

Prebiotics in human milk and infant formula: current scientific knowledge

Workshop report of a Scientific Group on Human Milk Research – a HiPP initiative –

CONTENT

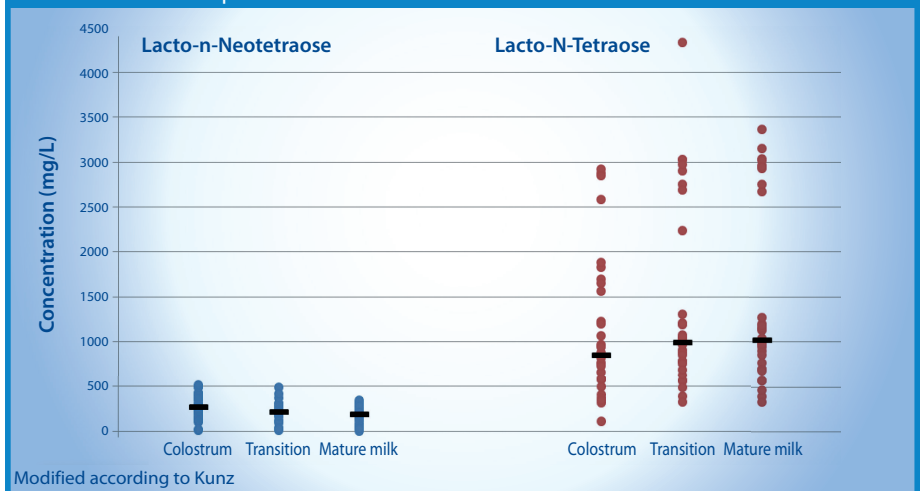
- Interaction of prebiotics and human milk oligosaccharides with the intestinal microbiota
- Clinical benefits of prebiotics in infant formula
- Benefits of prebiotic supplementation during pregnancy for neonates



The low infection rate of breastfed infants is closely connected to the prebiotically active oligosaccharides in breast milk. Over the last few years, a number of research groups have examined possible immunomodulatory effects of prebiotics. Within the framework of the “HiPP Scientific Group on Human Milk Research”, a European group of experts was invited and discussed recent findings on the importance of prebiotics in human milk and presented current data on efficacy and safety of prebiotics in infant formula and as a supplement for pregnant women.

In contrast to the extremely low levels of prebiotic oligosaccharides in cow's milk, human milk contains a large variety of complex oligosaccharides, ranging from 10–20 g/L. After lactose and lipids, human milk oligosaccharides (HMOs) are the third-most abundant component in human milk [1], explained Prof. Dr. Clemens Kunz of Gießen. The HMO concentration varies throughout lactation; colostrum has the highest content with approx. 20–25 g/L, while in mature breast milk the average concentration is 10–15 g/L [2, 3]. HMOs are composed of five monosaccharides: glucose, galactose, fucose, N-acetylglucosamine and sialic acid, mostly as N-acetylneuraminic acid. Until now, more than 150 different structures are found [4]. HMO can be subdivided into

Figure 1
Content of lacto-n-neotetraose (LNnT) and lacto-N-tetraose (LNT) in different lactation periods

