 or Dectin-2 did not modify DSS-induced colitis, double deletion of Dectin-1 and Dectin-2 significantly protected the mice. Supplementation of D-1/2KO mice with opportunistic fungal pathogens or antifungal treatment did not affect the protection against gut inflammation. Amplicon-based microbiota analysis of D-1/2KO fecal bacterial and fungal microbiota confirmed the absence of alteration of the mycobiota but a strong modification of the bacterial microbiota. The protection was largely mediated by the bacterial microbiota, as demonstrated by fecal transfer experiments. We showed that bacteria from the Lachnospiraceae family were at least partly involved in this protection and that treatment with it was enough to recapitulate the protection.

Conclusions: Deletion of both Dectin-1 and -2 receptors triggers a global change of the microbial gut environment and surprisingly mainly the bacterial population driving protective effects in colitis. Members of the Lachnospiraceae family seem to play a central role. This finding provides new insights into the role of Dectin receptors in intestinal physiopathology and potentially in IBD.

Topic: *AS12 DMH - Other*

THE MICROBIOME OF THE STOMACH IN CHILDREN FED THROUGH A GASTROSTOMY

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Background and Aims: To study the microbiome of the stomach in children fed through a gastrostomy

Methods: Gastric aspirates were taken from 22 children fed through a gastrostomy tube and studied by sequencing the amplified regions of the 16S RNA gene.

Results: There were identified 310 OTU (operation taxonomic unit), represented by genera of microorganisms, grouped into 23 bacterial phylums. The samples were dominated by Firmicutes phylum (45.53%±25.17), Proteobacteria (35.28%±24.37), Bacteroidota (10.06%±8.89). When the gastrostomy was in the stomach for less than 1 year, the overwhelming number of bacteria was represented by Proteobacteria (40.20%±26.03), followed by Firmicutes (33.01%±22.98), Bacteroidota was 13.32%±11, 12. When the gastrostomy was standing for more than 1 year, the Firmicutes dominated (53.9%±28.47) due to a decrease in the number of Proteobacteria (33.27% ± 20.37) and Bacteroidota (8.24%±11.62). Carriers of gastrostomy before 1 year had 7-14 phylums, the number of bacterial phylums in the other group was less - from 5 to 11. Shannon's index in samples with a gastrostomy for less than 1 year was 2.1±0.61, index in samples with a gastrostomy for more than 1 year was 1.53±1.01. Among pathogenic microorganisms Helicobacter pylori was detected in 46.6% of patients. The detection rate of this microorganism in children fed through a gastrostomy for less than 1 year was 33.3%, when fed through a tube for more than 1 year - 55.5%.

Conclusions: Thus, there are differences between the stomach microbiome in children fed through a gastrostomy for less than 1 year and more than 1 year.

Topic: *AS12 DMH - Other*

IDENTIFICATON OF LATIC ACID BACTERIA ISOLATED FROM COLOMBIAN CHEESE MADE BY TRADICIONAL SYSTEMS

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Background and Aims: Lactic Acid Bacteria (LAB) native play a key role in the production of traditional Colombian cheeses; their characterization has led to a better understanding of the ecosystem to optimize artisanal processes. To characterize the BAL present in traditional Colombian Quesillo cheese (CQC) produced by traditional systems in the Magdalena Valley area, by conventional culture methods and molecular methods to obtain an inventory of native LAB that characterize it.

Methods: 20 acid whey and quesillo samples were subjected to conventional microbiological quality analysis and the associated LAB were assessed by their ability to grow on selective media M17 and MRS. 50 isolates were selected for biochemical characterization through API®50CHL and molecular sequencing (16S rDNA genes and 23S, 16S rDNA).

Results: Coliforms, mesophile, molds and yeasts counts were higher in Acid Whey that Quesillo and the number of LAB grown was about 3.95×10^5 $CFUmL^{-1}$ in Whey and 1.69×10^4 $UFCg^{-1}$ Quesillo, where Lactoccoccus lactis sp lactis was more frequently bacteria isolated; sequences of amplified 16S rDNA gene also identified Leuconostoc mesenteroides, Lactobacillus fermentum and Enterococcus faecium in Quesillo and Whey samples; the presence of Klebsiella sp. isolates was evident.

Conclusions: The results suggest an important role of these bacteria associated with samples of Whey and Quesillo which are artisanal in Colombia. This information is a basis for the study of national indigenous products and to enhance their quality and industrial development. Technological characterization of the strains found is the first step in obtaining an autochthonous starter culture useful in industrial production of Quesillo.

Topic: *AS12 DMH - Other*

PREBIOTIC EFFECT OF PEANUT CONSUMPTION ON FECAL SHORT CHAIN FATTY ACIDS SEEMS TO IMPROVE LIPID PROFILE

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Background and Aims: Peanuts, rich in prebiotic fiber and polyphenols, may have a beneficial effect on the gut microbiota. Therefore, the aim of this study was to evaluate the prebiotic effect of daily intake of peanut products on microbiota and lipid profile.

Methods: ARISTOTLE study, a randomized controlled trial was conducted in 63 healthy young adults that consumed skin roasted peanuts (SRP), peanut butter (PB) or a control butter made with peanut oil and without polyphenols nor fiber, (CB) for six months. Plasma lipids, fecal short chain fatty acids (SCFAs: acetic, propionic and butyric acids) and gut microbial 16S rRNA sequencing data were analyzed at baseline and after intervention.

Results: A lower total cholesterol/HDL and LDL/HDL ratios were observed in SRP compared to CB ($p= 0,019$ and 0.008). SRP group produced significantly more SCFAs in feces ($p= 0,016$). Particularly, those who produced more propionic acid had lower LDL, total cholesterol/HDL and LDL/HDL ratios. Although no changes were observed in Bacteroidetes phylum abundance, those who produced more propionic acid had an increase in Prevotella and Prevotella-to-Bacteroides (P/B) ratio. Moreover, those with a higher abundance of Prevotella and P/B ratio, showed lower total cholesterol/HDL and LDL/HDL ratios. These associations were in all the cases moderate but significant (r around ±0.30 and $p < 0.05$).

Conclusions: A healthier lipid profile after intervention with peanut products seems to be related to higher fecal propionic acid as well as greater abundance of Prevotella and Prevotella-to-Bacteroides ratio.

Topic: *AS12 DMH - Other*

USING THE BOVINE GUT MICROBIOME AS A MODEL OF LOWER NOISE DATASETS TO EXPLORE PREDICTION OF FOOD INTAKE IN MAMMALS

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Background and Aims: Prediction of food intake and conversion of nutrients into desirable traits in bovine species such as milk production and meat quality is pivotal to optimize nutrient utilization. The identification of a core stable gut microbiome that contributes to optimum nutrient conversion can be an alternative pathway to study the prediction of food intake in mammals. Therefore, our objective was to 1) use the gut microbiome from cows under similar genetic and environmental conditions throughout their lactation cycle to predict food intake; and 2) integrate the bovine residual feed intake, the gold standard measurement of nutrient utilization efficiency, and gut microbiome to improve predictive models for feed intake.

