Nestlé Research on Human Milk Oligosaccharides: Latest Update

Abstracts accepted at WCPGHAN Congress 2020
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Objectives and Study:
A growing body of research, primarily through animal studies, has suggested that specific human milk oligosaccharides (HMOs), such as 2'-Fucosyllactose (2'FL), 3'-Sialyllactose (3'SL) and 6'-Sialyllactose (6'SL), may have a positive effect on neurodevelopment and cognitive ability. By leveraging the UNC/UMN Baby Connectome Project (BCP), an accelerated longitudinal cohort study designed to quantitatively map brain functional and structural development in typically developing children 0-5 years of age, and the BCP-Enriched study, an ancillary study with expanded scopes to explore nutritional impacts on early brain development, we aimed to determine whether correlations exist between specific HMOs and cognitive development in early childhood.

Methods:
A group of healthy children (n=99) in several sequential longitudinal and cross-sectional cohorts who were breastfed at the time of study visits (mean age = 9.88 months, ranging 2.9 – 24.3 months) were included in this analysis. The Mullen Scales of Early Learning (MSEL) was administered to assess the child’s cognitive development, with data points available for each month within the age range. The concurrent breast milk samples (n=191) were obtained and analyzed for specific HMOs including 2'FL, 3'FL, 3'SL, 6'SL, Lacto-N-tetraose (LNT), Lacto-N-neotetraose (LNNT), Lacto-N-fucopentaose I (LNFPI), and A-tetrasaccharide (A-Tetra). To assess the potential associations between HMOs and MSEL scores, age effects of all HMOs were removed first using spline regression, and then a log-transformation was applied for 3'SL to minimize heteroskedasticity and satisfy the linear assumption of a linear model. The associations between age-adjusted HMOs and concurrently collected age-adjusted MSEL (as the response variable) was tested using a random linear mixed effects model with both the child IDs and examiner IDs as random effects. The potential batch and study site effects were also controlled.

Results:
HMOs were quantified in all breast milk samples, except for A-Tetra which was undetectable in 129 samples from 68 mothers, and 44 samples from 19 subjects with 2'FL <11 mg/L. Specific HMOs (3'FL and 3'SL) positively and others (2'FL, LNT, LNNT, 6'SL and LNFPI) negatively associated with age. More importantly, age-adjusted 3'SL was positively associated with Receptive (partial correlation coefficient r=0.28, p<0.03) and Expressive (r=0.399, p<0.005) language t-scores in the subset with detectable A-Tetra. No correlation between age-adjusted 3'SL and any of the MSEL subscale scores was detected in the subset of undetectable A-Tetra. No other associations were detected between age-adjusted HMOs and MSEL scores.

Conclusion:
To the best of our knowledge, this is the first study reporting positive associations between sialylated HMOs and language development in early childhood. The results also indicated that interactions between specific HMOs may influence their effect on early cognitive development. Further research into the underlying biological mechanisms is warranted.
Objectives and Study:
Modulation of the gut microbiome via prebiotics or probiotics can improve memory or reduce anxiety. The mechanisms by which pre- and probiotics act are largely undiscovered. We have shown that oligosaccharides impact recognition memory in both an oligosaccharide- and memory type-specific manner. Herein, a sub-groups mediation analysis was performed to identify variables mediating the relationship between colonic bacterial genera and recognition memory in context of oligosaccharide intake in young pigs.

Methods:
Male pigs (n=70) were artificially reared from postnatal days 2-33 and provided milk replacers analyzed to contain: 0 g/L oligosaccharides (control [CON]); 5.8 g/L bovine milk oligosaccharides (BMOS); 1.2 g/L human milk oligosaccharides (HMO, 0.8 g/L of 2’fucosyllactose [2’FL] + 0.4 g/L of Lacto-N-neotetraose [LNnT]); BMOS and HMO (5.8 g/L BMOS + 1.0 g/L 2’FL + 0.5 g/L LNnT); 3.6 g/L oligofructose (OF); or 3.4 g/L OF + 1.1 g/L 2’FL (OF + 2’FL). All groups underwent magnetic resonance imaging (MRI) and the novel object recognition task, mRNA expression from hippocampus were analyzed, and colonic samples were collected for 16S sequencing. Using R, Ordinary Least Squares regression coefficients were constructed using the “stats” package. Mediation analyses were conducted with the package “Mediation”, with a bootstrap sample size of 2000 with 95% confidence interval estimates constructed using the percentile method. Predictor variables included microbial genera from colonic and fecal samples. Mediating variables included gene expression, MRI, and behavioral variables. The predicted variable was the recognition index from either a short- or a long-delay. All mediations were performed on each diet group by using a sub-group analysis.

