Human Milk Oligosaccharides: Where Do We Go from Here?

Satelite Symposium Abstracts ESPGHAN 2018

Nestlé Nutrition Institute

Human Milk Oligosaccharides: Where Do We Go from Here?

Dr. Norbert Sprenger | Switzerland
Physiological Significance: Why Are They in Mother's Milk?

Dr. Philippe Alliet | Belgium
Clinical Studies: What Is Known and What Needs Further Studies?

Welcome Note

Prof. Yvan Vandenplas
Universitair Ziekenhuis Brussel,
Vrije Universiteit Brussel | Belgium

Breastmilk is the natural and best food for babies which provides all energy and nutrients that the infant needs for the first months of life. Mother's milk continues to provide half or more of a child's nutritional needs during the second year of life. Compared to exclusively breastfed infants, formula-fed infants suffer more infectious diseases, such as gastroenteritis and acute otitis media and more immune mediated diseases such as allergic disease. The composition of breastmilk is very complex as it contains thousands of different biomolecules. Focussing on the carbohydrate composition of breast milk, two types of carbohydrates have been isolated: lactose and oligosaccharides. The major component is lactose, which is nutritionally very important. However, human milk oligosaccharides (HMOs) are the third most important component of breast milk, after lactose and lipids, and are composed of a very complex mixture of more than 200 components and are the non-digestible and non-nutritional carbohydrates present in human milk. Among the many compositional differences between human milk and cow milk based infant formula, one of the biggest quantitative difference is the presence of these unique carbohydrates structures in human milk and their virtual absence in cow's milk and it derivates such as infant formula. This brochure will help you to better understand the biology of human milk and its benefit on the infants, and how these effects can be mimicked to the best if breastfeeding is not possible.

Prof. Yvan Vandenplas
Chairperson

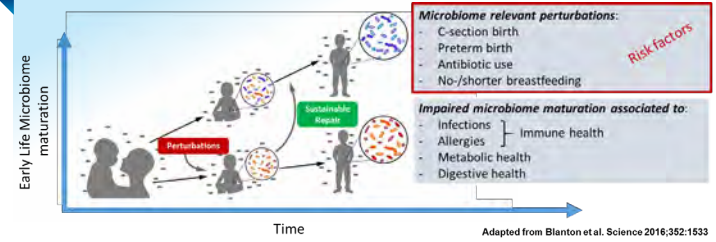
Physiological Significance of HMO: Why Are They in Mother's Milk?

Dr. Norbert Sprenger
Institute of Nutritional Science, Gut ecosystem department, Nestlé Research Center, Lausanne | Switzerland

Human milk oligosaccharides (HMO) have no nutritive value, yet mothers spend significant energy for their synthesis. So what do they do? Clinical observational studies together with basic research position HMO as multifunctional innate breastmilk component. They shape the establishing gut microbiota and supposedly help the development of appropriate immune competence.

The early-life gut microbiome establishes and matures sequentially during infancy and early childhood from an aerobic to anaerobic milk-oriented early life microbiome towards an adult like microbiome. Different phases of this maturation process can be characterized by a progression of microbiota communities, starting from *Enterobacteriaceae, Streptococcaceae* dominated aerobic communities over anaerobic *Bifidobacteriaceae* dominated communities to increasingly diverse *Bifidobacteriaceae* and *Lachnospiraceae* dominated communities. The age-appropriate microbiome maturation is considered important for normal digestive, immune competence and metabolic development (Blanton et al. Science 2016;22:713). Mode of delivery, antibiotic use and diet are probably most influential to this end. (Chart 1)

Chart 1



➡ *Which breast milk-specific components influence the early-life microbiota?*

Turroni F, et al. PLoS One 2012;7:e36957; Arrieta M-C, et al. Front Immunol 2014;5:427; Tamburini S, et al. Nat Med 2016;22:713–22; Gray J, et al. Sci Transl Med 2017;9: pii: eaaf9412; Blanton et al. Science 2016,352:1533

Breastfeeding is associated with a lower risk of gastrointestinal and respiratory infections, and possibly lower risk of diabetes and obesity, while the effect on allergies is less clear (Victora et al. Lancet 2016; 387:475). This suggests that breastmilk-specific components may contribute.