Methods: Eighteen first lactation Holstein dairy cows were enrolled in a prospective longitudinal study and samples from the rumen and feces were collected throughout different phases of the lactation cycle (Days 7, 21, 50, 90, and 130 after parturition). Bacterial DNA was sequenced by targeting the V4 region of the 16S rDNA using the Illumina MiSeq platform.

Results: The gut (rumen and feces) microbiome was associated with intake and nutrient utilization efficiency throughout lactation in dairy cows. Even after controlling for differences in intake an association between nutrient utilization was present. The integration of the microbiomes to the prediction equations improves by ~30% the accuracy of the predictive models.

Conclusions: The gut microbiome is associated with food intake and nutrient utilization, and predictions are improved with their integration into well-established models.

Topic: *AS12 DMH - Other*

OLEAF4VALUE – IN VITRO FERMENTATION AND CELL CULTURE MODELS FOR HEALTH BENEFIT SUBSTANTIATION OF OLIVE LEAF EXTRACTS

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Background and Aims: Olive production creates around 4.5 million tonnes of leaves each year. Although rich in bio-resources, only around 0.2% is currently used for extracts. The OLEAF4VALUE project has been setup to develop a valorisation system for olive leaves. It aims to extract high value bioactive compounds (polyphenols, triterpenoids, essential oils, lipids, lignocellulose)

Methods: Polyphenols can act as prebiotics on the gut microbiota. In addition, bacterial polyphenol metabolites may have beneficial effects. The olive leaf extracts will be screened for effects on gut microbiota composition and activity in the NIZO MicroColon technology, which is a high-throughput in vitro screening model constructed on the basis of human colon-like culture media. Polyphenols are indicated to possess anti-oxidant activity and suppress inflammation in intestinal cells. The human intestinal epithelial cell line Caco-2 in the absence/presence of human monocyte/macrophage cell line THP-1 will be used for testing immune modulatory effects of the extracts and their fermentation derived metabolites present in conditioned medium. Our technologies are being optimized to screen effects of well-known polyphenols. This includes spiking intestinal pathogens into MicroColon, combining immune with intestinal cells, and combining conditioned MicroColon medium with intestinal cells to study host responses. As readouts we will apply state-of-the-art techniques for determination of microbiota responses in composition, gene expression, metabolism, pathogen inhibition and intestinal immune responses: 16S and shotgun metagenomic profiling, qPCR, transcriptomics, SCFA analysis, polyphenol (metabolite) measurement, and cytokine profiling.

Results: Initial results show that spiked Clostridioides difficile can be maintained and controlled in MicroColon.

Conclusions: Results from the described plans will be presented.

Topic: *AS12 DMH - Other*

LARGE ANIMALS – CHARACTERIZATION OF LONGITUDINAL GUT MICROBIOME AS A STRATEGY TO INVESTIGATE TEMPORAL STABILITY OF MICROBES AND ITS ASSOCIATION WITH WEIGHT CHANGE IN MAMMALS

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Background and Aims: An association between obesity and gut microbiome has been identified in several mammals. The bovine offers an alternative approach to assess body weight changes throughout the lactation cycle that represents a diverse spectrum of energy demands and its potential associations with the gut microbiome. Therefore, our objective was to characterize temporal associations of the gut microbiome from cows under similar genetic and environmental conditions throughout their lactation cycle and its association with body weight change.

Methods: Eighteen first lactation Holstein dairy cows were enrolled in a prospective longitudinal study and samples from rumen and feces were collected throughout different phases of the lactation cycle (Days 7, 21, 50, 90, and 130 after parturition). Body weight was measured throughout the study. Bacterial DNA was sequenced by targeting the V4 region of the 16S rDNA using the Illumina MiSeq platform.

Results: Rumen and fecal microbiomes were stable throughout lactation. Both gut microbiomes were associated with body weight, while the changes in body weight were mostly associated with the rumen microbiome throughout the study.

Conclusions: The gut microbiome is stable throughout the lactation cycle in dairy cows and the rumen microbiome is associated with body weight and body weight change over time.

Topic: *AS12 DMH - Other*

IMPACT OF DIET AND AGE IN THE LEVEL OF SELECTED BACTERIAL PHYLA AND FAMILY IN FECES IN CHAROLAISE BULLS

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Background and Aims: The aim of the study was the assessment of the microbiological composition in the digestive system of ruminants depends on “host” individual differences, nutrition changes and age of animals. The microbiome of the digestive tract of ruminants contains microbial ecosystem that is affected by a numerous factors – diet, age, breed, geographical location or environmental conditions. In the study conducted on the change in microbiome composition in calves, the influence of individual factors, age, and two feeding periods were considered.

Methods: Fecal samples were collected from 10 Charolaise bulls at 3 months of age, before the introduction of the new diet and 5 months after its modification. The bacterial DNA isolation and qPCR were performed for selected main bacterial phyla and family Lactobacillaceae. The animals were living on the same environmental conditions, without disease symptoms, in good health condition and they were fed the same fodder. The conducted experiment consisted of measuring the weight gain of ruminants and recording changes in the level of tested bacteria, depending on the feed components provided.

Results: The daily body weight gains of the bulls were between 1100 and 1400 grams/day (according to accepted norms). After changing the feed components, the level of Bacteroidetes phylum increased significantly, while a decrease was noted in the case of Firmicutes phylum and Lactobacillaceae family.

Conclusions: The obtained qPCR results indicate that each animal has an individual microbiome composition, which changes under the influence both of the age and the applied diet.

Topic: *AS12 DMH - Other*

SMALL INTESTINE MICROBIOTA IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

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Background and Aims: The goal is to identify possible triggers for ASD by means of gas chromatography-mass spectrometry analysis using microbial blood markers.

Methods: The parietal microbiota of the small intestine was studied by GC-MC of blood (according to the author's method of Osipov G.A.). 39 children aged 1 to 10 years were examined. They had various functional gastroenterological complaints. 26 children (group 1) had no signs of autism and other neuropsychiatric problems according to the psychologist's opinion. 13 children (group 2) were followed up by a psychiatrist and diagnosed with ASD according to CARS. Statistical materials were processed on a personal computer with STATISTICA 12 software. ROC analysis was performed.

Results: There were revealed significant differences in the small intestine microbiota among children with gastrointestinal symptoms and ASD and children with gastroenterological dysfunctions only: prevalence of Clostridium perfringens $P=0.041$, Clostridium ramosum $P<0.001$, Eggerthella lenta $P=0.026$ and Nocardia asteroides $P=0.020$.

Conclusions: Clostridial overgrowth is representative for children with ASD.

Topic: *AS12 DMH - Other*

ASSESSMENT OF THE LEVEL OF SELECTED BACTERIAL GROUPS IN THE FAECES OF THREE BREEDS OF SHEEP.