Results:
Numerous bacterial genera in both the colon and feces were related to short- and/or long-term memory. Mediating variables frequently included GABA- and glutamatergic hippocampal gene expression. Other mediating variables included genes related to myelination, transcription factors, brain volume, and exploratory behavior. Importantly, these mediating variables differed by diets, with pigs fed HMO demonstrating a significant number of glutamatergic genes as mediators of short-term memory, and myelination related genes mediated long-term memory in pigs fed BMOS.

Conclusion:
Mediation analysis identified multiple pathways between the gut and brain, with a focus on genes related to excitatory/inhibitory neurotransmission. Emerging research suggests that multiple neurotransmitter pathways regulate the relationship between the gut microbiome and brain development, this research highlights the specificity of these systems to the type of oligosaccharide consumed in early life.
Objectives and Study:
Human milk oligosaccharides (HMOs) are a key component of breast milk and have been proposed to be one of the mediators of breast feeding beneficial effects on neurodevelopment and associated adult brain functions. Among the different types of HMOs, the sialylated HMOs are suggested to play a key role in brain development and functions. There is however a lack of understanding of the mechanisms of action for this long-term programming effects of sialylated HMOs. Here, we used a dysfunctional mutant of both beta-galactoside alpha-2,6-sialyltransferase 1 (St6gal1) and beta-galactoside alpha-2,3-sialyltransferase 4 (St3gal4) genes that results in the absence of 6’sialyllactose (6’SIL) and a reduction of about 80% of 3’sialyllactose (3’SIL) in dams milk respectively. To distinguish between the effects due to the HMOs deficiency in milk and those due to the mutations we used a full cross-fostering design and evaluated the adult mice in behavioural tests assessing attention and mnesic functions.

Methods:
At birth, we performed a full cross-fostering procedure involving C57BL/6J wild-type mice and double KO mice for St6gal1 (B6.129-St6gal1tm2Jxm/J) and St3gal4 (B6.129-St3gal4tm1.1Jxm/J) at birth. The mutation in this line rendered dysfunctional both St6gal1 and St3gal4 genes, thereby resulting in an absence of 6’SIL and a reduction of about 80% of 3’SIL in the milk. This design resulted in four treatment groups (dams x pups genotype): WT x WT (control group), MUT x WT (milk group), WT x MUT (mutant group) and MUT x MUT (milk+mutant group). Adult offspring (> post-natal day 65), were tested for their spatial and recognition memory, and attention. Data were analyzed using factorial ANOVA.

Results:
Mice receiving HMOs deficient milk exhibited a reduced performance in prepulse inhibition and in working memory assessed in the T-maze. Both the mice receiving HMOs deficient milk and the mutant exhibited a reduction of retention of spatial memory assessed in the Barnes maze. Furthermore, mice receiving HMOs deficient milk showed a reduced attentional performance compared to control group in the attentional set-shifting task. There was no effect of milk or mutant group on recognition memory assessed in the novel object recognition task or anxiety assessed in the elevated zero maze. Finally, we also observed an increase of maternal behavior in the mutant mice and a reduction of body weight in the mutant offspring.

Conclusion:
This study further confirms the previous findings using single KO cross-fostering strategies for either 6’SIL or 3’SIL, that absence of early life source of dietary sialylated HMOs lead to impaired cognitive functions, specifically attention, and spatial and working memory.
Objectives and Study:
Human milk oligosaccharides (HMOs) are key components of breast milk. Presence of 6'Sialyllactose (6'SL) in pre-weaning diet has been shown to program adult attentional processing and mnemonic functions. Here we investigated the mechanisms of action mediating these effects in a full cross-fostering design using wild-type mice and beta galactoside alpha 2,6 sialyltransferase 1 (St6gal1) KO mice by measuring: i) caecal microbiota composition and function, ii) brain gene expression and iii) ex vivo long-term potentiation (LTP).

Methods:
We performed a full cross fostering of C57BL/6J wild-type mice with B6.129-St6gal1tm2Jxm/J transgenic mice at birth. The mutation results in a dysfunctional St6gal1 gene, essential for the synthesis of 6'SL and therefore results in an absence of 6'SL in the milk. A four treatment groups study was designed (dams x pups genotype): WT x WT, MUT x WT, WT x MUT and MUT x MUT. We evaluated caecal microbiota composition and function by shotgun metagenomics both at post-natal day 10-11 (eye opening) and in adulthood. RNAseq analyses of gene expression was performed on samples from the prefrontal cortex and the hippocampus collected either at PND 10-11 or 180. LTP was assessed ex vivo using brain slice from the four experimental groups. A factorial ANOVA analysis was performed using two between subject factors (dams and pups genotype).