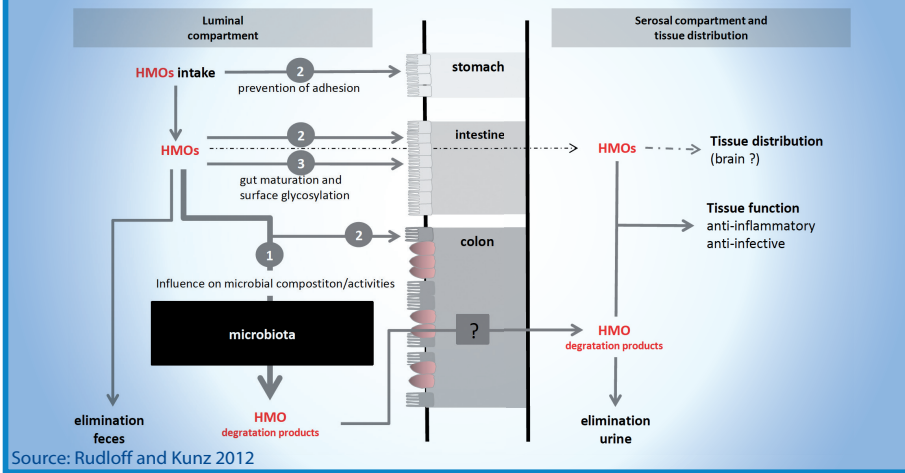


two main groups: about two thirds of HMO are neutral, fucosylated structures, the remaining third are acidic, sialylated compounds. Within the lecture Kunz presented human milk analyses showing concentrations of LNnT (lacto-N-neotetraose) and LNT (lacto-N-tetraose) during the first weeks of lactation (colostrum, transition and mature milk) (Fig. 1). The total content of these short-chain, neither fucosylated nor sialylated compounds, changed very little, despite large individual variations. However during lactation major changes in other milk oligosaccharides occur.

Considerable structural differences among individuals exist in the composition of HMO, because their existence is connected to genetically determined fucosyltrans-

ferases (FucT). These compounds are also involved in the synthesis of blood group antigens in the Lewis blood group system, and the release of blood group characteristics in body fluids (secretor status). Therefore, every human milk sample shows a specific oligosaccharide pattern [2, 3, 5] depending on the Lewis blood group and the secretor status of the mother. With over 70%, the Lewis blood group b secretors (Se+Le+) are the most common group in Europe. 2'-fucosyllactose is the most abundant HMOs among secretors, which, on the other hand, cannot be found in milk of non-secretors. One biological effect of HMOs is most likely their positive influence on the intestinal microbiota. However, only in vitro-study exist at present.

Figure 2
Proposed functions of human milk oligosaccharides



Postulated beneficial effects (Fig. 2):

- HMOs function as natural prebiotics; meaning that positive intestinal bacteria are stimulated to grow. However, this effect does not apply to all strains of bacteria. The interaction between HMOs and bacteria depends on the bacterium's genetic configuration
- A number of studies have shown that HMO interact with different pathogens directly. Because the structure of the HMO is similar to the receptors which are involved in adhesion of pathogenic bacteria, viruses and toxins to the intestinal cell wall, HMOs may function as a receptor decoys. Consequently, HMOs may prevent the adhesion of pathogenic microorganisms to the intestinal mucosa.
- The intestinal cell response is directly affected by HMOs, intervening in the gene expression of intestinal epithelial cells. This leads to changes in cell surface glycans and other cell response as shown in vitro and in some animal studies.

HMOs are seen by Prof. Kunz as an extremely promising future supplement for infant formula. At the time of the workshop, however, apart from animal studies, no data from human studies were available that proved the safety and benefits of using HMOs in infant formulae.

Conclusion: As human milk is the gold standard for future infant formula development, adding HMO to infant formulae is one key topic for improving nutrition of non-breastfed children.