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Background and Aims: The microbiome in most animals plays a key role in maintaining homeostasis. Its composition can be influenced by factors such as diet, physiology and genetics. Therefore, the aim of the study was to assess the basic microbiome of three different breeds of sheep kept in the same environment

Methods: Three breeds of sheep (n = 10 for each group, the same age, sex and environment) were included in the experiment, did not show any disease symptoms. DNA extraction was from stool, then the quality of the performed DNA isolations was checked using the NanoDrop 2000. Next, qPCR analysis (Bio-Rad CFX Connect 96) at a volume of 10 µL in 3 technical repetitions (with NTC). The qPCR analysis strategy was based on the amplification of specific amplicons for the tested phyla (Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria) against the reference amplicon for all bacteria (16S rDNA). The references were universal eubacterial amplicon. The data were then processed using the CFX Maestro software and the Statistica ver. 13.1.

Results: The obtained results showed statistically significant ($P \leq 0.05$) differences in the level of the Firmicutes cluster between studies breed ($P=0.044127$). Correlation analysis was also performed, which showed its occurrence between Bacteroidetes and Firmicutes in the case of one of the studied sheep breeds (Świniarka sheep) - positive correlation ($r_s = 0.9512$).

Conclusions: The obtained results are probably related to the long period of living the animals at the station, and what is related to the possible adaptation of the microbiome to the environment. The obtained results require further analysis.

Topic: *AS12 DMH - Other*

A NOVEL IN VITRO APPROACH TO IDENTIFY MICROBIAL AND CELLULAR SIGNATURES OF SHORT BOWEL SYNDROME

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Background and Aims: Short bowel syndrome (SBS) is a severe condition characterized by a bowel length of < 200cm, due to congenital defects or surgical resection, resulting in intestinal failure and requiring parenteral nutrition. After bowel resection, spontaneous morphological changes (adaptation) occur in the mucosa, to increase the nutrient absorption, hormonal secretions and microbial modulation. Yet the exact mechanisms still remain poorly understood. Here we aim at developing an in vitro approach to identify microbial, metabolic and cellular hallmarks of SBS.

Methods: A Simulator of the Human Intestinal Microbial Ecology (SHIME), including small intestinal and colonic microbiota, was optimized to simulate an intestinal resection. Microbial composition and metabolic activities will be studied in resected and non-resected SHIME by metagenomic and metabolomic analysis. In parallel, two triple cell cultures models of intestinal epithelium are under development and being challenged with SHIME extracts, to assess the effect of resection-altered microbiota on permeability, mucus production and endocrine secretion, by immunostaining.

Results: Data analysis is still ongoing. Preliminary results indicated (I) an altered microbial metabolic activity in the colon compartment, upon resection, with an enrichment in chain elongation processes and alteration in short chain fatty acid (SCFA) profile; (II) an increase in MUC2 gene expression in colon compartment upon resection, possibly due to the altered SCFA profile.

Conclusions: Here we provide a first proof-of-concept that a SBS in vitro model is achievable and microbial and cellular alterations identifiable. In future, the microbial alterations identified will be validated against a cohort of SBS patients and potentially lead to novel therapeutic approaches.

Topic: *AS12 DMH - Other*

MICROBIOMESUPPORT: TOWARDS COORDINATED MICROBIOME R&I ACTIVITIES IN THE FOOD SYSTEM TO SUPPORT (EU AND) INTERNATIONAL BIOECONOMY GOALS

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Background and Aims: Microbiomes have crucial roles in maintaining life on Earth, and their functions drive human, animal, plant and environmental health. The microbiome research landscape is developing rapidly and is performed in many different science fields using similar concepts but mostly one (eco)system at-a-time. Thus, we are only starting to unravel and understand the interconnectedness of microbiomes across the (eco)systems.

Methods: MicrobiomeSupport is a Coordination and Support Action with the overall objective to establish an international network of experts and stakeholders in the field of microbiome food systems research and assess applicability and impact of the microbiomes on the food system.

Results: Key outcomes include - database containing information on microbiome activities, programmes and facilities along the food chain and beyond in the EU and worldwide - recommendations for an internationally agreed microbiome definition, best practices and standards, as well as consistent protocols in research - establishment of a dialogue between multiple stakeholders (i.e. representatives from science, industry, policy, funding and regulatory bodies) - publications showcasing microbiomes potential and current hurdles for their full exploitation - educational materials for the general public

Conclusions: Detailed information on the project and link to resources can fou found at www.microbiomesupport.eu or @MicrobiomeEU.

Topic: *AS12 DMH - Other*

DEVELOPMENT OF THE SMALL INTESTINAL MICROBIOTA IN AN IN VITRO GUT MODEL

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Background and Aims: To date, the small intestinal microbiota remains mainly unexplored despite its importance to human health and immune system development. Its characterization is challenging, due to invasive sampling techniques and ethical restrictions. These limitations could be resolved by use of in vitro models, mimicking both physiological and microbial environment, such as the Simulator of the Human Intestinal Ecosystem (SHIME). This is a dynamic semi-continuous model which simulates the gastrointestinal tract, optimized to study the colon microbiota. Yet, expanding the model by including also the small intestinal microbiota, would boost knowledge on functionality, dynamics and composition. Furthermore, this could serve as platform to study small intestinal diseases linked to microbial alterations, such as Crohn's ileitis.

Methods: To reach this goal, the small intestinal and colon microbial communities were established in a SHIME model by use of an antegrade colonization with salivary microbiota and a retrograde colonization with fecal microbiota. The microbial community was regularly monitored through 16S rRNA amplicon sequencing, cell counting and metabolomic quantifications.

Results: The first results show an enrichment in Streptococcus and Veillonella when inoculating the saliva, a metabolic shift to more lactate and formate compared to the colon and a clear impact of the retrograde inoculation on the community composition with introduction of Bacteroides.

Conclusions: The development of this model can break ground by its personal approach through inoculation with fecal and saliva samples. As next step, the model will be validated against patient's samples

Topic: *AS12 DMH - Other*

CORRELATION BETWEEN GUT MICROBIOTA COMPOSITION AND SHORT-CHAIN FATTY ACID (SCFA) LEVELS IN DIARRHEOGENIC ESCHERICHIA COLI INFECTIONS

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Background and Aims: Background: The gut microbiota and its metabolites are responsible for the maintenance of intestinal homeostasis and the prevention of colonization by enteric pathogens, such as diarrheagenic Escherichia coli (ECD). Short-chain fatty acids (SCFA) are the most abundant products of bacterial fermentation, and their role in the development of diarrheal disease is controversial. We determined SCFA levels and gut microbiota composition in stool samples from children under 5 years of age, with and without diarrhea, and their correlation with the presence of SCFA-producing bacteria.

Methods: Methodology: 40 diarrheal samples positive for a DEC pathotype and 43 stool samples from healthy children were analyzed. Gut microbiota composition and SCFA concentration were determined by 16S rRNA sequencing and HPLC, respectively.

Results: A distinctive composition of the gut microbiota was observed in both groups. In diarrhea samples, higher levels of acetate, propionate, and butyrate compared to samples from healthy children were found. Correlation analysis showed that acetate production is positively correlated with Blautia genus, while propionate and butyrate production is positively correlated with Coprococcus genus in children with diarrhea by ECD. Butyrate production was positively correlated with Faecalibacterium and Roseburia genera in both the ECD and control groups, and Bacteroides was negatively correlated with acetate and propionate production in the control group.