Among such breastmilk specific components are the non-digestible human milk oligosaccharides (HMO), the third largest solid breastmilk component. Chemically, HMOs are elongations of the milk sugar lactose by galactose, N-acetyl-glucosamine, fucose and sialic acid. Most HMOs such as the fucosyl-oligosaccharides are not present in farmed-animal milks and therefore absent from animal milk based nutrition products. In structure and composition the HMO resemble and have identical epitopes like mucosal surface glycans that are at the interface between the mucosal cells and the intestinal microbiome.

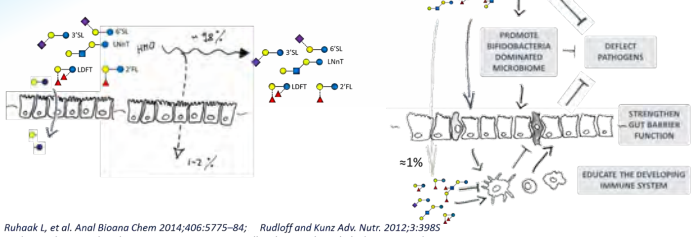
In contrast to HMOs, generic prebiotics like Fructo-oligosaccharides (FOS, inulin) are elongations of the table sugar sucrose by fructose units. These are typical plant storage glycans usually consumed from weaning with the introduction of solid plant based complementary food.

Mothers spend a considerable amount of energy to form HMOs in milk at an estimated 5 to 15 g/L. The HMO composition varies primarily due to the maternal genotype for the Secretor and Lewis gene encoded fucosyltransferases as well as stage of lactation. While most HMOs decrease in concentration with time of lactation, some increase. This means with age-dependent growing milk intake infants consume relatively constant amounts of most HMOs per day and increasing amounts for some. Generally, HMOs are non-digestible and hence have no nutritive value per se. Small amounts of HMOs can go systemic and are mostly excreted in urine, while the bulk of HMOs remains in the gut lumen. (Chart 2)

Chart 2

- *HMOs are generally not digested*
- *Small amounts of HMOs are absorbed and excreted in urine*
- *HMOs are differentially fermented*

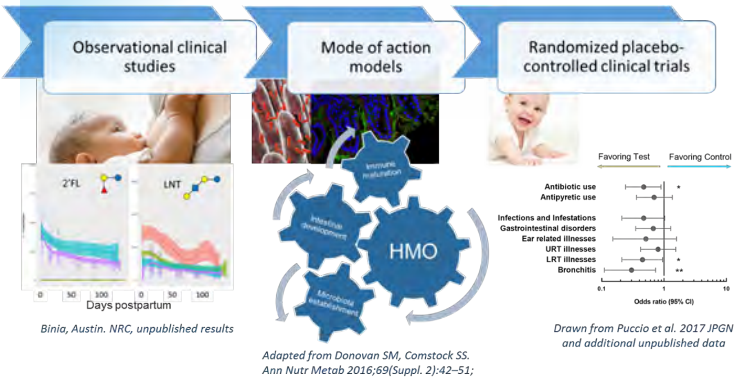
So what do HMOs do?



