Intestinal microbiology in early life: specific prebiotics can have similar functionalities as human-milk oligosaccharides\textsuperscript{1–4}

Raish Oozeer, Kees van Limpt, Thomas Ludwig, Kaouther Ben Amor, Rocio Martin, Richele D Wind, Günther Boehm, and Jan Knol

ABSTRACT

Human milk is generally accepted as the best nutrition for newborns and has been shown to support the optimal growth and development of infants. On the basis of scientific insights from human-milk research, a specific mixture of nondigestible oligosaccharides has been developed, with the aim to improve the intestinal microbiota in early life. The mixture has been extensively studied and has been shown to be safe and to have potential health benefits that are similar to those of human milk. The specific mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides has been found to affect the development of early microbiota and to increase the Bifidobacterium amounts as observed in human-milk–fed infants. The resulting gut ecophysiology is characterized by high concentrations of lactate, a slightly acidic pH, and specific short-chain fatty acid profiles, which are high in acetate and low in butyrate and propionate. Here, we have summarized the main findings of dietary interventions with these specific oligosaccharides on the gut microbiota in early life. The gut ecophysiology in early life may have consequences for the metabolic, immunologic, and even neurologic development of the child because reports increasingly substantiate the important function of gut microbes in human health. This review highlights major findings in the field of early gut colonization and the potential impact of early nutrition in healthy growth and development. Am J Clin Nutr 2013;98(suppl):561S–71S.

INTRODUCTION

Exclusive human-milk feeding is widely recommended as the first choice for infant nutrition. Human milk provides not only optimal nutrition but also bioactive components that are important to optimize gut microbial colonization, immune maturation, metabolic development, and even cognitive development. The beneficial effects of human milk have recently been reviewed by the ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology and Nutrition) committee, and it is acknowledged that it can, for example, reduce the risk of infectious diarrhea and acute otitis media (1). Emerging evidence also indicates a potential role in lowering the risk of chronic diseases such as obesity, allergy, cardiovascular disease, or diabetes (2, 3).

Because human-milk feeding may not always be possible, human-milk substitutes have to provide nutritional and functional properties as close as possible to those of human milk. Major analytic efforts have been made to characterize the composition of human milk to identify relevant bioactive molecules. Human milk differs substantially from cow milk, which is generally the basis for infant formulas. Although nondigestible oligosaccharides (NDOSs)\textsuperscript{5} are virtually absent from cow milk, they represent the third most abundant fraction after lactose and lipids in human milk. Human-milk oligosaccharides (HMOSs) from an individual sample of human milk can already contain a repertoire of >1000 distinct molecules (4). The first safe infant formulas based on cow milk were introduced in the early 19th century; however, NDOSs were not incorporated until the end of the 20th century. The concept of prebiotic, defined as nondigestible oligosaccharides that reach the colon intact and known for their ability to selectively stimulate the growth and activity of bacteria that exert positive health effects, has been added to a new generation of infant nutrition (5). The first specific mixture was composed of short-chain galacto-oligosaccharides (scGOSs) and long-chain fructo-oligosaccharides (lcFOSs). Since 2002, several clinical investigations with scGOS+lcFOS have been performed. These showed the safety and efficacy of this specific prebiotic mixture toward the prevention of allergies and infections in newborns, with effects that lasted beyond the intervention period. Results of these studies provided evidence of the clinical efficacy of scGOS+lcFOS in different groups of infants (Table 1) (4). In summary, it has been shown that scGOS+lcFOS can support the development of the immune system as shown in clinical studies with reduced incidence of atopic dermatitis and decreased episodes of infections and antibiotic use (15, 16, 18, 22). Clinical data also showed that supplementation with scGOS+lcFOS increased stool frequency and stool softness in both infants and healthy volunteers (22).

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\textsuperscript{3}All experiments described in this article were funded by Danone Research.

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\textsuperscript{5}Abbreviations used: HMOS, human-milk oligosaccharide; lcFOS, long-chain fructo-oligosaccharide; NDOS, nondigestible oligosaccharide; SCFA, short-chain fatty acid; scGOS, short-chain galacto-oligosaccharide; SFB, segmented filamentous bacteria; sIgA, secretory IgA.