Results:
Absence of 6'SL from the milk resulted in a decrease of Actinobacteria (phylum) and Oscillibacter (genus) and an increase of Muribaculum (genus). Deletion of St6gal1 gene induced an increase of Lachnospiraceae (family), Turicimonas and Romboutsia (genus) levels and a reduction of Eubacterium (genus). To understand the functional impact of these alteration of microbiota composition, we investigated the orthologies with the Kyoto Encyclopedia of Genes and Genomes, 14 modules (functional unit of genes) were significantly modified in the milk group and 22 in the mutant group. When evaluating the gene expression, the samples showing the strongest gene expression alteration were those collected from the prefrontal cortex at PND 10-11, with pups receiving milk without 6'SL being characterized by a downregulation of 53 genes and an upregulation of eight genes. Pathway analyses showed that myelination related genes were downregulated in the absence of 6'SL. Finally, mice receiving milk without 6'SL exhibited an increase of LTP.

Conclusion:
These results support a strong impact of presence of 6'SL on several microbiota species, in particular Actinobacteria, strengthening the evidence of a mediator role of microbiota in sialylated HMO related effects. Beyond microbiota mediation, we also reported a prefrontal cortex specific alteration of gene expression associated with myelination at PND 10-11 only, suggesting that brain connectivity is impacted by early life presence of 6'SL. The reported increased LTP could be a functional result of this connectivity alteration. While increased LTP is usually associated with increased memory, several studies in disease models or genetically modified mice have associated an increased LTP from impaired inhibition of the post-synaptic potential with deficits in memory.
Objectives and Study:
Breast-feeding provides the optimal early life nutrition associated with increased cognitive functions. Recently, human milk oligosaccharides (HMOs) were proposed to be mediator of the cognitive benefit associated with breastmilk duration. To understand the role played by some of these HMOs, we evaluated the impact of early life supplementation with three different blends of HMOs on the behaviour of overweight Göttingen Minipigs before weaning, after weaning and at 1 year of age. The HMOs blends were containing either sialylated HMOs, neutral HMOs or both.

Methods:
Female Göttingen Minipigs (n=48, Ellegaard, Denmark) were randomly allocated to be artificially reared with milk substitutes containing blends of either sialylated (2-HMOs: 3'SL and 6'SL; 0.68 g/L), neutral (4-HMOs: LNnT, LNT, 2'FL and di-FL; 4 g/L) or both (6 HMOs: 4 g/L) HMOs and compared to control animals recieving lactose (C: 4 g/L) from 10 days to 11 weeks of age (weaning). From weaning onwards, piglets were offered equal controlled amounts of a mild-obesogenic diet (13% protein, 14% fat, 33% cho, 4.4 MCals GE/kg) up to 48 weeks of age. A naturally-reared reference group of piglets (NR; n=8) was kept with the sow until weaning and fed the same mild-obesogenic diet afterwards. The minipigs were subjected to a series of behavioural tests (open field test followed by a novel object test, runway test and holeboard task) during 3 consecutive periods: Period I between 3 and 9 weeks of age (pre-weaning), Period II between 16 and 21 weeks of age (post-weaning), and Period III between 39 and 45 weeks of age (adulthood).

Results:
Early life HMOs supplementation improved working and reference memory scores in the holeboard task during reversal learning in Period II, and during acquisition of the task in Period III. In addition, early life HMOs supplementation tended to increase exploratory activity in the open field test. We did not observe effects of either blend on locomotor activity in the open field, indexes of anxiety-related behaviours in the novel object test (vocalization and standing alert), heart rate, salivary cortisol or motivation for a food reward in the runway test.

Conclusion:
These results support a positive impact of early life dietary supplementation with HMOs on the development of cognitive functions. The three HMOs blend supplementation had a similar positive impact on cognitive flexibility in Period II (post-weaning). The positive impact of the HMOs mixes on cognition was still visible in Period III (adulthood), but, was observed on memory performance during acquisition of the spatial task at this age and not during reversal learning. The enhancing impact of the HMOs mixes on cognitive performance could result from a beneficial effect of the mixes on brain maturation in early life, leading to an earlier capacity of the piglet to exhibit executive function and, therefore, improve memory in adulthood. The mechanism of action of the HMOs is most likely mediated by alterations of microbiota composition, which is currently being analysed.
Objectives and Study:
It has been hypothesized that human milk oligosaccharides (HMOs) could have a relevant role in stimulating growth during infancy. HMO-like oligosaccharides were shown to restore growth in models of infant undernourishment, while they have been recently reported to associate with infant body composition. Here we evaluated the long-term impacts of early life supplementation of HMOs on growth, organ development, and body fat distribution using a preclinical model of obesity.