Ruhaak L, et al. Anal Bioana Chem 2014;406:5775–84; Rudloff and Kunz Adv. Nutr. 2012;3:398S Underwood MA, et al. Pediatr Res 2015;77:229–35. Albrecht S, et al. Carbohydrate Research 2011;346:2540;

Generally, we base our hypothesis on possible roles of HMOs for healthy infant growth and development on observational clinical association studies. In a first step towards the establishment of causality, we investigate the formulated hypothesis using basic research mode of action models. In a second step, we run randomized clinical intervention trials. (Chart 3)

Chart 3



Binia, Austin. NRC, unpublished results

Adapted from Donovan SM, Comstock SS. Ann Nutr Metab 2016;69(Suppl. 2):42–51;

Drawn from Puccio et al. 2017 JPGN and additional unpublished data

In cohorts of breastfed infant-mother dyads, specific HMOs correspond with infant gut microbiota, allergies, morbidity, infectious diarrhea and respiratory infection. (Chart 4)

Chart 4

Microbiome	2'Fucosyl-HMOs are associated with bifidobacteria dominated early life microbiota in breastfed infants
Growth & development	2'Fucosyl-HMOs seem not alter infant growth through 4 months of age, but maybe body composition through 6 month
Allergies	2'Fucosyl-HMOs are possibly related of IgE allergy manifestation through 2 years of age in C-section born infants at high risk, and other HMOs possibly to cow milk through 18 months
Infections	2'Fucosyl-HMOs are related to lower incidence of infectious diarrhea through 9 months, lower morbidity through 4 months and lower acute respiratory infections through 6 months

Lewis et al. Microbiome 2015; 3:13; Smith-Brown et al. PlosONE 2016; 11:e0161211; Matsuki et al. Nature Comm 2016; 7:11939; Sprenger et al. PlosONE. 2017; 12:e0171814; Alderete et al. AJCN. 2017; doi: 10.3945/ajcn.115.115451.; Sprenger et al. Eur J Nutr. 2017; 56:1293; Seppo et al. (2017) JACI 139:708; Morrow AL, et al J Pediatr 2004;145:297-303; Davis JC, et al. Sci Rep 2017;7:40466; Sakwinska and Binia et al. NRC, unpublished results

In the gut lumen, HMOs modulate the establishing gut microbiome through the promotion of a bifidobacteria-dominated microbiome. Basic research studies identified different bifidobacteria strains that can use HMOs for growth, either after internalization or after extracellular breakdown. Interestingly, specific HMOs also boost the metabolic activity of specific bifidobacteria seen primarily as higher formation of the short chain fatty acid (scfa) acetate that is not necessarily seen with prebiotics that lead to similar growth. Metabolites from the HMO stimulated bifidobacteria provide immune protection from inflammation and pathogen invasion in preclinical models. The bifidobacteria-dominated early life microbiome, likely through its metabolites and acidification of the gut lumen, leads to colonization resistance against the intrusion of new and potentially harmful microbes and thus represents a major intestinal barrier.

Because HMOs modulate the maturation of the early life microbiome and because the microbiome presumably affects food efficiency and energy harvest, variation in HMO composition might affect infant growth and body composition. We did not observe growth differences through 4 months of age in a cohort of 25 boys and 25 girls in relation to 2'Fucosyl-HMOs (Sprenger et al. PlosONE. 2017; 12:e0171814). However, another group observed lower fat mass related with higher breastmilk content of one specific 2'Fucosyl-HMO in a cohort of 25 infants (Alderete et al. AJCN. 2017; doi: 10.3945/ajcn.115.115451). It will be interesting to further study HMO relations to body composition in larger cohorts.