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TABLE 1
Overview of results of randomized, double-blind, placebo-controlled trials that used a prebiotic scGOS+lcFOS mixture (9:1) in term infants who received nutritional intervention, including gut microbiota monitoring, during the first year of life

<table>
<thead>
<tr>
<th>Intervention and study groups (country)</th>
<th>Results</th>
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<tr>
<td>IF with partially hydrolyzed protein, 0.8 g scGOS+lcFOS/100 mL, high β-palmitic acid and starch or control formula without prebiotics; 3 mo; 102 healthy term infants; breastfed group as reference (Germany)</td>
<td>Higher bifidobacteria counts ($P = 0.002$) and softer stools ($P = 0.005$) in scGOS+lcFOS group; no clinically significant differences in blood biochemical and amino acid values</td>
<td>Schmelzle et al, 2003 (6)</td>
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<td>IF with 0.8 g scGOS+lcFOS/100 mL; $6 \times 10^{15}$ CFU/L Bifidobacterium animalis strain Bb-12 or control without prebiotics; 16 wk; breastfed group as reference; 120 healthy infants (Netherlands)</td>
<td>Higher fecal acetyl ratio ($P &lt; 0.05$) and higher fecal lactate concentration and lower pH ($P &lt; 0.05$) for scGOS+lcFOS groups; scGOS+lcFOS group similar to HM-fed group</td>
<td>Bakker-Zierikzee et al, 2005 (7)</td>
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<td>IF with 0.8 g scGOS+lcFOS/100 mL or control without prebiotics; 6 wk; HM-fed group used as reference; 68 healthy term infants aged 28–90 d (Germany)</td>
<td>Trend toward higher fecal sIgA concentrations in prebiotics group; highly variable sIgA in probiotics-fed infants was not significantly different from standard cow-milk-formula–fed group; sIgA concentrations in HM-fed infants higher than those in formula-fed infants</td>
<td>Bakker-Zierikzee et al, 2006 (8)</td>
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<tr>
<td>IF with 0.8 g scGOS+lcFOS/100 mL, high β-palmitic acid and starch or control</td>
<td>Significant increase in lactobacilli in scGOS+lcFOS and HM-fed group; Lactobacillus species distribution in scGOS+lcFOS group similar to that in HM-fed infants</td>
<td>Haarman and Knol, 2006 (9)</td>
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<td>with partially hydrolyzed protein, 0.8 g scGOS+lcFOS/100 mL, or control without prebiotics; 1 mo; 90 healthy term infants (Italy)</td>
<td>Higher mean proportion of bifidobacteria ($P &lt; 0.05$) and lower stool mean pH ($P &lt; 0.001$) in scGOS+lcFOS group; increased proportion of acetate ($P &lt; 0.001$) and lactate ($P &lt; 0.001$) and decreased proportion of propionate ($P &lt; 0.001$) with scGOS+lcFOS</td>
<td>Knol et al, 2005 (10)</td>
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<td>IF with 0.4 g scGOS+lcFOS/100 mL, 0.8 g scGOS+lcFOS/100 mL, or control without prebiotics; 1 mo; 97 healthy term infants (Netherlands)</td>
<td>Increase in the total amount of fecal bifidobacteria in the prebiotics group ($P = 0.047$); relatively high amounts of Bifidobacterium breve, Bifidobacterium longum subsp. longum/infantis</td>
<td>Haarman and Knol, 2005 (11)</td>
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<td>IF with 0.4 g scGOS+lcFOS/100 mL or control; breastfed group as reference; 3 mo; 97 healthy term infants (Hungary)</td>
<td>Dose-dependent increase in bifidobacteria ($P &lt; 0.01$) and decrease in fecal pH ($P &lt; 0.05$); increase in lactobacilli in both supplemented groups ($P &lt; 0.001$); dose-dependent change in stool consistency toward softer stools; increase in stool frequency with high doses ($P &lt; 0.001$)</td>
<td>Moro et al 2002, 2003 (12, 13)</td>
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<td>Hypoallergenic formula with extensively hydrolyzed cow-milk whey protein with 0.8 g scGOS+lcFOS/100 mL or control without prebiotics; 6 mo; 259 term infants at risk of atopy (Italy)</td>
<td>Significantly higher bifidobacteria ($P &lt; 0.05$) in scGOS+lcFOS group; no difference in atopic manifestations</td>
<td>Decsi et al, 2005 (14)</td>
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<td>Five-year follow-up in 89 children showed lower incidence of AD, recurrent wheezing, and allergic urticaria were lower in the intervention group ($P &lt; 0.05$); fewer episodes of physician-diagnosed overall and upper respiratory tract infections ($P &lt; 0.01$), fewer fever episodes ($P &lt; 0.00001$) and fewer antibiotic prescriptions ($P &lt; 0.05$)</td>
<td>Reduced incidence of AD ($P = 0.014$) in prebiotics group; subgroup analysis ($n = 98$) showed higher fecal bifidobacteria at 3 and 6 mo ($P &lt; 0.0001$); significantly increased stool frequency and softer stools at 3 and 6 mo; significantly less regurgitation and crying</td>
<td>Arslanoglu et al, 2007 (16)</td>
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<td>May 2005 (11)</td>
<td>Fewer episodes of infections (all types) ($P = 0.01$); trend for fewer episodes of upper respiratory tract infections ($P = 0.07$) and trend for fewer infections requiring antibiotics ($P = 0.10$) in scGOS+lcFOS groups</td>
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<td>Two-year follow-up in 134 infants showed that cumulative incidence of AD, recurrent wheezing, and allergic urticaria were lower in the intervention group ($P &lt; 0.05$); fewer episodes of physician-diagnosed overall and upper respiratory tract infections ($P &lt; 0.01$), fewer fever episodes ($P &lt; 0.00001$) and fewer antibiotic prescriptions ($P &lt; 0.05$)</td>
<td>Reduction in plasma concentrations of total IgE, IgG1, IgG2, and IgG3 ($P &lt; 0.01$ in all cases) in the scGOS+lcFOS group; no effect on IgG4 was observed; cow milk protein–specific IgG1 decreased ($P &lt; 0.05$)</td>
<td>Arslanoglu et al, 2008 (18)</td>
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<td>Five-year follow-up in 89 children showed lower incidence of AD, allergic rhinitis, and allergic urticaria were lower in the intervention group ($P &lt; 0.05$ for all); no difference in recurrent wheezing</td>
<td>No statistically significant differences in atopic manifestations and quality of life</td>
<td>Arslanoglu et al, 2012 (19)</td>
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(Continued)
term and preterm infants, similar to what is observed in human-milk–fed infants (12).