Methods:
Female Göttingen Minipigs (n=48) were randomly allocated to be artificially reared with milk substitutes without (Control) or with blends of either sialylated (2-HMOs: 3'SL and 6'SL; 0.68 g/L), neutral (4-HMOs: LNnT, LNT, 2'FL and di-FL; 4 g/L), both sialylated or neutral (6 HMOs: 4 g/L) human milk oligosaccharides (HMOs) from 10 days to 11 weeks of age. Starting at weaning, piglets were offered equal controlled amounts of a mild-obesogenic diet (13% protein, 14% fat, 33% cho, 4.4 MCals GE/kg) to achieve average BW gains of ~1.5 kg/week up to week 48 of age. Body weight and length as well as abdominal circumference were measured along the experiment. At 48 weeks of age, animals were euthanized and organs, semitendinosus muscle as well as omental, mesenteric and perirenal/retroperitoneal fat depots were dissected and weighed. In addition, subcutaneous adipose tissue thickness was measured in the back area at the level of the last rib at both sides of the spine.

Results:
No significant differences in body weight, length and abdominal circumference were detected between Control group and any of the HMO groups at the end of the supplementation period or at the end of the experiment. Similarly, no differences between Control group and the 2-, 4-, or 6-HMOs groups were detected in the relative weight (g/kg BW) of the heart (Median ± SE: 53.6 ± 1.5 vs. 52.8 ± 0.7; 54.6 ± 0.9; 53.3 ± 0.7, respectively), liver (15.9 ± 0.9 vs. 14.9 ± 0.6; 15.9 ± 0.3; 16.2 ± 0.6) and kidneys (3.1 ± 0.1 vs. 3.1 ± 0.1; 3.4 ± 0.2; 3.1 ± 0.2) at 48 weeks of age. However, pancreas relative weight increased significantly in the 6-HMOs group (1.9 ± 0.1, P<0.05) but not in 2- and 4-HMOs relative to Control group (1.7 ± 0.1). There were no significant differences between Control and the 2-, 4- or 6-HMOs groups in the relative weight of semitendinosus muscle (3.3 ± 0.5 vs. 3.2 ± 0.5; 3.5 ± 0.6; 3 ± 0.2, respectively), omental (3.3 ± 0.3 vs. 3.3 ± 0.3; 3.4 ± 0.3; 4.5 ± 0.5), mesenteric (7.8 ± 0.7 vs. 7.6 ± 0.6; 7.3 ± 1.5; 8.0 ± 0.3) retroperitoneal/perirenal (5.7 ± 0.4 vs. 6.4 ± 0.9; 6.5 ± 1.2; 6.7 ± 1.1) adipose fat or back fat thickness (23 mm ± 2.6 vs. 24.5 mm ± 1.7; 23 mm ± 2.9; 26 mm ± 1.9, respectively).

Conclusion:
We demonstrated that consumption of different blends of HMOs in early life did not alter weight gain or linear growth or had an impact on the development of different organs in our pre-clinical model. Furthermore, consumption of HMOs did not alter the pattern of adipose tissue accretion or muscle growth up to young adulthood.
Objectives and Study:

Human milk oligosaccharides (HMOs) are known as non-nutritive components of human milk exerting their immune protection related functions to some extent through establishing the microbiome. Because of the relation of the microbiome with nutrient intake and obesity, we explored possible HMO associations with early growth and body composition of predominantly breastfed infants.

Methods:

We quantified 20 major HMO in milk of healthy mothers (N=221) over the first 4 months of lactation across 7 European countries using a validated method. Milk samples were identified as being secretor positive (Se+) or negative (Se-) depending on the concentration of 2'-fucosyllactose (2'FL). We performed statistical analyses to test for associations of infant anthropometry (N=213) and body composition (N=72 infants) with secretor status and individual HMOs across 6 time-points (2, 17, 30, 60, 90, and 120 days).

Results:

Weight, length and head circumference did not differ by secretor status over time. Individual HMO Area Under the Curve (AUC) calculated over all 6 visits was not associated with any infant growth or fat mass parameters at visit 6. PCA analysis on HMOs and HMO AUC did not reveal any distinctive clusters in the population that could be associated to fat accretion, total and fat mass index. Previously reported HMO correlations with infant growth and fat mass12 were not confirmed in our study cohort. Instead, we report significant correlations of Lacto-N-difuicosyl-lactose (LFDT) (r=-0.336, P=0.015) and Monofucosyllacto-N-hexaose-III (MFLNHIII) (r=0.306, P=0.027) with % fat mass at 120 days. Finally, infant weight was only correlated with Sialyllacto-N-tetraose-c (LST-c) and Disialyllactose-N-tetraose (DSLNT) at 30 days (r=-0.192, P=0.002 and r=-0.144, P=0.024 respectively) and 3’sialyllactose (3'SL) at 120 days (r=-0.185, P=0.007).