Numerous environmental, including microbiome and nutrition, and genetic factors affect allergies. Among them are breastmilk bioactives, and possibly HMOs. In a cohort of 266 mother infant dyads with hereditary allergy risk, 2'Fucosyllactose (2'FL) concentrations in breastmilk related to lower risk to manifest IgE-eczema through 2 years of age in the C-section born infants only (Sprenger N, et al. Eur J Nutr 2017;56:1293–1301). This indicates that 2'FL might have an immune effect via the promotion of specific bifidobacteria that were at lower prevalence in the C-section born infants up to 6 months in this cohort (Kuitunen et al. JACI 2009;123:335). Noteworthy, specific *Bifidobacterium breve* were related to lower eczema risk and 2'FL related to abundance of specific *B. breve* strains and their metabolic activity (Pediatric Allergy and Immunology 2016; 27:838. Matsuki et al. Nature Comm 2016; 7:11939). Further, HMOs like 2'FL and another larger fucosyl-HMO (LNFP III) interact with the dendritic cell lectin DC-SIGN, which might modulate immune development. Another group found LNFP III related to cow milk allergy (CMA) at 18 months of age in a cohort of 39 mothers with CMA infants and 41 mothers with healthy infants (Seppo et al. (2017) JACI 139:708). They speculated that LNFP III might have acted via DC-SIGN. In a mouse model on food allergy, 2'FL and the sialyllactose 6'SL were tested and both reduced symptoms via the modulation of mast cell response (Castillo-Courtade L et al. (2015) Allergy. 70:1091).

Because HMOs resemble mucosal glycans and largely escape complete fermentation, they can act as soluble decoys preventing pathogen adhesion to mucosal cells. Besides glycans the mucosa and immune cells are also rich in lectins (glycan binding proteins) and some were shown to bind specific HMOs. Examples are DC-SIGN, galectins and Siglecs. Whether observed changes in epithelia and immune cells upon treatment with specific HMOs is mediated via such lectins remains to be shown. Together, such proposed mechanisms including the aforementioned colonization resistance and importance of the microbiome for immune development suggest that HMOs contribute to protection from infections in the gastrointestinal tract and likely at other mucosal sites such as the respiratory tract.

In a cohort of Mexican mothers and infants (n=93), higher 2'Fucosyl-HMOs in breastmilk related to lower incidence rate of infectious diarrhea through 9 months of age primarily caused by *Campylobacter jejuni*, but also by Calicivirus (Morrow AL, et al J Pediatr 2004;145:297–303). In a smaller cohort of 33 infant mother pairs 2'Fucosyl-HMOs related to lower morbidity in the first 4 months of age (Davis JC, et al. Sci Rep 2017;7:40466). These

observations are corroborated by findings from different preclinical models, where 2'FL (i) prevented adhesion and reduced disease index by *Campylobacter jejuni* (Ruiz-Palacios et al., JBC 2003; 278:14112; Yu et al. J Nutr 2016; 146:1980) and (ii) helped from pathogenic *E. coli* by strengthening barrier function (He et al., Gut 2016; 65:33; Angeloni et al. Glycobiology 2005; 15:31-41).

Recently, we observed an association between 2'Fucosyl-HMO positive milk and a 1.4 times reduced risk of acute respiratory infections during the predominant breastfeeding period through 6 months of age in a cohort of 220 mother infant pairs in Bangladesh (Sakwinska and Binia et al. NRC, unpublished results). We currently further investigate etiology. From preclinical models there is data indicating that specific HMOs might block adhesion of respiratory pathogens and that HMOs, among them 2'FL, modulate immune response to influenza virus infection and immunization (Sprenger, et al. NRC, unpublished results; Xiao, et al. Frontiers in Immunology 2018. 9:452). Whether the connection between the gut and the lung is mediated by specific microbial metabolites like the scfa or trafficking immune cells as proposed from studies in mouse models has to be established still for HMOs.

Conclusion

Together, such clinical observations corroborated by experimental basic research data suggest that HMO act in a multifunctional way, affecting the (i) establishment of the early-life microbiota dominated by bifidobacteria, (ii) resistance to pathogens and (iii) mucosal barrier and immunity. Interestingly, several basic research models indicate that specific HMO may not only modulate the gut-lung axis, but also modulate brain and cognitive development, via the gut-brain axis.

Chart 5

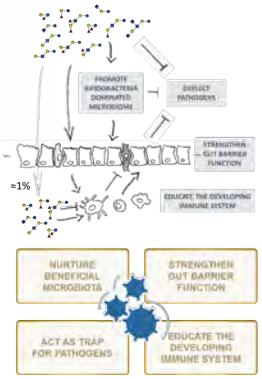
Why are HMOs in mother's milk?