Here we review the effects of scGOS+lcFOS on the developing gut microbial ecosystem in infants. Similarities in the impact of human milk and scGOS+lcFOS on the gut microbiota composition and functionality may explain the clinical benefits of this specific prebiotics mixture.

HUMAN MILK AND FORMULAS CONTAINING scGOS+lcFOS AFFECT THE EARLY MICROBIOTA COMPOSITION AND ACTIVITY IN A SIMILAR MANNER

The human body hosts ~100 trillion bacteria, most of which find their niche in the intestine. The intestinal microbiota represents 10 times the number of cells of the human body and comprises ~50% of fecal matter (23–25). At birth and immediately thereafter, microbes colonize the digestive tract, reaching high numbers within a few days, with the highest amounts in the distal part of the colon (26). The establishing microbiota is considered to be important for intestinal homeostasis. It has been found to affect the concomitant development of the immune system, a cross-talk that is critical in building the host-microbiota symbiosis. This symbiosis is important even in later life because the microbes contribute to the degradation of food-derived and endogenous constituents, to the supply of metabolites serving as an energy source for enterocytes, to detoxification of xenobiotic compounds, to bioactivation of beneficial constituents such as polyphenols, to the basal stimulation of the immune system and epithelium, and to the prevention of the colonization by, or excessive development of, pathogenic microorganisms (27, 28).

Early-life microbial colonization can be regarded as crucial for healthy development. First, adverse environmental factors can irreversibly affect changes in tissue structure, gene expression patterns, tolerance induction, and physiologic functions postnatally and increase the risk of disease later in life (29, 30). Second, the first microbial colonizers may shape the early and later ecosystem and setting the base for the core components of the microbiota in later life (31).

Because human milk is the reference for infant nutrition, its impact on microbial colonization and the consequence on the gut physiology have been extensively studied over the past decades; and nutritional strategies that may mimic the functionalities of human milk have been investigated (32, 33). As mentioned above, specific oligosaccharides, which act as selective microbiota substrates, have been considered to be the most efficient in modulating microbial composition and activity in a way that is similar to human milk.

Early development of intestinal microbiota in newborns

Because the newborn immune system progressively develops after birth, the digestive tract is a highly permissive environment where diverse bacterial populations quickly develop. Nevertheless, bacterial colonization follows a relatively consistent and ordered pattern, under the influence of exogenous and endogenous factors. Exogenous factors include exposure to microorganisms from maternal origin such as gut, vaginal canal, or skin but also the environment in general. A few studies have suggested that maternal milk could convey microorganisms from mother to infant (34–37). Exogenous factors also encompass the mode of delivery (vaginally or via cesarean section), the type of feeding, and antibiotic or drug use (38–43).