Conclusion:

Our results show only modest correlations between HMO concentrations and infant growth and body composition in healthy growing and exclusively breastfed infants. Longer follow-ups in cohorts of infants and toddlers are needed to demonstrate if there is any impact of HMO intake for the future growth and metabolic health.

Objectives and Study:
The infant intestinal microbiota ferments human milk oligosaccharides (HMOs) into diverse repertoire of microbial products, which have a role in regulating a variety of host functions, including intestinal epithelial barrier function. A dysregulated intestinal epithelial barrier function has been implicated in the pathogenesis of many highly prevalent infant inflammatory diseases, such as infections and allergy. Here, we investigated the capacity of HMO related microbial products derived from infant microbiota fermentation to modulate the epithelial barrier function and its response to inflammatory challenge in-vitro.

Methods:
Aliquot of freshly-voided fecal sample from an exclusively breast-fed infant was cultured in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®; Prodigest) culture system. The culture system was left untreated for two weeks after inoculation to obtain stable infant microbial communities. Thereafter, the culture system was daily fed with a mix of 6 HMOs (HMO6: 2’Fucosyllactose, 2’FL; di-Fucosyllactose, diFL; Lacto-N-tetraose, LNT; Lacto-N-neotetraose, LNnT; 3’Siallylactose, 3’S; 6’Siallylactose, 6’S) or a mix of 2’FL and LNnT (2’FL+LNnT) or 2’FL alone. Controls were either left untreated or fed with lactose. Two days after feeding, culture supernatants were collected, centrifuged and filtered. The same culture supernatants were used to pre-treat in-vitro a co-culture epithelial system (Caco2 and the mucus-producing HT29-MTX cell-lines) for 48 hours prior to interferon (IFN)-γ and tumor necrosis factor (TNF)-α exposure. Epithelial barrier function was assessed by evaluating the transepithelial electrical resistance (TEER) and permeability to the inert macromolecule FITC-labeled dextran (FD4; 4 KDa) from apical to basolateral compartment after the inflammatory challenge.

Results:
Culture supernatants of HMO6, 2’FL+LNnT or 2’FL increased TEER before the inflammatory challenge, and significantly dampen the effects of TNF-α and IFN-γ on TEER compared to culture supernatants of lactose. Similarly, culture supernatants of HMO6, 2’FL+LNnT or 2’FL showed decreased FD4 translocation compared to all controls after inflammatory challenge.

Conclusion:
HMO-related microbial products are efficacious in reinforcing the epithelial barrier and prevent the deleterious effects of TNF-α and IFN-γ on epithelial barrier function in vitro. These data extend the beneficial effects of HMOs on the gastrointestinal homeostasis and in conferring protection against inflammation-related epithelial barrier dysfunctions.
Objectives and Study:
Human milk strongly influences the early life development and functioning of the intestinal epithelial barrier. Perturbations in the intestinal barrier play a role in many infant diseases such as infections, allergy and necrotizing enterocolitis. Here, we investigated the efficacy of the human milk oligosaccharides (HMOs), an important component of breast milk, to reduce susceptibility against inflammatory-induced epithelial barrier dysfunction.

Methods:
An in-vitro co-culture of Caco2 and the mucus-producing HT29-MTX cell-lines was pre-treated for 48 hours with either single HMOs (2′Fucosyllactose, 2′FL; di-Fucosyllactose, diFL; Lacto-N-tetraose, LNT; Lacto-N-neotetraose, LNnT; 3′Siallylactose, 3′SL; 6′Siallylactose, 6′SL) or a mix of the six HMOs (HMO6) prior to interferon (IFN)-γ and tumor necrosis factor (TNF)-α exposure. Control cells were either left untreated or treated with lactose. Epithelial barrier function was assessed by evaluating the transepithelial electrical resistance (TEER) and permeability to the inert macromolecule FITC-labeled dextran (FD4; 4 KDa) after the inflammatory challenge.

Results:
HMO6 pretreatment increased TEER before the inflammatory challenge in a dose-dependent manner, compared to lactose-treated and non-treated control cells. None of the single HMOs significantly increased TEER before the inflammatory challenge nor ameliorated the effects of TNF-α and IFN-γ on TEER. In contrast, 2′FL and, to a lesser extent, LNT significantly decreased FD4 translocation compared to lactose-treated and non-treated control cells.