Interaction of prebiotics with intestinal microbiota

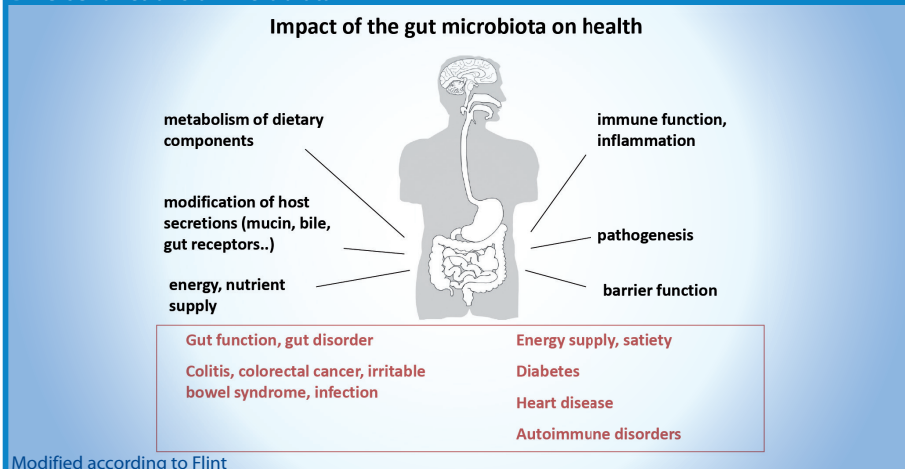
More than 95% of the intestinal microbiota, once known as intestinal flora, in an adult is anaerobes (1,011 bacteria/g stool). Over 80% of the microbiome belong to the group of gram-negative Bacteroidetes and gram-positive Firmicutes [6]. Together with Proteobacteria and

Actinobacteria these bacteria make up 99% of intestinal microbiota. The intestinal microbiome is responsible for various tasks in humans. Major function is to support digestion, but also modulation of the immune system is of great importance, as Prof. Dr. Harry J. Flint of Aberdeen, Great Britain, emphasised (Fig. 3). That includes, most importantly, metabolic functions, such as the formation of short-chain fatty acids (SCFA) and the maintenance of an intact intestinal barrier, thereby preventing the colonization and invasion of pathogenic bacteria in the intestine [6].

Apart from the type of delivery, genetic influences, the environment and possible antibiotic therapies, nutrition during the first months of life plays an essential role in the composition and functionality of the microbiome. The intestinal flora of a breastfed infant is dominated by bifidobacteria, whereas infants who receive formula have a higher percentage of representatives of the Bacteroides genus, which belong to the Bacteroidetes family [7, 8, 9]. The composition of the intestinal flora in breastfed infants leads to a variety of health benefits. [10]. The microbial composition of formula-fed infants is rather similar to the microbial intestinal communities in adults. During weaning and the introduction of solid food, the intestinal flora of breastfed and formula-fed infants becomes increasingly similar. At the end of the second year, a relatively stable microbiota composition is reached in both. Fluctuations between individuals, however, remain [7].

Prebiotics, which are of dietary origin, are of great importance with numerous positive effects on the intestinal microflora [11]. Prebiotics are non-digestible carbohydrates, that beneficially affect the host by selectively stimulating the growth and/or activity of colonic bacteria. [8, 9, 12, 13, 14]. Bifidobacteria (Actinobacteria) and lactobacilli (Firmicutes) metabolize them into mono-, di- or trisaccharides which

Figure 3
Diverse functions of microbiota



serve commensal, apathogenic intestinal bacteria as nutritive substrates [16]. In addition, fermentation of the prebiotics produces short chain fatty acids (SCFA) such as butyrate, acetate and propionate [8, 11, 13]. SCFA supply the colonic epithelium with energy, stimulate the proliferation and differentiation of the intestinal epithelial cells, and influence the mucosal blood flow and the production of mucus [11, 14]. The reduction of the intestinal pH inhibits the growth of pathogens. Prebiotics, therefore, provide a diversity of health-promoting effects through the promotion and stabilisation of the diversity of the microbiota, the prevention of an increase in pathogens and the formation of SCFA.