Between the ages of 2 and 3 y, a more functionally stable microbiota that is more similar to that of adults is established (44). Early colonization is driven by a sequential process, and in general, the “pioneer” organisms that colonize an infant’s intestine are facultative anaerobic bacteria (Escherichia coli and Streptococci) that gradually consume all oxygen in the environment (45, 46). Bifidobacterium, Bacteroides, and Clostridium are then able to grow in the gut 1 wk after birth. Generally, in human-milk–fed infants, Bifidobacterium species become dominant, and infants are also colonized to a lesser extent by facultative anaerobes. Recent investigations have not always confirmed the bifidogenic effect of human milk (39, 47). However, these controversial observations may result from technical challenges, because the DNA sequencing tools not properly detect Bifidobacterium species and may require further optimization (48).

The gut microbiota of formula-fed infants, not supplemented with prebiotics, is generally not dominated by Bifidobacterium species, which leaves a niche for the colonization of a more diverse microbiota, which can include bacterial groups such as Bacteroides, Clostridium, and Enterobacteriaceae (26, 43, 49).

Influence of scGOS+lcFOS on early microbial colonization

Since 2002, studies have investigated the effect of a specific mixture of prebiotics, scGOS+lcFOS, on the composition of the intestinal microbiota in preterm, term, and weaning infants (4, 6, 12, 50, 51). These studies showed that scGOS+lcFOS affects the

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**TABLE 1 (Continued)**

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<td>IF with 0.4 g scGOS+lcFOS/100 mL or control without prebiotics; 66 healthy term infants (Greece)</td>
<td>Increase in <em>Bifidobacterium</em> genus (<em>P</em> = 0.0001); decrease in <em>Bacteroides</em> groups (<em>P</em> = 0.02) and <em>Clostridium coccoide</em> group (<em>P</em> = 0.01) in the prebiotics group</td>
<td>Magne et al, 2008 (21)</td>
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<td>IF with 0.6 g scGOS+lcFOS/100 mL or control without prebiotics; 82 healthy term infants (Algeria)</td>
<td>Higher stool frequency (<em>P</em> = 0.031) and softer stools (<em>P</em> = 0.026) in the scGOS+lcFOS group; fecal clostridia lower (<em>P</em> = 0.042); no significant changes in bifidobacteria and <em>Escherichia coli</em> counts</td>
<td>Costalos et al, 2008 (20)</td>
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1 AD, atopic dermatitis; CFU, colony forming units; HM, human milk; IF, infant formula; lcFOS, long-chain fructo-oligosaccharide; scGOS, short-chain galacto-oligosaccharide; sIgA, secretory IgA.
early microbial pattern in a way similar to human milk with an intestinal microbiota enriched with *Bifidobacterium* and *Lactobacillus* (12). A more detailed analysis of these bacterial genera has shown that, even at the species level, the microbial composition in human-milk–fed and scGOS+lcFOS-fed infants was very similar but different from that in formula-fed infants (9, 11, 52). The microbiota of formula-fed infants contains relatively more *Bifidobacterium catenulatum* and *Bifidobacterium adolescentis*, 2 species that are commonly found in adults (Figure 1). Similarly, infant formula is more favorable for *Lactobacillus delbrueckii* but promotes less *Lactobacillus acidophilus* and *Lactobacillus paracasei* compared with human milk. The selective increase in specific species of *Bifidobacterium* seems to be crucial for gut physiology because ingestion of *Bifidobacterium animalis* species alone results in a microbial metabolic activity that more closely resembles that in formula-fed infants (7). *Bifidobacterium* species are the first utilizers of NDOs (53), but the resulting metabolites from this primary fermentation step could affect the ecosystem through the cross-feeding process between different microbes. Compared with formula-fed infants, several bacterial groups such as *Clostridium coccoides/Eubacterium rectal cluster, Clostridium*, and *E. coli* species are significantly decreased in human-milk–fed infants (49). The numbers of several pathogenic bacteria present in the subdominant population are also significantly less when formula containing scGOS+lcFOS is consumed (51).