Conclusion:
Tested HMOs blend reinforced the epithelial barrier and partially prevent the negative effects of TNF-α and IFN-γ on epithelial barrier function in vitro. This effect may be partially explained by the presence of 2′FL and LNT in the blend. These data reinforce the key role of specific HMOs on the gastrointestinal homeostasis and support a mediating role of these compounds on the beneficial effects of breastmilk on the promotion of gut health and protection against intestinal inflammatory disorders. Further in-vivo studies could support the relevance of HMOs in reinforcing the epithelial barrier and in conferring resistance against inflammation-related epithelial barrier dysfunctions.

1. Nestlé Research, Lausanne, Switzerland
Objectives and Study:
Scientific evidence suggests that human milk oligosaccharides distinctly influence gastrointestinal (GI) microbiome in support of immune development in early life. Our objective was to evaluate the effect of infant formula supplemented with 2′fucosyllactose (2′FL) on the abundance of beneficial and pathogenic bacteria.

Methods:
Healthy infants <14 days old (n=289) from Italy and Belgium were randomized in a double-blind manner to receive cow’s milk-based infant formula containing Lactobacillus reuteri at 1x10⁷ CFU/g (Control) or the same formula with 1.0 g/L 2′FL (Test) until 6 months of age. A non-randomized breastfed (BF) group served as a reference (n=60). As part of secondary objectives, stool samples were collected at study baseline (age <14 days), 1, 2 and 3 months of age for the assessment of fecal bacterial species and enterotoxin targets via qPCR.

Results:
Analyses of the fecal microbiome showed that, at age 1 month, Clostridium difficile was significantly lower in the Test group compared with the Control group (mean ± SEM 16.0×10⁴ ± 5.3×10⁴ vs. 29.2×10⁴ ± 8.3×10⁴ copies/mg, p=0.047). However, only numerically lower abundances in the Test group (vs. Control) were observed for C. difficile at 2 and 3 months of age, and for Clostridium perfringens at all three time points, with the Test group closer to that of the BF group which had the lowest abundance throughout the study. In the subgroup of caesarian delivered infants, the Test group had significantly lower abundance of Klebsiella pneumonia than the Control group at 1 month of age (7.6×10⁴ ± 5.2×10⁴ vs. 37.8×10⁴ ± 19.0×10⁴ copies/mg, p=0.011). No difference in abundance was found between the Test group and Control group for Campylobacter coli or jejuni, enteropathogenic Escherichia coli, enterotoxigenic E. coli heat-labile and heat-stable enterotoxins and Salmonella species. These bacterial and enterotoxin targets were below the limit of detection in the majority of infants in all feeding groups. During the study, bifidobacteria abundance in the Test group tracked towards the abundance in the BF group while the abundance in the Control group was lowest.

Conclusion:
Infant formula with 1 g/L 2′FL is associated with a lower abundance of pathogenic bacteria during early infancy, and may play a role in shifting gut microbial pattern towards that of breastfed infants.
Objectives and Study:
Human Milk Oligosaccharides (HMOs) were reported to promote growth of specific component of the infant gut microbiota, such as bifidobacteria, as well as production of health-related bacterial metabolites. In the present work, we examined for the first time the impact of HMO or their combination of on toddler gut microbiota and its metabolites.

Methods:
Three different HMO preparations (B1, B3 and B5) were tested and compared to lactose: B1= 2’FL; B3= 2’FL, DiFL, LNT and B5= 2’FL, DiFL, LNT, 3’SL and 6’SL. All preparations and lactose were used at 5 g/L final concentration. HMOs were first added to sugar-depleted nutritional medium and tested in an ex vivo short-term colonic fermentation model inoculated with a freshly collected fecal samples from 5 healthy 2 to 3 year old toddlers. Incubations were done in closed vessels over 48 h, at 37°C, under continuous mixing and anaerobic conditions. HMOs preparations were also tested with stool from one donor in another ex vivo model designed to evaluate long term (3 weeks) impact of ingredients (M-SHIME® Prodigest, BE). The microbiota composition, Short Chain Fatty Acids (SCFAs), Branched Chain Fatty Acids (BCFAs) and ammonium derivatives were measured at different time points in both systems.