Possibly,

- to help the development of appropriate immune competence likely in part through the support of age-appropriate microbiome maturation

Outlook,

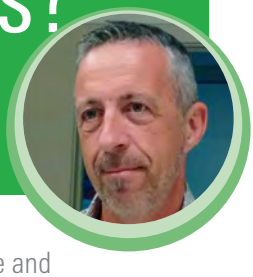
- Well defined observational studies for confirmation and further hypothesis generation including metabolic and nervous system development
- Randomized controlled intervention trials to establish causality and evidence based nutrition



Notes

Clinical Studies: What Is Known and What Needs Further Studies?

Dr. Philippe Alliet
Jessa Hospital
Department of Paediatrics
Hasselt | Belgium



HMO are the third most important component in human milk, more important as solid than protein fraction. And that is a big difference with infant formula and cow's milk, in which this part is not present.

The oligosaccharides are very complex,very abundant and the proportion is not similar during the whole period of lactation.

More than 150 different HMOs have been identified. To more simplify the 150 we can according to the different monosaccharides present them in three categories .(Chart 1)

Two of those are more abundant: 2`FL and LNnT. And from in vitro studies we know that there is accumulating evidence suggesting that they may support gastrointestinal and immune functions of breastfed infants. The mechanisms that have been put forward are the effect of the microbiota growth, function and establishment, protection from infection and effects on allergy and immune competence. One of the ways why people think that they might help against infections and bacterial infections is that they have similarities with some receptors of certain gastrointestinal bacteria and they could interact or prevent the adherence of those microbes to the receptors in our gut.

We performed in two centers in Palermo, Italy, and in Hasselt, Belgium, this randomized controlled trial with infant formula supplemented with 2 synthetic human milk oligosaccharides (Alliet et al JPGN 2017; 64: 624-631). The overall objective of that safety study was the effect of this formula supplemented with these 2 synthetic human milk oligosaccharides on growth, digestive tolerance and morbidity in healthy infants. For the microbiota related objectives we compare microbiota profile and metabolic signature among the infants given the test formula (EF) and the control formula (CF) and there was also a non-randomized exclusively breastfed infants group (BF).

The hypothesis was that microbiota profile and metabolic signature in the infants fed EF (vs. CF) will be closer to that of BF infants.

Infants were enrolled before the age of 2 weeks. After informed consent they got a medical examination and anthropometry. Then they were randomized towards the test or the control formula. (Chart 2)

They were seen at 1, 2, 3, 4 and 6 months, which was the end of the treatment. All infants received a standard follow-up formula from 6-12 months and were seen again at 12 months. Complementary food was only started at 4 months of age, digestive tolerance and adverse effect were recorded in a parent's diary. (Chart 3)

Baseline characteristics

175 infants were included, most of them were followed up at 6 and 12 months. Baseline characteristics were comparable between EF and CF groups. Mean difference in weight gain (EF vs. CF) was -0.30 g/day (95% CI -1.94–1.34, p=0.72), within the non-inferiority margin. Infants receiving EF (vs. CF) did not differ in weight, length, head circumference, BMI, or corresponding z-scores through 12 months.

Digestive Tolerance Measures of digestive tolerance were generally similar between groups:

- no difference in stool consistency, except at 2 mo (softer in EF vs. CF)
- no difference in stool frequency between EF and CF groups
- GI symptoms and behavioral patterns were similar between groups

Chart 1: Human Milk Oligosaccharides (HMOs)