The introduction of solid foods at the weaning period coincides with an increase in some species in the microbiota, such as plant polysaccharide degraders (eg, *Bacteroides*) (54). Even in a more complex microbial environment during the weaning period, the addition of scGOS+lcFOS to solid foods induces an increase in the fecal proportion of *Bifidobacterium* in the intestinal microbiota (55). More recently, it has been shown that the microbial changes associated with scGOS+lcFOS during the first 6 mo of life may have a long-lasting effect. Significant differences continue to be observed after the weaning period, at age 1 y, without further supplementation (56). These data show that dietary intervention in a period of life when the microbiota is still developing may have substantial consequences on microbial equilibrium. The so-called programming of the microbiota is of major interest because the composition of the stable adult microbiota is increasingly associated with susceptibility to disease (57–60).

**Changes in early-life microbial composition by scGOS+lcFOS consumption coincides with metabolic modulation of the microbiota**

Bacterial fermentation of nondigestible carbohydrates and proteins in the colon results in the production of short-chain fatty acids (SCFAs) and lactate, which is directly associated with the amount of luminal pH. The early microbiome is already enriched in genes facilitating lactate utilization and in functional genes involved in different types of polysaccharide metabolism (54, 61). Several studies have shown major differences in the profile of SCFAs in feces of human-milk–fed infants compared with formula-fed infants. Feces from human-milk–fed infants contain mainly acetic and lactic acid, essentially derived from the metabolic activity of bifidobacteria. Whereas small amounts of propionic acid and butyric acid are detected in human-milk–fed infants, these SCFAs are abundant in formula-fed infants due to high numbers of bacteria capable of producing propionate or butyrate from lactate (44). The impact of scGOS+lcFOS on microbial composition coincides with changes in SCFAs, lactate, and pH in the infant gastrointestinal tract that resemble the fermentation pattern generated by human milk (10). The development of tools such as metagenomic sequencing allows the identification of the complete gene repertoire of the human intestinal microbiota in a given sample (23). These tools provide for the first time large-scale information on a functional level, even though the presence of genes is not necessarily indicative of the gene products being present and active. An understanding of the gene repertoire of the human intestinal microbiota allows the design of novel tools for functional mapping at the level of

![Figure 1. Bifidobacterium species in the stools of infants fed with either human milk, scGOS+lcFOSs, or a standard infant formula. The lower pie chart represents the proportion of the Bifidobacterium as a percentage of total bacterial load measured with fluorescence in situ hybridization. The upper pie charts show the proportion of different species after the subtraction of Bifidobacterium infantis, which is a dominant species with similar proportion in all groups. Analyses were performed by real-time polymerase chain reaction in 10 infants of 1–3 mo per each group. The intervention period was 6 wk. Significant differences were observed between human-milk or prebiotics groups and standard infant formula groups for Bifidobacterium catenulatum and total Bifidobacterium (paired-samples t test, P < 0.05). Adapted with permission from reference 51.](https://academic.oup.com/ajcn/article-abstract/98/2/561S/4577351/564S)

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**FIGURE 1.** *Bifidobacterium* species in the stools of infants fed with either human milk, scGOS+lcFOSs, or a standard infant formula. The lower pie chart represents the proportion of the *Bifidobacterium* as a percentage of total bacterial load measured with fluorescence in situ hybridization. The upper pie charts show the proportion of different species after the subtraction of *Bifidobacterium infantis*, which is a dominant species with similar proportion in all groups. Analyses were performed by real-time polymerase chain reaction in 10 infants of 1–3 mo per each group. The intervention period was 6 wk. Significant differences were observed between human-milk or prebiotics groups and standard infant formula groups for *Bifidobacterium catenulatum* and total *Bifidobacterium* (paired-samples *t* test, *P* < 0.05). Adapted with permission from reference 51.
metagenome, metatranscriptome, and metaproteome. Further investigation by using so-called omics tools (62) is needed to get a complete picture of the potential of the microbiota and of its consequences on health (54, 61).

It can be concluded that human milk and the specific mixture of scGOS+lcFOSs significantly affect the microbial ecosystem during early life in a similar manner. As a result, and through microbe-microbe interactions, a very specific ecosystem enriched with acetate and lactate and with a low pH and limited microbial diversity is established before the introduction of solid foods. This early modulation of the microbiota may imprint on the body’s ecosystem because the effects may still be maintained in later life and will have a significant impact on host physiology during infancy and beyond.