Results:
The fermentation of HMOs strongly increased production of lactate and acetate (for all five donors), typical metabolites produced by bifidobacteria, which were increased by at least 1 log unit for each treatment and 4 out of 5 donors. This observation was confirmed in the long-term model for one donor. A strong luminal pH decrease was observed with values dropping from 6.6 to 5.6 for all HMO treatments. The observed acidification is likely to be attributed to increased levels of SCFA and/or lactate which was also observed.
A strong shift in the microbiota was noted in mucus compartment and lumen of the colon segment with a promotion of the Actinobacteria and a decrease in the relative abundances of Firmicutes and Bacteroidetes. In the Actinobacteria group mainly Bifidobacterium and particularly Bifidobacterium pseudocatenulatum increased. Bifidobacteria are amongst the primary users of lactose and HMOs, which relates in the model used here with the production of SCFAs and reduction of ammonium and branched SCFA levels in all modeled intestinal regions.

Conclusion:
The present data provide the first evidence that HMOs drive toddler gut microbiota growth and metabolic activity shifting their metabolism from a proteolytic to a saccharolytic metabolism. The formed SCFA are related to health beneficial effects.
Objectives and Study:
Breastmilk contains a large number and diversity of Human Milk Oligosaccharide (HMO) structures. These can be grouped in 3 main categories: core structures (e.g. LNnT, LNT), fucosylated- (e.g. 2'FL, DiFL) and sialylated structures (e.g. 3'SL, 6'SL). Different HMOs were reported to promote growth of specific bifidobacteria, as well as production of health-related bacterial metabolites. Here, we examined the impact of HMO diversity on infant gut microbiota and its metabolites in ex vivo fermentation systems with infant stool samples.

Methods:
Three HMO blends (B1, B2 and B6) with increasing HMO diversity were tested in comparison to lactose: B1= 2'FL; B2 = 2'FL and LNnT in a 2:1 ratio and B6 = 2'FL, LNnT, LNT, diFL, 3'SL, 6'SL. All blends and lactose were studied at 5 g/L final concentration. HMOs were first added to sugar-depleted nutritional medium used in an ex vivo short-term colonic fermentation model inoculated with a freshly collected fecal sample from a four months old and exclusively breast-fed healthy infant. Incubations were performed in closed vessels over 48 h, at 37°C, under continuous mixing and anaerobic conditions. The same HMO blends were also tested in a long term (3 weeks) ex vivo model designed to evaluate the impact of ingredients on the microbiota (SHIME® Prodigest, BE). Microbiota composition, Short Chain Fatty Acids (SCFAs), Branched Chain Fatty Acids (BCFAs) and ammonium derivatives were measured at different time points in both systems.

Results:
In both short- and long-term model all three HMOs blends increased the microbial diversity, increased production of beneficial metabolites, reduced production of potentially harmful metabolites and decreased proteolytic activity. Noteworthy, increase in butyrate production positively correlated with HMO blend diversity, with B6 resulting in the highest final butyrate concentration in cultures of infant fecal microbiota, followed by B2 and B1 used at same concentrations. Conversely, production of BCFAs was inversely correlated to HMOs blend diversity.

Conclusion:
Although only a simplified model with a single microbiota donor, the data indicate that microbiota dynamics are driven in an HMO diversity dependent manner. Future observational and interventional clinical studies may establish to what extent our observations replicate and translate to health benefits in clinical settings.
**Objectives and Study:**

Human Milk Oligosaccharides (HMOs) were reported to promote growth of specific component of the infant gut microbiota, such as bifidobacteria, as well as production of health-related bacterial metabolites. This may be through individual strains able to use specific HMOs, possibly combined with cross-feeding in the infant gut ecosystem. The aim of the present work is to examine in vitro the ability of various bacteria strains, either isolated from infant stool or used as probiotic in infant nutrition, to use HMOs for growth and metabolic activity.

**Methods:**

Probiotics and commensal bacteria were incubated with HMOs, 2'FL, DiFL, LNT, LNnT, 3'SL, 6'SL or classic prebiotic oligosaccharides, fructo- or galacto-oligosaccharides (FOS, GOS). Fifteen bifidobacteria strains, nine lactobacilli and four other commensal bacteria isolated from a healthy baby were incubated for 24 and/or 48h at 37°C in anaerobic conditions in minimal media supplemented with the different carbohydrates. Lactose and Glucose were used as controls. Bacteria growth and production of Short Chain Fatty Acids (SCFAs) were measured.