In recent years, a link has been discovered between the imbalance (dysbiosis) of individual main strains of bacteria and metabolic diseases such as adiposity and diabetes mellitus. According to the data from different studies referred to by Flint, obese patients exhibit a microbiota that differs from a normal flora. Changes in Firmicutes and Bacteroidetes are observed which leads to an altered enzymatic capacity [15, 16]. Consequently, the resulting digestion might be more efficient, thus increasing energy intake from food. It is not yet clear, whether the changes in the microbiota composition are the cause or the result of obesity or whether targeted administration of prebiotics is a potentially successful therapeutic approach. Clinical examinations of type-2 diabetic patients indicate that moderate microbial dysbiosis goes hand in hand with a reduction of butyrate-producing species and an increase in various opportunistic strains [17]. The authors of the study postulated that the microbiota could be a useful marker for the classification of type 2 diabetes. Also in patients with chronic inflammatory bowel disease such as Crohn's disease and Colitis ulcerosa, a severely altered microbiota can be found along with a

reduction of bacteria variety which results in stimulation of pro-inflammatory processes in the intestine [18, 19, 20].

Conclusion: Indigestible carbohydrates influence the composition of the intestinal flora and thus play a significant role on infant's health. Human milk oligosaccharides (HMOs) foster the diversity of the intestinal flora more than specific strains. SCFA are highly important for the organism, which can be modulated through the dietary supply of prebiotics.

Clinical benefit of prebiotics in infant formula

Supplementation of infant formula with prebiotics is an important field of research. Accordingly, over recent years, numerous studies in this field have been published. Prof. Dr. Hania Szajewska of Warsaw, Poland, as well as Prof. Dr. Seppo Salminen of Turku, Finland introduced the most relevant studies.

Systematic literature research by the Nutrition Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) examined 23 randomised, controlled trials on prebiotic supplementation in infant formulae. Typically, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), acidic oligo-saccharides (AOS), polydextrose (PDX) or mixtures of these are used [21]. According to the studies, there are no safety concerns with respect to the use of prebiotics. However, the Committee's conclusion is that the amount of data available at the moment is insufficient to recommend the routine use of prebiotic supplements in infant formula. In the future, validated clinical outcome measures assessing the effects of supplementation of formulae should be used in well-designed and carefully conducted trials, with relevant inclusion/exclusion criteria and adequate sample sizes as well as long-term follow-up observations.

To what extent prebiotics could be important in the prevention and/or therapy of some major diseases was the question of various trials presented by Szajewska and Salminen. Main topic were the influence of prebiotics in the development of allergies, the therapy and prophylaxis of diarrhoea and a possible influence on the immune system. The experts followed the opinion of the Cochrane Analysis carried out by Osborn et al. [22] (4 studies, n=1,428). For prebiotic supplements, there were some indications of a preventive effect on atopic eczema. It is unclear whether the use of prebiotics should be restricted to infants at high risk of allergy or may have an effect in low risk populations, or whether it may have an effect on other allergic diseases including asthma. The authors' conclusion is that, in summary, further studies are needed before routine use of prebiotics can be recommended for prevention of allergy in formula fed infants. To what extent early use of prebiotics could prevent intestinal and extraintestinal infections in healthy infants was also subject of Szajewska's and Salminen's lecture. Due to a number of methodological limitations to the study neither for the preventive nor for a therapeutic use a recommendation can be given.

A recently published meta-analysis from Srinivasjois et al. examined the safety and efficacy of infant formulae enriched with prebiotics in preterm infants (<37th week of pregnancy) [23]. Seven studies with a total of 417 preterm infants were taken into consideration, five studies (n=345) related to the incidence of a necrotizing enterocolitis (NEC) and three examined (n=295) the incidence of late-onset sepsis as the primary study parameter. A statistically significant difference was shown concerning the bifidobacterial colonization in the infants that were given prebiotics-supplemented formula, versus the control group (p<0.00001). The incidence of NEC or sepsis was not influenced by prebiotics.