**COLONIZATION RESISTANCE TO PATHOGENS INDUCED BY THE COLONIC FERMENTATION OF HMOs AND SCGOSs+LCFOSs**

It has been acknowledged for several decades that the intestinal microbiota has an important function in protecting the host from colonization by enteric pathogens (63). The development of a well-adapted microbial community in the gut is the result of a dynamic equilibrium between the different gut microbes and available nutrients. Any exogenous microbes that attempt to colonize the gut environment will have to face a barrier effect established by the resident microbiota, which is defined as “colonization resistance” (64). Several studies that used gnotobiotic mice, which are colonized by few bacterial species, have shown that the amount of exogenous microbes needed to colonize the gut of these animals compared with the conventional amount can be 1000- to 100,000-fold lower (65). This “colonization resistance” concept has been reviewed (63, 66), but its basic mechanisms remain to be elucidated.

As described above, the use of human milk or formula with prebiotics for infant nutrition can reduce the number of enteric pathogens (51). Interestingly, the decreased amount of pathogens correlates with positive clinical outcomes on acute diarrhea incidence and use of antibiotics (22, 67). The mechanisms behind the higher colonization resistance observed in infants supplemented with human milk or the specific prebiotics could result from direct inhibition of pathogens in the gut or the stimulation of the innate and adaptive immunity response through commensal bacteria.

Human milk contains many antimicrobial factors, such as partially digested or fermented peptides, milk-borne fatty acids, human lactoferrin, lysozyme, and secretory IgA (sIgA) (68–70). These are well-known factors that may decrease the prevalence of pathogens in the gut’s ecosystem in infants. The broad range of NDOSs specifically found in human milk but not in other mammals’ milk (32, 71) is a major factor in the prevention of pathogen growth in the gastrointestinal tract. First, specific HMOs may mimic ligands by binding to certain cell surface glycan epitopes of the intestinal epithelial cells or mucus. Many enteric pathogens of bacterial and viral origin need these binding sites to initiate their pathogenesis. The presence of these glycans in human milk has been shown to reduce the risk of diarrhea as well as the incidence of respiratory diseases (72). Similarly, GOS has been shown to display antiadhesive properties against enteropathogenic *E. coli*, *Salmonella*, *Vibrio cholerae* toxin binding and *Chronobacter sakazakii* (73–76).

However, the largest impact of HMOs on human health derives from their function as substrates for specific endogenous bacteria such as *Bifidobacterium* species commonly found in the distal gastrointestinal tract of infants (77, 78), such as *Bifidobacterium longum* subsp. *infantis*. The genomic analysis of different bifidobacteria species such as *Bifidobacterium breve*, *Bifidobacterium bifidum*, and *B. longum* subsp. *longum* has shown that these species are efficient with regard to the use of intact HMOs (53, 79), whereas other bifidobacteria species not commonly found in infants, such as *B. animalis* and *B. adolescentis*, are less efficient in degrading HMOs (80). Most of the microorganisms in the gastrointestinal tract do not have the enzymatic potential to use HMOs, except for the *Bacteroides* genus, which harbors metabolic pathways and metabolizes mucous components. Interestingly, prominent neonatal gut *Bacteroides* species such as *Bacteroides thetaiotaomicron* and *Bacteroides fragilis* have been described to use the mucus-utilization pathways to metabolize HMOs (78, 81).

To determine how the fermentation of HMOs or prebiotics can affect the microbial ecosystem in infants and thereby contribute to colonization resistance, we have mimicked, in vitro, the ecosystem commonly found in human-milk–fed infants by combining SCFAs and lactate at several concentrations and at different pH conditions. The unpublished observations (K. van Limpt, 2007) from these experiments show that the combination of low pH (5.5) with individual SCFAs and l-lactate induces a highly dose-dependent inhibition on potential pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Acinetobacter* sp., or *Streptococcus agalactiae*. Overall, the strongest inhibitory effect was observed with a combination of acetate and a pH of 5–5.5, as commonly found in human-milk–fed infants (7, 44, 82–87). Interestingly, the growth of different *Bifidobacterium* species commonly found in infants, such as *B. breve* strains, was instead stimulated by the ecosystem commonly found in human-milk–fed infants. In summary, these unpublished data support the notion that the physiologic conditions (high acetate and low pH), which result from the direct colonic fermentation of HMOs in the colon, are a major driver of the bacterial composition at the species or even the strain level.