Conclusions: Conclusion. We found high levels of SCFA in stool samples from children with DEC infection compared to samples from healthy children. The SCFA levels correlate with the presence of specific genera of SCFA-producing bacteria in both groups.

Topic: *AS12 DMH - Other*

METHACRYLAMIDE-MODIFIED GELATIN AND SILK FIBROIN AS SCAFFOLD MATERIALS FOR EPITHELIAL CELLS IN AN IN VITRO MODEL FOR THE SMALL INTESTINAL ECOSYSTEM.

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Background and Aims: The small intestinal (SI) environment is a key site for host-microbiota interaction. Yet, it remains difficult to explore and study. In this context, we aim to develop an innovative in vitro model to recreate the SI microenvironment with relevant physiological parameters. We started by screening for potential biocompatible materials that can be used as a scaffold for SI epithelial cells, allowing cell attachment and mechanical support, as well as enable diffusion of nutrients and metabolites. We characterized two materials, methacrylamide-modified gelatin (gel-MOD) and electrospun silk fibroin (SF) for this aim.

Methods: Two types of SI epithelial cells (Caco-2 and LS174T) were seeded in a 90:10 ratio on gel-MOD membranes. The cell viability was then monitored over a period of 14 days by resazurin assay. Afterwards, cell morphology was examined using confocal microscopy. In addition, the diffusion of different-sized molecules (Lucifer yellow, FITC-dextran 4kDa and 10kDa) through gel-MOD and SF membranes was tested and from these results, the apparent permeability coefficients were calculated.

Results: indicated that (I) gel-MOD allowed the adhesion of the cells and the development of a confluent cell layer, preserved over 14d and (II) both gel-MOD and SF show adequate diffusion of the tested compounds through the membrane.

Conclusions: Gel-MOD and SF were identified as suitable scaffold materials. Next, we will introduce 3 essential key-players (epithelial cells, immune cells and microbial community) into an in vitro model using either gel-MOD or SF as scaffold material for epithelial cells and study the interaction by looking at cell differentiation and gene expression.

Topic: *AS12 DMH - Other*

IMPACT OF FERMENTATION SUPERNATANTS OF B-GLUCANS PLEUROTUS ERYNGII – RICH MUSHROOMS ON INTESTINAL PERMEABILITY IN AN EX-VIVO MODEL

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Background and Aims: Some studies have elucidated the beneficial effect of β-glucans containing foods, after their fermentation, on an impaired gut barrier. However, the effect of these glucans from Pleurotus mushrooms origin has not been fully explored. The aim of the study was to investigate the ability of P. eryngii (PEWS) fermentation products (FS) to counteract induced intestinal hyperpermeability in human colonic tissues in an ex vivo system.

Methods: Fresh colonic biopsies from healthy adult subjects were stimulated with FS-PEWS in Ussing chambers, after inducing increased gut permeability with sodium deoxycholate (SDC). Paracellular and transcellular permeability were measured by quantifying FITC-dextran passage and horseradish peroxidase (HRP) passage, respectively. Based on the normality of the data, Students' t-test for paired comparisons or Wilcoxon matched-pairs signed rank test was applied.

Results: Our results indicate that the administration of FS-PEWS did not affect the paracellular and transcellular permeability. Biopsies stimulated with SDC only exhibited increased transcellular ($p=0.012$) and paracellular ($p=0.005$) permeability. Supplementation of FS-PEWS managed to maintain transcellular permeability similar to that of the unchallenged colon specimens. The most remarkable difference was observed in the preservative effect on transcellular permeability of the pre-incubation of colon tissues with FS-PEWS followed by stimulation with SDC compared to SDC ($p= 0.020$). However, the same treatment compared to SDC didn't reach statistical significance when testing paracellular permeability.

Conclusions: In conclusion, our data displayed the potential protective effects P. eryngii mushrooms on intestinal barrier integrity, specifically on transcellular permeability fueling further efforts to elucidate their manifold role, prebiotic included, in human health.

Topic: *AS12 DMH - Other*

CURRENT MICROPLASTIC POLLUTION LEVELS IMPACT WILDLIFE GUT MICROBIOMES

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Background and Aims: Microplastics pollute environments across the globe and are ingested by countless species from different trophic levels, but corresponding effects on organism health are unclear. A key dimension of health that may be affected is the microbiome, which describes the symbiotic relationship between microorganisms and hosts that harbor them. Gut microbiota are essential for host digestion, access to nutrients, immune function, and interactions with the nervous system. Thus, disruptions to gut microbiomes have been implicated in several diseases. Here, we tested the hypothesis that microplastics are associated with changes in the microbiome, and that these changes occur in different microbiomes throughout the GIT and in different species.

Methods: We sequenced the V4 region of the 16S rRNA gene of the microbiota swabbed from the proventriculus and cloaca of 85 individuals belonging to two seabird species that chronically ingest microplastics, northern fulmars (Fulmarus glacialis) and Cory's shearwaters (Calonectris borealis), for which we also collected (micro)plastic debris from their GIT.

Results: Our results show that microplastics affect the proventricular and cloacal microbiomes in both species, and that changes in alpha diversity are microbiome location- but not species-specific, whilst changes to beta diversity are microbiome location- and species-specific. An increase in microplastics was associated with a decrease in abundance of resident microbiota associated with healthy hosts and an increase in pathogens, antibiotic resistant bacteria, plastic-degrading microbes, and bacteria which have been proposed to be zoonotic pathogens.

Conclusions: These results illustrate for the first time that microplastics at existing, environmentally-relevant concentrations impact the gut microbiomes in wild seabirds.

Topic: *AS12 DMH - Other*

USING FECAL IMMUNOCHEMICAL TUBES FOR THE ANALYSIS OF GUT MICROBIOME HAS POTENTIAL TO IMPROVE COLORECTAL CANCER SCREENING

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Background and Aims: Colorectal cancer (CRC) is an important and challenging public health problem whose successful treatment depends on the early detection of the disease. Recently, colorectal cancer-specific microbiome signatures have been proposed as an additional marker for CRC detection. A desirable aim would be the possibility to analyze the microbiome from the fecal samples collected during CRC screening programs into FIT tubes for fecal occult blood testing.

Methods: We investigated the impact of Fecal Immunohistochemical Test (FIT) and stabilization buffer (DNA/RNA Shield) on the microbial community structure in stool samples from 30 volunteers and compared their communities to fresh-frozen samples highlighting also the previously published cancer-specific communities. Altogether 214 samples were analyzed including positive and negative controls using 16S rRNA gene sequencing.

Results: The variation between individuals is greater than the differences introduced by the collection strategy. The vast majority of the genera are stable up to 7 days. None of the changes observed between fresh frozen samples and FIT tubes are related to previously shown colorectal cancer-specific bacteria.

Conclusions: Overall, our results show that FIT tubes can be used for profiling the gut microbiota in colorectal cancer screening programs as the community is similar to fresh frozen samples and stable at least for 7 days. Sample material from FIT tubes could be used in addition to fecal immunochemical tests for future investigations into the role of gut microbiota in colorectal cancer screening programs circumventing the need to collect additional samples and possibly improving the sensitivity of FIT.