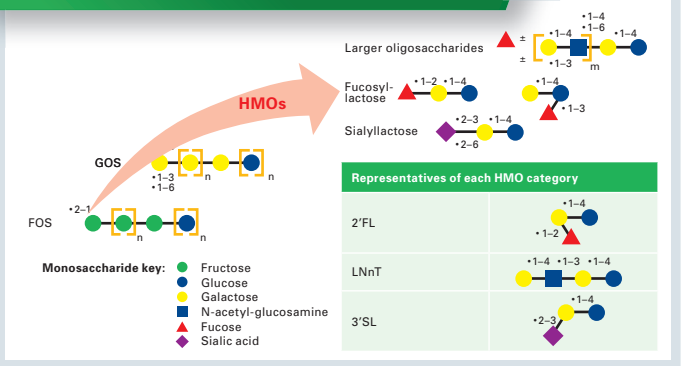
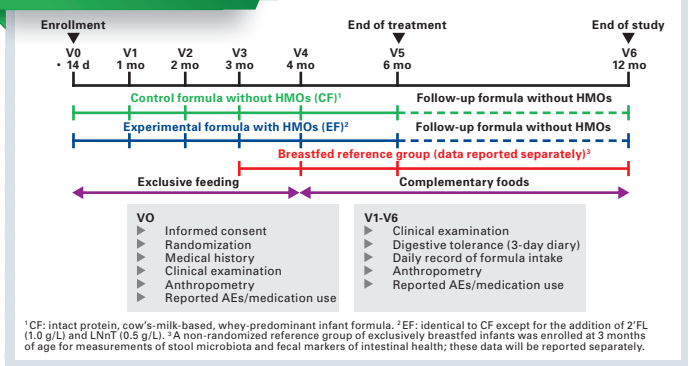


Chart 2: Study design



¹CF: intact protein, cow's-milk-based, whey-predominant infant formula. ²EF: identical to CF except for the addition of 2'FL (1.0 g/L) and LNnT (0.5 g/L). ³A non-randomized reference group of exclusively breastfed infants was enrolled at 3 months of age for measurements of stool microbiota and fecal markers of intestinal health; these data will be reported separately.

The bacterial composition

There was a big distinction between EF and CF groups.

- 2'FL and LNnT-containing formula shifted the global gut microbiota composition closer to that of breastfed infants. (Chart 4)
- Formula with 2'FL and LNnT promoted the colonisation of potentially beneficial Bifidobacterium and reduced taxa with potentially pathogenic members.
- Stool metabolic signature of EF was closer to BF, consistent with the findings in bacterial composition and diversity.
- Altered bacterial composition may result in reduced protein fermentation in EF vs. CF.

Clinical results

The only way we could look at the clinical results in this study was by using the reported adverse events,identified a priori, and medication use.

- Infants receiving EF (vs. CF) were less likely to experience bronchitis through 4 months (OR 0.16, 95% CI 0.02–0.78, p=0.010), 6 months (p=0.005) and 12 months (p=0.004), and lower respiratory tract infection through 12 months (OR 0.45, 95% CI 0.21– 0.95, p=0.027).
- Infants receiving EF were also less likely to receive antipyretics through 4 months (OR 0.44, 95% CI 0.20-0.98, p=0.032), and antibiotics through 6 months (OR 0.53, 95% CI 0.27-1.02, p=0.047) and 12 months (p=0.016).

Of course we have to be careful with the interpretation of these results since they were secondary endpoints of the study.

Fecal community types in infants at 3 months

If you put all data of the enrolled infants together you can find 3 types of fecal community type (FCT), namely

- FCT A: low bifidobacteria, high enterobacteriaceae/ lachnospiraceae
- FCT B: high bifidobacteria and low others
  FCT C: medium bifidobacteria and also the others

When we now looked in the three groups in our study (EF, CF, BF) we see again a big difference between the breastfed and formula fed. But it seems that in the test group (EF) the FCT B was higher and the FCT C was lower than in the control group (CF). (Chart 5)

Chart 3: Reporting of AE (morbidity)