**MODULATION OF GASTROINTESTINAL PHYSIOLOGY BY COLONIC FERMENTATION**

The nondigestibility of the scGOS+lcFOS mixture implies, in principle, ample opportunity of direct interaction with the digestive tract’s surface as described by Georgi et al elsewhere in this supplement (88). With regard to the direct physical interaction of prebiotics as reviewed by Jeurink et al (89), it has been shown, for HMOs, that these can directly modulate the function of immune cells (90, 91). For scGOSs+lcFOSs, it is well recognized that these have the ability to beneficially shape host microbe interactions. This is most probably linked to their structural similarity to receptors on intestinal epithelial cells (32, 75, 92). As such, it has been found in vitro that GOS reduces the adherence of enteropathogenic *E. coli* and *Cronobacter sakazakii* to intestinal epithelial cells and cholera toxin to its receptor (32, 74, 75). However, little is still known about possible direct short- and long-term effects on the gastrointestinal tract itself.
As described above, the scGOS+lcFOS mixture significantly affects gut microbiota functionalities and metabolites. The latter are known to directly affect the host’s digestive system and metabolism. The total and relative amount of SCFAs that are generated from fermentable fibers such as scGOSs and lcFOSs inside the intestine are modulated by various factors, such as the site of fermentation, the local microbiota composition, its specific functionality, and other dietary factors (93). The fermentation of prebiotics takes place mainly in the proximal colon, and fecal concentrations of SCFAs may thus depend partly on gut transit time (94, 95). Due to the rapid absorption of SCFAs, their luminal concentrations inside the gut are usually low (95–97) and provide an indication regarding the amount of SCFAs produced by the microbiota.

Furthermore, supplementation with scGOSs+lcFOSs has been proven in several clinical trials to induce a fecal SCFA pattern in formula-fed infants closer to that in human-milk–fed infants (10) with relatively more acetate and less propionate. Given the distinct properties of different SCFAs, the observed changes are particularly relevant. The biological effect depends equally on the type of a specific acid but also on its absolute concentration inside the intestine are modulated by various factors, such as the colonic epithelium, which explains its rather low concentrations in the portal vein (104–106). Butyrate has been shown to have beneficial effects on gut biology, ranging from inhibition of inflammation and carcinogenesis to improving gut barrier function (94, 107–109). Direct colonic application of physiologic concentrations of SCFAs (103) and provide an indication regarding the amount of SCFAs produced by the microbiota. Butyrate is, for example, metabolized predominantly by the colonic epithelium, which explains its rather low concentrations in the portal vein (104–106). Butyrate has been shown to have beneficial effect on gut biology, ranging from inhibition of inflammation and carcinogenesis to improving gut barrier function (94, 107–109). Direct colonic application of physiologic concentrations of butyrate via enemas appears to decrease visceral sensitivity even in healthy volunteers (110). Note that fecal butyrate concentrations in human-milk–fed infants have been found to be lower than in formula-fed infants, and therefore the activity related to butyrate may be not appropriate for an immature gut (10). The significance of this finding remains to be elaborated, however, because SCFAs have been found to have a broad impact on metabolism and gut physiology beyond local effects. Most surprisingly, this can even be observed on parenteral administration of SCFAs (111–116). As such, it has been shown that parenteral SCFA administration stimulates glucagon-like peptide 2 from enteroendocrine cells (117). Glucagon-like peptide 2 has been credited as a driver of intestinal proliferation and differentiation (118). It can thus be hypothesized that the production of SCFAs does not only positively influence local metabolic processes in the large intestine but that it likely has a major effect on the development of the digestive system as such.

Compared with the extensively reported beneficial local and metabolic effects of butyrate, the knowledge on the functional impact of propionic and acetic acid is rather concise. The relative higher amount of fecal acetate in scGOS+lcFOS-fed infants compared with that in formula-fed infants without this prebiotic mixture is highly interesting in this context (10). As such, the unpublished observations mentioned above as well as those by Fukuda et al (119), have shown that the protective effect against enteropathogens correlates with the production of acetate. Several findings indicate, in addition, that acetate may contribute substantially to the de novo lipid synthesis in enterocytes, equal to that of butyrate, and that this effect could be antagonized by propionate (120–122). Acetate may thus contribute significantly to several aspects of gut health (98, 123). However, many aspects of the complex interplay of SCFAs and their effect on gastrointestinal development and metabolic imprinting in young infants remain to be elucidated (124).

### THE MODULATION OF EARLY-LIFE MICROBIOTA CORRELATES WITH IMPROVED IMMUNE MATURATION

The intestinal microbiota and the host form a symbiotic relation and have coevolved, such that proper immune development and function rely on colonization of the gastrointestinal tract with commensal bacteria. It seems clear that the intestinal microbiota is a vital source of microbially driven immune regulation. Alterations of the normal bacterial colonization pattern may therefore change the outcome of immune development and cause a predisposition to certain immune-related disorders later in life. The ability of the normal intestinal microbiota to resist invasion by exogenous microorganisms and to prevent the overgrowth of potential pathogens present in very low number in the gut has already been described above.