Topic: *AS12 DMH - Other*

GUT METAGENOME ASSOCIATIONS WITH EXTENSIVE DIGITAL HEALTH DATA IN A VOLUNTEER-BASED ESTONIAN MICROBIOME COHORT

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Background and Aims: Microbiome research is starting to move beyond the exploratory phase towards interventional trials and therefore well-characterized cohorts will be instrumental for generating hypothesis and providing new knowledge.

Methods: As part of the Estonian Biobank (EstBB), we established the Estonian Microbiome Cohort (EstMB) which includes stool, oral and plasma samples from 2,509 participants and is supplemented with multi-omic measurements, questionnaires, and regular linkages to national electronic health records (EHRs). In this study, we analyzed stool data from deep metagenomic sequencing together with rich phenotyping, including 71 diseases, 136 medications, 21 dietary questions, 5 procedures, and 19 other factors.

Results: The data revealed numerous relationships (n = 3262) with different microbiome features. Additionally, we present that long-term antibiotic usage, independent from recent administration, has a significant impact on the microbiome composition, partly explaining the common associations between various diseases.

Conclusions: This study extends the understanding of microbiome–host interactions and facilitates the development of microbiome-related studies.

Topic: *AS12 DMH - Other*

BACTEROIDES FRAGILIS PRODUCES THREE HMUY HOMOLOGS BUT WITH DISTINCT PROPERTIES

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Background and Aims: Porphyromonas gingivalis (Bacteroidetes phylum), considered as the main etiologic agent of chronic periodontitis, requires heme and iron for growth and proliferation. To acquire these nutrients, the bacterium uses heme uptake system encoded by a hmu operon composed of six genes. Among them is a hmuY gene encoding a hemophore-like protein. Heme acquisition systems based on the HmuY protein are characteristic not only for P. gingivalis. Other member of Bacteroidetes, Bacteroides fragilis, expresses three HmuY homologs termed BfrA, BfrB, and BfrC. The aim of this study was to perform structure-function analysis to characterize the proteins in regard to three-dimensional structure, heme binding, and heme sequestration from host hemoproteins.

Methods: Proteins were overexpressed in Escherichia coli and purified using affinity and size exclusion chromatography. Heme binding and sequestration experiments were carried out using UV-vis spectroscopy. Three-dimensional structure of Bfr proteins was examined using crystallography.

Results: B. fragilis BfrA and BfrB bind heme and protoporphyrin IX but in a manner different to P. gingivalis HmuY. BfrC is not able to bind these ligands. BfrA is able to sequester heme from human serum albumin and hemopexin under reducing conditions only. Crystallization analysis demonstrated three-dimension structures similar to HmuY, with differences in the heme-binding pocket.

Conclusions: Our data shed more light on molecular mechanism of heme uptake by B. fragilis, and enabled characterization of new members of HmuY family of proteins.

Topic: *AS12 DMH - Other*

EVALUATION OF PROBIOTIC PROPERTIES OF BACTERIA REPRESENTATIVE OF THE MICROBIOTA OF COLOMBIAN DOUBLE CREAM CHEESE

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Background and Aims: Double Cream Cheese (DCC) is a traditional Colombian product, made through artisanal practices and is usually manufactured with a mixture of fresh and acidified cow milk. This mixture has a native microbiota, mostly species belonging to Lactic Acid Bacteria (LAB), including Lactococcus, Streptococcus, Lactobacillus, Pediococcus and Leuconostoc. They could be potential probiotics that exert a health benefit to the consumer. The aim of this work was to evaluate the probiotic properties of LAB isolated from Colombian traditional DCC.

Methods: Three LAB strains (Pediococcus pentosaceus, Weissella viridescens, Lactobacillus casei) were evaluated for probiotic properties including resistance to pH and bile salts conditions, exopolysaccharide production, antimicrobial activity against pathogens, autoaggregation, cell surface hydrophobicity, sensibility to antibiotics and adhesion ability to human cell line SW620.

Results: All bacterial strains were not hemolytic and catalase negative. The isolates were able to survive under acidic pH and bile salts conditions. In addition, these strains inhibited the growth of E. coli ATCC25922, S. typhimurium ATCC14028 and S. aureus ATC25923. Atypical antibiotic resistances were not observed, except for chloramphenicol resistance in W. viridescens. They also exhibited autoaggregation and cell surface hydrophobicity with two solvents with percentages ranging from 22% up to 53%. The three strains efficiently adhered to the SW620 cells with similar and higher values than the control strain Lb. rhamnosus GG.

Conclusions: According to the in vitro results, all strains can be proposed as potential probiotics. Besides, the isolated strains from indigenous Colombian foods like DCC could be used to develop a novel probiotic product or functional food.

Topic: *AS12 DMH - Other*

ROLE OF THE GUT MICROBIOME IN GASTROINTESTINAL PROBLEMS OF PATIENTS WITH MYOTONIC DYSTROPHY

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Background and Aims: Myotonic dystrophy (MD), an autosomal dominant genetic disorder, is a disease that is found in high numbers in some regions of Québec. In type 1 of this disorder, there is an unstable trinucleotide repeat expansion containing CTG, located in the 3' untranslated region of DMPK, the gene encoding the DM protein kinase on chromosome 19q13.3. The core pathogenic feature of MD1 is the intra-nuclear blockage of RNA-binding proteins with the toxic RNA repeat, resulted in a wide array of nonfunctional proteins. Although MD1 is primarily characterized by progressive muscular weakness, multisystem involvement from cognitive deficits, cardiac conduction abnormalities, diabetes and cataracts, endocrine, and reproductive problems is often present. Furthermore, involvement of the gastrointestinal (GI) tract is frequent and may happen at any level from the pharynx to the anal sphincter. However, it is not clear if the mentioned symptoms for GI are caused by biomechanical problems of the intestine due to the genetic predisposition or if the intestinal microbiome is involved. Therefore, in this research work, we assessed the role of the gut microbiome in the gastrointestinal problems of MD1 patients.

Methods: For this purpose, 16 stools samples from MD1 patients and their close family members as control were collected. These samples were sequenced by MiSeq and analyzed by DADA2 to generate taxonomic signatures

Results: Our analysis indicated that the status of MD1 significantly changes the bacterial structure of the gut microbiome.

Conclusions: Top bacterial taxa with significant differences between control groups and MD1 patients were Firmicutes, Bacteroidota, and Actinobacteriota Phylum.

Topic: *AS12 DMH - Other*

DETERMINATION OF ANTIBIOTIC RESISTANCE OF BACTERIA REPRESENTATIVE OF THE MICROBIOTA OF DOUBLE CREAM CHEESE

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Background and Aims: Antibiotic resistance is a global public health problem. Indiscriminate antibiotic use is a major contributor in the introduction of selective pressures in our natural environments. Lactic acid bacteria (LAB) are deliberately introduced into the food chain as starter cultures and due to their health benefits on the host also as probiotics. Multiple studies have confirmed the beneficial effects of probiotic use in the health of both livestock and humans. Concerns have been raised in the use of some probiotics strains that carry antibiotic resistance genes themselves, as they have the potential to pass the antibiotic resistance genes to pathogenic bacteria through horizontal gene transfer. Then it is important to select probiotics that do not contribute to the spread of antibiotic resistance and not carry transferable antibiotic resistance. The aim of this work was to evaluate the resistance of strains of LAB representative of the microbiota of Double Cream Cheese (DCC) to antibiotics.