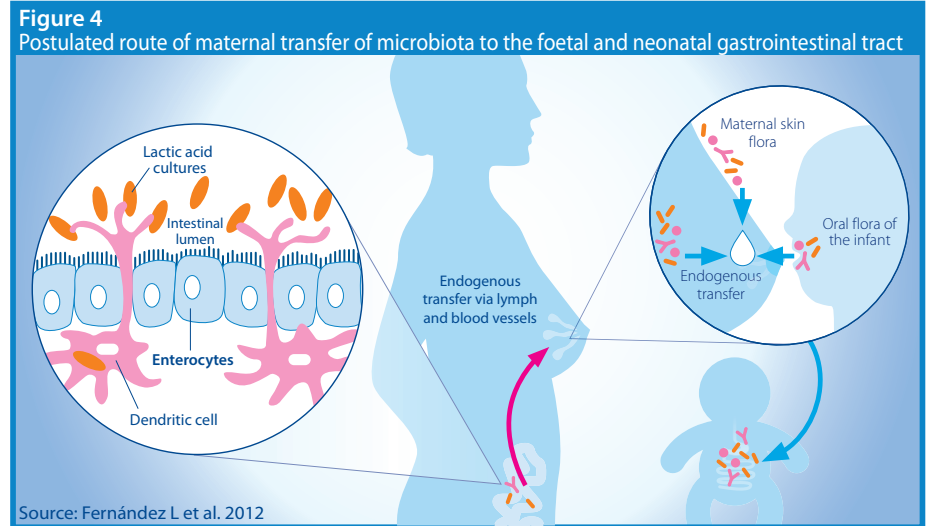
Conclusion: Prebiotics used at the moment are safe. There are also promising indications of positive effects. Prebiotics are an interesting field of research. Currently, there are too few reliable studies in order to recommend prebiotics in general. Results of long-term observations are also still unavailable.

Supplementing pregnant women with prebiotics and benefits for the newborn

About 10 years ago, it was assumed that the intestine of the foetus was microbiologically sterile, and that bacterial colonization only occurred during vaginal birth through contact with the maternal vaginal and rectal flora. Prof. Dr. Catherine Michel of Nantes, France, presented recent data indicating that a child's intestinal colonisation already begins during pregnancy [24, 25, 26]. Bacteria from the maternal gastrointestinal tract and/or urogenital tract, such as *Enterococcus* species and *Lactobacillus* species, were detected in umbilical cord blood as well as in amniotic fluid without any clinical evidence of infection [26, 28, 29]. These findings are an indication that the maternal microbiota is transferred to the foetus via the placental barrier. Current knowledge states that four phases of development and maturation of foetal/neonatal microbiota can be identified, which partially depend on maternal factors (Fig. 4) [24]:

- trans-placental transfer of microbiota to the foetus,
- foetal ingestion of amniotic fluid,
- bacterial colonization during birth,
- intake via breast milk.

The exact mechanisms of the transfer of maternal microbiota to the foetus and breast milk have not yet been fully clarified [24]. A considerable role is assumed to play the dendritic cells in the Peyer's patch and the lymphocytes [24, 27]. Maternal bacteria could translocate through the intestinal epithelial barrier and migrate to the mammary



glands via an endogenous cellular route. Through the lymphocytes circulating within the mucosa, the maternal microbiota gets into the bloodstream and reaches the foetus through the mammary glands and the placental barrier (enteromammary pathway).

These findings raised the question of how prebiotic supplementation during pregnancy could be a possible option for positively influencing the maternal intestinal microbiota during the peri- and postnatal periods and, in this way, to achieving a long-term benefit for the child's health. Currently, however, there are only a few human studies which confirm this hypothesis. In a randomised, double-blind and placebo-controlled trial with 48 pregnant women, Shahid et al. examined the influence of administration of different concentrations of GOS/FOS or maltodextrin (placebo) from the 25th week of pregnancy until delivery, on maternal and neonatal microbiota and child immunity [28]. The prebiotic supplementation showed a significant bifidogenic effect on the maternal microbiota, but this was not transferred to the neonate. In addition, infant's immune markers, evaluated by various immunological parameters in umbilical cord blood, were similar in both groups. Birth weight and length were also not different. A Japanese study, on the other hand, showed a statistically significant influence on immunoregulatory parameters after the administration of fructo-oligosaccharides to pregnant and breastfeeding women. The

supplementation led to a significant increase in interleukin (IL) 27, which is involved in the formation of B and T lymphocytes in breast milk [29].