- Parent kept a diary to record infant's illness, fever, symptoms, and medication use
- This information was reviewed and confirmed by study physicians at each study visit before being recorded as an AE
- AEs were then coded and categorized by a single physician (not involved in study conduct) using SOC categories and PTs
- Several AE PTs were identified a priori of interest, i.e., upper respiratory infection, bronchitis, otitis media, and gastroenteritis
- Three AE clusters were identified: upper respiratory infection, lower respiratory tract infection, and otitis/ear infection
- Medication use were categorized into groups as appropriate, including antibiotics, antipyretics, and GERD medications

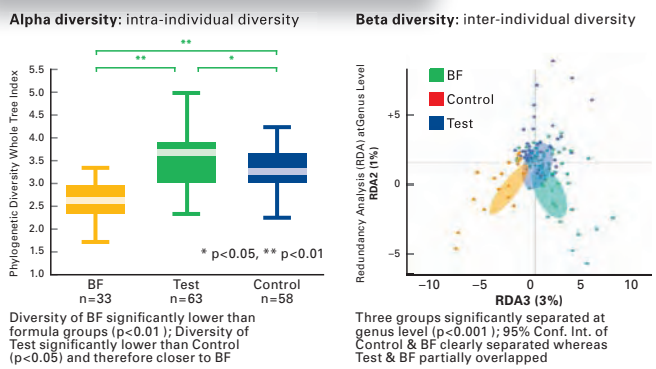
AE: adverse event; SOC: system, organ and class; PTs: preferred terms; GERD: gastroesohageal refl ux disease

And if we compare that to the antibiotic use it looked that especially the FCT C was more prominent in the group that was receiving the antibiotics. All those data seem to go into the same direction and are concordant to the in vitro data.
But this of course need further research in order to confirm these results.

Conclusion

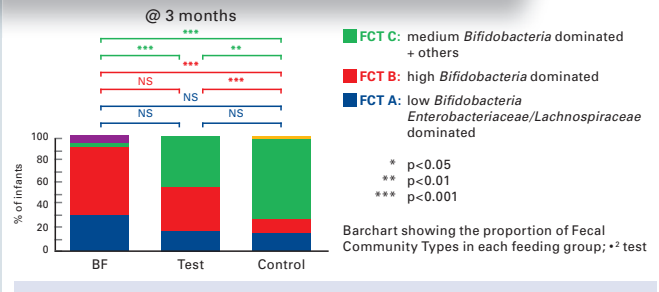
- First clinical data demonstrate that synthetic HMOs are safe and well tolerated (structurally identical to those found in human milk)
- First clinical data show that formula with 2'FL + LNnT promotes the Fecal Community Type B seen in breastfed infants.
- The reported reduced likelihood of antibiotic use with 2'FL + LNnT may be linked to the Fecal Community Types
- After these fi rst promising health outcomes, further studies are needed to establish efficacy

Chart 4: Bacterial diversity at 3 months



2'FL and LNnT-containing formula shifted the global gut microbiota composition closer to that of breastfed infants

Chart 5: Distribution of fecal community types



Formula with 2'FL+LNnT decreased the number of infants with FCT C and increased those with FCT B, which was the majority in the BF group

Nestlé Nutrition Institute

Your **trusted partner** for **scientific** nutrition information and **education**

The Nestlé Nutrition Institute (NNI) is an organization that exists to advance the science for nutrition. Since 1981, we have been committed to supporting the academic community, stimulating discussion and building knowledge.

Our commitment today is as strong as ever.

By sharing science-based information and education, the Institute fosters "Science for Better Nutrition" to contribute to the enhancement of the quality of people's lives all over the world.

LEARN

Access content from some of the world's most renowned experts through more than 3,000 nutrition publications and hundreds of exclusive video presentations.

UNDERSTAND

By providing a wide range of online e-learning courses, we bring you up to speed with the latest evidence-based scientific information.

GROW

Exclusive post-graduate training fellowships available in leading institutions, to support the development of the next generation of young health professionals.

Sign up today for free, and join a community of fellow healthcare professionals.

www.nestlenutrition-institute.org

Connect with a world of resources