Methods: The minimum inhibitory concentration (MIC) of six antibiotics (gentamycin, penicillin, vancomycin, chloramphenicol, tetracycline, and ampicillin) to twelve LAB strains was determinated by micro dilution broth method according to EFSA guidance.

Results: None of the strains were resistant to penicillin, tetracycline, and ampicillin; some strains were resistance to gentamycin and vancomycin (Leuconostoc spp. and Enterococcus spp.), Leuconostoc spp., Weissella spp. and Lactobacillus spp. presented resistance to chloramphenicol.

Conclusions: The LAB representative of the microbiota of DCC showed resistance to some antibiotics so it is necessary to determine if the resistance is intrinsic or acquired before being used as probiotics.

Topic: *AS12 DMH - Other*

ADHESION ABILITY OF LACTIC ACID BACTERIA REPRESENTING OF THE MICROBIOTA OF DOUBLE CREAM CHEESE TO HUMAN INTESTINAL CELL LINE

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Background and Aims: Double Cream Cheese (DCC) is a traditional Colombian product made in the regions of Ubaté and Chiquinquirá valleys. DCC microbiota is composed of Lactic Acid Bacteria (LAB), including the genera Lactococcus, Streptococcus, Lactobacillus, Pediococcus and Leuconostoc, some of which are recognized by their probiotic properties. Adhesion to intestinal epithelial cells is one of the most important parameters for probiotic evaluation because it increases their residency time in the intestine allowing them to exert their beneficial effects and interact with the immune system. The aim of this work was to evaluate the adhesion of three LAB isolated from DCC to the human cell line SW480, as well as to determine the effect of probiotic inoculum to the adhesion.

Methods: For this, 3 cell inocula were evaluated to determine with which of them a confluent monolayer could be obtained and finally adhesion was evaluated comparing the contents of the CFU/mL after contact with the bacteria on MRS agar when using 5 different bacterial inocula.

Results: By using an initial inoculum of 500,000 cells/mL and culturing them under standard conditions, a confluent monolayer that simulates the intestinal epithelium was obtained. All bacterial strains adhered to the cell line with adhesion percentages that varied between strain and according to the bacterial inoculum found added.

Conclusions: In conclusion, the three strains were able to adhere to the cell line, indicating that they could be potential candidates of probiotics.

Topic: *AS13 Oral - The origin and development of the oral microbiome*

THE IMPACT OF OROFACIAL CLEFTS ON THE MATURATION OF THE ORAL MICROBIOME WITHIN THE FIRST WEEKS OF LIFE

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Background and Aims: Cleft lip and cleft palate (CLP) either manifest as unilateral (UCLP), bilateral (BCLP) or as isolated cleft palate (CPo)/ cleft lip (CLo) and present a potential risk factor for dysbiosis of oral microbiota. Postnatal development of oral microbiota seems to play a pivotal role in health and disease. However, the intricacy of the oral microbiome in CLP neonates has not yet been studied using 16S rDNA sequencing.

Methods: In order to investigate the impact of CLP, smear samples from 15 neonates with CLP (UCLP=5, BCLP=4, CPO=5, CLO=1) and 20 neonates without CLP were collected from two oral metaniches (tongue, cheek). To investigate early alterations of oral microbiota, samples were collected at two time points, i.e. at first consultation after birth (T0: Ø4.8d CLP group; Ø2.4d control group) and 4-5 weeks later (T1: Ø32d CLP group; Ø31d control group). Subsequently, the samples were processed and analyzed using next-generation sequencing.

Results: We detected a trend towards higher alpha diversity from T0 to T1. Significant differences in beta diversity were seen comparing CLP vs. control (regardless of site and time point), time points (T0 vs.T1) and group/time point combinations. Interestingly, ordination analysis showed a cluster for the CLP group at T1 separated from the other categorical groups without metaniche-associated characteristics.

Conclusions: Our findings revealed differences in beta diversity between neonates with CLP compared to controls after birth and within the first weeks of life with a distinct microbial clustering after five weeks which demonstrates an impact of orofacial clefts in the maturation process of oral microbiota.

Topic: *AS13 Oral - The origin and development of the oral microbiome*

CONTROLLED HUMAN INFECTION WITH NEISSERIA LACTAMICA IN LATE PREGNANCY TO MEASURE HORIZONTAL TRANSMISSION AND MICROBIOME CHANGES IN MOTHER-NEONATE PAIRS: A SINGLE-ARM INTERVENTIONAL PILOT STUDY

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Background and Aims: Neonatal upper respiratory microbiota are derived at least in part from the maternal respiratory tract, and certain microbiota are associated with altered risk of childhood infections and asthma. This raises the question of whether altering maternal upper respiratory microbiota by controlled bacterial inoculation may offer a means of favourably altering neonatal microbiota. Neisseria lactamica is a common and harmless pharyngeal commensal in young children, and is associated with reduced carriage and invasive disease by N. meningitidis. Nasal inoculation with N. lactamica in over 400 healthy adults has been shown to safely and reproducibly reduce N. meningitidis carriage. We present a protocol for nasal inoculation of pregnant women with N. lactamica, to establish if neonatal pharyngeal colonisation occurs after birth, and to characterise microbiome evolution in mother-infant pairs.

Methods: 20 healthy pregnant women will receive nasal inoculation with 10^5 colony-forming units N. lactamica (strain Y92-1009) at 36-38 weeks gestation. Nasopharyngeal, oropharyngeal and saliva samples, as well as optional breastmilk, umbilical cord blood and infant venous blood, will be collected from mother-infant pairs over one month post-partum. We will assess safety, N. lactamica colonisation (by targeted polymerase chain reaction) and longitudinal microevolution (by whole genome sequencing), and microbiome evolution (by 16S rRNA gene sequencing).

Results: This study has received local and national ethical approval. Recruitment will begin in October 2021, and we expect primary outcome data by April 2022.

Conclusions: This proof-of-concept human challenge study may offer a valuable model for conducting interventional microbiome research in mother-infant pairs.

Topic: *AS15 Oral - Oral microbiome and oral diseases*

A CASE STUDY OF SALIVARY MICROBIOME IN SMOKERS AND NON-SMOKERS IN HUNGARY: ANALYSIS BY SHOTGUN METAGENOME SEQUENCING

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Background and Aims: To investigate the role of cigarette smoking in disease-development through altering the composition of the oral microbial community. Periodontitis and oral cancer are highly prevalent in Hungary; therefore, the salivary microbiome of smoker and non-smoker Hungarian adults was characterized.