Conclusion: Further research is needed to answer the question as to what extent intervention with prebiotics during pregnancy influences infant's health.

Further perspectives

As limited data on the benefit of prebiotic-enriched infant formulae is available, further, better designed studies with clearly defined outcome parameters are needed. This task is being addressed by the Consensus Group on Outcome Measures Made in Paediatric Enteral Nutrition Clinical Trials (COMMENT). Participants from the Early Nutrition Academy and the Child Health Foundation, in collaboration with the Committee on Nutrition, Hepatology and Nutrition (ESPGHAN) settled six working groups to improve evidence on the effects of nutritional interventions in infants and young children. Aim is to identify and define criteria for assessing key outcomes as well as update a list of core data that should be reported in nutritional trials (30).

Prebiotics, in addition to probiotics, provide another beneficial way of modulating the intestinal flora to positively influence health. However, prebiotics will only provide growth to those strains that are already present in the intestine. Since the effects of prebiotics are partially non-specific, a possibility to improve infant formula is to supplement both pre- and probiotics. First

HiPP initiative: HiPP Scientific Group on Human Milk Research

Since human milk supports the growth and development of infants in the most natural way, it has always been the gold standard for the manufacturing of infant formula. For that reason, the human milk research group, which was initiated by the HiPP infant food Company, focuses on the composition and health benefits of human milk with the goal to develop infant formula products that in its functionality and composition are closer to human milk than ever before. In recent years, since the initiation of the research group, the knowledge gained has been integrated in the development of new HiPP products. The research group meets regularly to address current topics presented by invited speakers. Together with

representatives of the HiPP Nutrition and Product Development Department, Prof. Dr. Clemens Kunz from the department of human nutrition at the University of Giessen in Germany, Prof. Dr. Harry J. Flint from the Microbiology Group at the Rowett Institute at the University of Aberdeen in Great Britain, Prof. Dr. Seppo Salminen Director of the Functional Foods Forum at the University of Turku in Finland; Prof. Dr. Hania Szajewska medical Director of the children's hospital at the medical school in Warsaw, Poland und Prof. Dr. Catherine Michel from the Université de Nantes in France met to discuss the topic of prebiotics in human milk and their relevance and significance in infant nutrition and development.

HMOs to infant formula is desirable. However, at present HMOs are not available commercially to be added to infant formula in the amounts needed. Therefore, currently infant formula is enriched with either oligosaccharides extracted from cow's milk called galacto-oligosaccharides or from oligosaccharides derived from plants called fructo-oligosaccharides. The structure and mechanism of these prebiotic substances is not comparable to human milk oligosaccharides, but nevertheless can be classified as safe. With regard to the effectiveness of prebiotics, further studies, especially those with long-term follow-ups, are desirable.

Imprint

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Speakers:

Prof. Dr. Clemens Kunz,
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Prof. Dr. Harry J. Flint,
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Prof. Dr. Seppo Salminen,
Turku, Finland

Prof. Dr. Hania Szajewska,
Warsaw, Poland

Prof. Dr. Catherine Michel,
Nantes, France

studies show that the combined addition of pro- and prebiotics to infant formulae is safe and effective [31, 32].

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Human milk contains both lactic acid bacteria and human milk oligosaccharides (HMOs). At present HMOs are not available in sufficient amounts to add them to formulae. Therefore, infant formula is enriched with either oligosaccharides extracted from cow's milk called galacto-oligosaccharides or from oligosaccharides derived from plants called fructo-oligosaccharides. The structure and mechanism of these prebiotic substances is not comparable to human milk oligosaccharides, but nevertheless can be classified as safe. With regard to the effectiveness of prebiotics, further studies, especially those with long-term follow-ups, are desirable.

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Conclusions

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