**Results:**

Only the two Bifidobacterium bifidum tested out of the tested 15 bifidobacteria were able to grow on all the individual HMOs and their combinations. Five out of the six tested B. longum strains were able to grow on LNT, two grew on 2'FL and one on LNnT. One of the B. longum strains belong to the subspecies infantis and grew on 2'FL and LNT. Of the two B. breve strains that we tested both grew on LNnT and one also grew on LNT. The B. suis grew only on LNT and none of the three tested B. adolescentis grew on any of the HMOs. On the other hand, most of the tested bifidobacteria grew on GOS and about half on FOS. Among the other commensal bacteria, the Bacteroides distasonis grew on almost all HMOs except DiFL. Interestingly, Clostridium perfringens grew on some HMOs, namely LNnT, 3'SL and 6'SL. Apart from the two tested Lactobacillus acidophilus strains, which grew on 2'FL, none of the other seven Lactobacillus strains nor the 2 Staphylococcus strains grew on any of the tested HMOs. Generally, we observed the SCFA acetate increased by any of the growing bifidobacterial.

**Conclusion:**

HMO use by bifidobacteria seems highly strain specific and HMO-structure specific. LNT followed by LNnT and 2'FL were the HMO structures most broadly used as sole carbon source. FOS and GOS seem to be more broadly used by different bifidobacteria strains but can also be broadly used by other commensal infant bacteria. The SCFA acetate may exert beneficial effects directly and favor a favorable gut ecosystem through acidification.
Objectives and Study:
Visceral hypersensitivity (VHS) plays a major role in the aetiology of pain-related functional gastrointestinal disorders (FGID) including infantile colic, and has been associated with alterations in the bidirectional microbiome-gut-brain axis communications. Human milk oligosaccharides (HMOs), the third most abundant component of human milk, can modify the microbiota composition and metabolism, and modulate brain functions. In this study, we investigated 1) whether a blend of six HMOs (HMO6) highly represented in human milk decreased VHS triggered by chronic psychological stress (brain-gut driven) or antibiotic treatment (microbiota dysbiosis - gut-brain driven) and 2) the impact on stress-driven VHS of simpler, HMO6-derived sub-blends.

Methods:
Two series of experiments were conducted in 6 to 8-week-old, male C57/Bl6 mice (n=12/group). In a first series, mice were submitted to daily sessions of water avoidance stress (WAS) (1 h/day) for 9 days or received non-absorbable antibiotics (bacitracin, neomycin, and pimaricin) in drinking water for 10 days (ATB). In each model (WAS and ATB) half of the mice received AIN93M diet (Cont) and the other half AIN93M containing 1% (w/w) HMO6 (2′FL+DiFL+LNT+LNnT+3′SL+6′SL), from 3 days before starting and during the WAS or ATB challenges. Similar diets were provided to unchallenged mice (Sham-Cont; Sham-HMO6). In a second series, four HMO sub-blends (2′FL+DiFL; 2′FL+DiFL+LNT; LNT+6′SL; 3′SL+6′SL) were tested in the WAS model and compared to WAS-Cont and Sham-Cont groups. At the end of the experimental procedures, visceral sensitivity to stepwise colorectal distension (CRD) was assessed by recording the mouse abdominal muscle electromyographic (EMG) activity.

Results:
In mice fed Cont, both WAS and ATB challenges significantly increased the EMG activities to most individual CRD volumes applied as well as the area under the curve (AUC, mVxml/s) of EMG activity across all CRD volumes (AUC = 5.53 ± 0.23 in Sham-Cont, 9.46 ± 0.50 in WAS-Cont, 7.84 ± 0.57 in ATB-Cont; p<0.01). HMO6 diet significantly reduced the WAS-mediated rise of the EMG activities at most CRD volumes and the overall AUC (AUC = 6.12 ± 0.50 in WAS-HMO6; p<0.0001 vs WAS-Cont). By contrast, HMO6 failed to reduce the EMG activities in ATB mice (p>0.05). HMO6 feeding did not affect basal visceral sensitivity in non-challenged mice (p>0.05 for both individual distension volumes and AUC). Among the tested sub-blends, 2′FL+DiFL and LNT+6′SL significantly decreased the EMG activities close to the values found in Sham-Cont (AUC = 5.74 ± 0.28 in Sham-Cont, 8.21 ± 0.41 in WAS-Cont, 5.77 ± 0.30 in WAS-2′FL+DiFL, 6.07 ± 0.45 in WAS-LNT+6′SL; p≤0.001).

Conclusion:
A blend of six major HMOs - in a proportion mimicking the natural occurrence in maternal milk - alleviates VHS triggered by chronic psychological stress but not by antibiotic treatment. Two simpler sub-blends -2′FL+DiFL and LNT+6′SL- were particularly efficient in normalising stress-driven VHS. These results suggest that interventions with specific HMOs may help to manage symptoms in abdominal pain related FGID in early life. They also support the hypothesis that the HMOs effect on VHS may require a relatively intact gut microbiota to help restoring brain-gut axis disturbances induced by a chronic psychological stress.
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