Methods: Shotgun metagenome sequencing of salivary DNA samples from 22 individuals (11 non-smokers and 11 current smokers) was performed using the Ion Torrent PGM™ platform. Quality-filtered reads were analysed by both alignment-based sequence similarity searches and genome-centric binning.

Results: Prevotella, Veillonella and Streptococcus were the predominant genera in the saliva of both groups. Although the overall composition and diversity of the microbiota were similar, Prevotella was significantly more abundant in salivary samples of current smokers compared to non-smokers. Members of the genus Prevotella were implicated in the development of inflammatory diseases and oral cancer. The abundance of the genus Megasphaera also increased in current smokers, whereas the genera Neisseria, Oribacterium, Capnocytophaga and Porphyromonas were significantly reduced. The data generated by read-based taxonomic classification and genome-centric binning mutually validated the two distinct metagenomic approaches.

Conclusions: Smoking-associated dysbiosis of the salivary microbiome in current cigarette smokers, especially increased abundance of Prevotella and Megasphaera genera, may facilitate disease development.

Topic: *AS15 Oral - Oral microbiome and oral diseases*

ORAL MICROBIOTA COMPOSITION IN EDENTULOUS INFANTS WITH A HISTORY OF ORAL CANDIDIASIS – A PILOT STUDY

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Background and Aims: Oral candidiasis (OC) is a common condition in infants. It may negatively affect feeding practices by causing pain in the affected infant and breast infection of the breastfeeding mother. As research on the influence of OC on infant oral microbiota development is scarce, this pilot was executed.

Methods: Sixteen edentulous infants (2-9 months old) participated in this cross-sectional study. The tongue dorsum, buccal mucosa, and saliva were sampled. Pacifier use, reflux and OC in the past three months were questioned. Microbial composition was assessed by 16S-rRNA-gene amplicon sequencing (Illumina). Microbiota profiles were analysed using Principal Component Analysis and PERMutational ANalysis Of VAriance. Relative abundances at genus level were calculated and tested using Mann-Whitney-U test.

Results: Of the 16 infants, 3 had experienced OC. Microbial composition of the tongue ($F=2.47$, $p=0.03$) and buccal mucosa ($F=1.98$, $p=0.03$), but not of saliva ($F=1.67$, $p=0.06$) differed significantly between infants who experienced OC and healthy infants. Infants who experienced OC had significantly lower Shannon diversity on the tongue ($p=0.014$) but not on buccal mucosa ($p=0.11$) and in saliva ($p=0.36$). The relative abundance of Haemophilus, Prevotella, Gemella, and Porphyromonas, but not Streptococcus ($p=0.16$), on the tongue was significantly lower ($p<0.039$) in infants who experienced OC.

Conclusions: Differences in oral microbiota in infants with a history of OC compared to healthy infants remained present after clinical presentation of OC, especially on the tongue. Large-scale longitudinal research including mycobiome assessment is necessary to confirm our results and to clarify the long term influence of OC on oral microbiota development in infants.

Topic: *AS15 Oral - Oral microbiome and oral diseases*

SALIVARY AND SUBGINGIVAL ENDOTOXIN ACTIVITIES AS REAL-TIME BIOMARKERS FOR PERSONALISED PERIODONTAL CARE

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Background and Aims: The objectives of this study were to analyse salivary and subgingival lipopolysaccharide profiles and its endotoxin activities in periodontal health and disease, to compare these with clinical parameters and to evaluate the use of the recombinant factor C assay as a novel, LPS-based biosensor for personalised, point-of-care periodontal therapy.

Methods: Saliva and subgingival plaque samples were collected from healthy individuals and periodontitis patients before and after non-surgical periodontal therapy. LPS was extracted by the phenol-water method and the chemical composition of lipid A moieties was determined by ESI-Mass Spectrometry. Endotoxin activity of LPS extracts was assessed using the recombinant factor C assay, and their inflammatory potential was examined in THP-1-derived macrophages by measuring TNF-α and IL-8 production.

Results: Characteristic lipid A molecular signatures, corresponding to over-acylated, bi-phosphorylated lipid A isoforms, were observed in diseased samples. Healthy and post-treatment subgingival samples were characterized by lower m/z peaks, related to under-acylated, hypo-phosphorylated lipid A structures. Endotoxin activity levels and inflammatory potentials of subgingival LPS extracts from periodontitis patients were significantly higher compared to healthy and post-treatment samples. Interestingly, salivary LPS architecture, endotoxin activity and inflammatory potential did not change after periodontal therapy and showed similarities to diseased samples.

Conclusions: This is the first study to consider structure-function-clinical implications of different lipid A isoforms present in the saliva and subgingival plaque and it sheds new light on molecular pathogenic mechanisms of oral microbial communities. Salivary and subgingival endotoxin activity could be a reliable, bacterially derived biomarker and a risk assessment tool for personalised periodontal care.

Topic: *AS16 Oral - Oral microbiome and implications on pregnancy*

THE CHARACTERISTIC OF ORAL MICROBIOME IN PRETERM DELIVERY

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Background and Aims: Preterm delivery (PTD) is one of the leading causes of perinatal morbidity and mortality and can cause lifelong health problems. Despite the reported link between the oral microbiome, periodontitis (PD) and PTD, studies so far have not been conclusive. We performed an independent case-control study carried out on the Slovenian population, where we re-evaluated the characteristics of the oral microbiome in PTD.

Methods: We obtained oral swab samples from 152 Caucasian women who delivered at either term (≥38 0/7 weeks, n = 91) or preterm (≤36 6/7 weeks, n = 61), of which some delivered extremely preterm (≤27 6/7 weeks, n = 15). Total bacterial DNA was isolated from the swabs and 16S ribosomal RNA (rRNA) gene sequencing of the V3-V4 region was performed on the MiSeq Illumina platform to determine the composition of oral microbiome. Microbiome composition was compared between the preterm and term, as well as the extremely preterm and term delivery groups.

Results: Our analyses identified the presence of >10.000 OTUs showing high diversity of the oral microbiome in pregnant women. Top identified taxa were concordant with previously reported oral microbiome compositions. Whereas there was no significant difference in abundance of taxa when comparing the preterm with term delivery group, the extremely preterm group showed decreased abundance of Proteobacteria phylum compared to the full-term group.

Conclusions: Compared with term deliveries, oral microbiome in extremely preterm deliveries is changed, which may be due to a small number of subjects. Further research is needed.

Topic: *AS17 Oral - Oral microbiome and systemic health*

DELETERIOUS IMPACT OF PORPHYROMONAS GINGIVALIS PERIODONTAL BACTERIA AND ITS LPS ON INFLAMMATORY AND METABOLIC MARKERS IN ADIPOCYTES

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Background and Aims: A link between obesity, type 2 diabetes and periodontitis has been reported. Porphyromonas gingivalis, a major periodontal bacteria, and its LPS endotoxins may translocate into the bloodstream, reach the adipose tissue and exacerbate inflammation and oxidative stress, promoting insulin resistance and diabetic status. This study aimed to evaluate the effect of P. gingivalis bacteria or its LPS on inflammatory and metabolic markers in mature adipocytes.

Methods: Murine 3T3-L1 differentiated adipocytes were exposed to P. gingivalis whole bacteria or its purified LPS for 3-48h. Then, the production of inflammatory and metabolic markers was determined by RT-qPCR, western-blot, ELISA and colorimetric assays.

Results: P. gingivalis bacteria and LPS induced a pro-inflammatory response in adipocytes, by up-regulating TLR2/TLR4-NFκB pathway as well as COX-2, iNOS, IL-6 and MCP-1 production. Bacteria and LPS also caused oxidative stress by increasing intracellular ROS levels and deregulating time-dependently the expression of genes encoding redox factors such as NOX2, Cu/ZnSOD, MnSOD, catalase and Nrf2. In parallel, bacteria and LPS altered the production of the adipogenic mediators C/EBPα, PPARγ and FAS, and insulin-mediated lipid droplet accumulation in adipocytes. Of note, P. gingivalis bacteria exerted a more detrimental effect than that of LPS on inflammatory and metabolic markers.

Conclusions: This study demonstrates the deleterious impact of periodontal bacteria on the inflammatory and metabolic response of adipose cells. It will be needed to evaluate the link between periodontal bacteria-induced inflammation and lipid metabolism alteration during obesity and related insulin resistance.

Topic: *AS18 Oral - Other*

AN INVESTIGATION INTO THE EFFECTS OF FREEZING ON THE MICROBIOME OF HUMAN CADAVERS

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Background and Aims: The potential forensic applications of human microbiome studies have grown exponentially, particularly with regards to estimating postmortem interval based on microbial shifts during decomposition. These studies at human taphonomy facilities often use previously frozen cadavers, which are thawed before the start of any experiment. However, the effects of freezing and thawing on the process of decomposition and long-term development of the (thanato)microbiome remain largely unexplored. Two studies involving animal and human cadavers have shown temperature related changes to the microbiome. Hence, understanding how freezing human cadavers may affect the microbiome is crucial, as these studies suggest that it could potentially skew experimental results and interpretation.

Methods: For this project, we collected swab samples from five anatomical locations of nine cadavers (oral, rectum, hand, foot, and neck). Swabs were collected from the cadavers prior to freezing at –20 °C, and after freezing and thawing. Analysis of the samples involves DNA extraction using Qiagen's PowerSoil Pro kit. Microbial species identification will be carried out using DNA metabarcoding, targeting the 16S rRNA gene.

Results: Processing of these samples is currently ongoing; however, results will be ready to be presented to the audience before the conference.

Conclusions: The results from this study will be important in relation to standard operating protocols and sampling procedures at human taphonomy facilities worldwide and will provide insight into the validity of using previously frozen cadavers in microbiome studies. Finally, the results may provide useful information on the abundance and composition of microbiota for forensic case investigations involving frozen bodies.

Topic: *AS18 Oral - Other*

EFFECT OF SOUR CHERRY ANTHOCYANINS ON HEALTHY HUMAN ORAL MICROFLORA (A PILOT CLINICAL STUDY)

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Background and Aims: The chewing gums can be improved by addition of natural active materials like anthocyanins of sour cherry, which can have bactericidal actions against caries causing oral bacteria.

Methods: 20 healthy people were put into two age group: young adult (20-35) and adult (35-45). We investigated the effects of sour cherry containing chewing gum usage (around 2 weeks, daily 3 times) on the human oral microflora by collection of saliva samples. After one week control period a scaling was made, then 10 patients changed their toothbrush (Br), another 10 did not (no_Br). 16S rRNA sequencing was started with DNA isolation by Inhibitor Removal Technology. Sequencing was done with IlluminaMiSeqSystem. Taxonomic classification was based on the Human Oral Microbiome Database (HOMD, http://homd.org/). Alpha and beta diversity analyses were measured in the QIIME 2 pipeline. The effects of the toothbrush change shown on a heat-tree was constructed with Metacoder R package. Changes of the most common family and genera were shown on bar chart.

Results: The effect of scaling and administration of sour cherry containing chewing gum led to a more diverse microflora almost remaining during the whole experimental period. Weighted unifrac analysis showed clustering of the groups (Br and no_Br).

Conclusions: Sour cherry chewing gum usage has beneficial effects on the human oral microflora by the addition of natural active ingredients supplemented by the physical action of chewing and salivary flow.

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Topic: *AS18 Oral - Other*

SARS COV-2 ASYMPTOMATIC INFECTION IN HISPANIC PREGNANT WOMEN

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Background and Aims: We studied SARS-Cov2 positivity in pregnant Hispanic women. Hispanics are more likely to contact the virus and have serious consequences

Methods: We measured antibodies to SARS-Cov2 in women, using an investigator developed antibody ELISA. These women were enrolled at their first or second prenatal visit and followed up several times in their pregnancy. A cytokine multiplex panel was used to determine concentration of IFN- γ, IL2, IL10, Il4, IL6, IL17, and TNF alpha. Stool samples were collected in Omnigene kits and brought to the lab, where DNA was extracted and the V4 region of the 16SrRNA gene was sequenced on the MiSeq. We also collected demographic information and a symptom questionnaire.

Results: We measured 149 women at their first prenatal visit, and then followed them up until birth. Fifty-five women were positive for COVID-19 (37%). Three additional women seroconverted during the study. Only one woman reported being previously diagnosed with COVID-19. Positive women were mildly symptomatic or asymptomatic. A Mann-Whitney U analysis of cytokines found that IL-10 was significantly lower in the COVID positive group ($Z=-2.12$, $P=0.03$). Microbiome is currently under analysis, but the first plate showed a surprising lack of Coprococcus. COVID positive women were more likely to give birth preterm (<37 weeks gestation) $\chi 2$ (3, 168)=7.7, p=.05.)

Conclusions: Hispanic pregnant women have a high rate of asymptomatic COVID-19 infection. Our preliminary data suggest that asymptomatic infection may be associated with immune and microbial changes and risk of preterm birth.

Topic: *AS18 Oral - Other*

IS ORAL MICROBIOTA LINKED TO TASTE PERCEPTION IN HUMAN?

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Background and Aims: Eating behaviour is a key determinant of human health and inappropriate behaviours can be at the origin of some of the major pathologies affecting the modern societies (obesity, cardiovascular diseases, diabetes). Among the biological factors known to influence eating behaviour, sensory perception (including the sense of taste) plays an important role. Taste perception varies strongly between individuals but the factors at the origin of this variability are not fully understood. For example, different events occurring at the vicinity of the taste receptors on the tongue could modulate taste perception. Our group has recently suggested that the microbiota at the surface of the tongue could be involved by controlling the taste compounds concentration in the lingual film (the biological material covering the tongue). The aim of this work is to evaluate the contribution of the oral microbiota in taste.

Methods: To do this, taste sensitivity (5 basic tastes) was determined in 100 healthy adult subjects and the microbiota of their lingual film and saliva was characterized using quantitative metagenomics.

Results: The first results show associations between bacterial species and taste sensitivity and highlight the importance of the location of microbiota in these associations.

Conclusions: This work opens new perspectives on the implication of the oral microbiota physiological functions occurring in the oral cavity.