In addition to conferring protection against infections by the so-called colonization resistance concept, the intestinal microbiota positively influences immune responses and protects against the development of inflammatory or allergic diseases. Cells of the immune system develop in proximity to large communities of microorganisms in the intestinal lumen, and therefore normal commensals should be discriminated from potentially pathogenic bacteria (125). In the gut, specific homoeostatic mechanisms protect resident immune cells against hyper-activation and the accompanying inflammation. Induction of an inflammatory response by the normal gut microbiota should be prevented, but an adequate immune response to pathogens should be maintained. Induction of sIgA expression is important in the establishment of host-microbiota homeostasis and in inducing immunologic tolerance toward the gastrointestinal microbiota (126).

sIgA is the dominant antibody produced in mammals. It can be produced in response to pathogens but also in response to commensal bacteria, and it is secreted by the mucosal immune system into the (gut) lumen. The intestinal sIgA repertoire represents the dominant species currently present in the intestine and mirrors the bacteria found the gut (126). The regulation of the composition of the intestinal microbiota by dietary supplementation with scGOSs+lcFOSs has been shown to correlate with the
production of sIgA (127), positioning scGOSs+lcFOSs as immunomodulatory components of the mucosal immune system.

However, there is strong preclinical evidence that indicates that supplementation with scGOSs+lcFOSs can also induce systemic immune effects (128, 129). The beneficial effect of this prebiotic mixture, which has been observed on variables associated with allergy, infection, and inflammation in both animal studies and clinical trials with oligosaccharides, are well documented (15–18, 128, 129) and are reviewed by Jeurink et al (89) elsewhere in this supplement.

Hence, the specific prebiotic mixture described here has a complementary capacity to interact directly with immune cells in a microbiota-independent mechanism as suggested by Vos et al (130) but also specifically promote a large set of specific microorganisms that may directly or indirectly through specific metabolites modulate immune responses.

HMOs and other NDOSs have been reported to bind to specific receptors on cells of the immune system as summarized by Georgi et al (88) and others (90, 131–133). Furthermore, in vitro evidence has been obtained for the epithelial transport of HMOs (131).

The prominent function of the microbiota on immune maturation and its homeostasis has been widely reviewed (134), and several microorganisms such as B. fragilis, segmented filamentous bacteria (SFB), or Faecalibacterium prausnitzii or their metabolites have been shown to affect the immune system (135–137). In addition to the important function of SCFAs for colonization resistance and gut physiology, as described earlier, there has been increasing interest in the idea that SCFAs may represent an important link between diet, the gut microbiota, and the body’s inflammatory responses.

The antiinflammatory properties of SCFAs on both the colonic epithelium and immune cells have been widely acknowledged and have been shown to be mediated by the G protein–coupled receptor GPR43 (138, 139), and several microorganisms such as B. fragilis, segmented filamentous bacteria (SFB), or Faecalibacterium prausnitzii or their metabolites have been shown to affect the immune system (135–137). In addition to the important function of SCFAs for colonization resistance and gut physiology, as described earlier, there has been increasing interest in the idea that SCFAs may represent an important link between diet, the gut microbiota, and the body’s inflammatory responses.

The antiinflammatory properties of SCFAs on both the colonic epithelium and immune cells have been widely acknowledged and have been shown to be mediated by the G protein–coupled receptor GPR43 (138, 139). Maslowski et al (140) recently showed that germ-free mice, devoid of bacteria and thus having few SCFAs, showed a dysregulation of certain inflammatory diseases, and this study also showed that GPR43 is the sole functional receptor for SCFAs on neutrophils, which act as chemoattractants. Therefore, any intervention directed toward a modulation of the intestinal microbiota could be expected to influence immune and inflammatory responses. SCFA-GPR43 interactions could represent a key mechanism to account for effects of prebiotics on immune responses. It may represent new avenues for understanding and potentially manipulating immune responses.

As mentioned above, in addition to SCFAs, other metabolites or bacteria are known to positively modulate the immune system. Several examples show how intestinal bacteria prime immune responses by promoting either inflammatory (ie, SFB) or anti-inflammatory (Faecalibacterium prausnitzii, Bacteroides fragilis, Lactobacillus and Bifidobacterium) conditions (141).

Antiinflammatory properties observed with F. prausnitzii and B. fragilis might be exerted via the suppression of the transcription of inflammatory factors (142, 143) by the following mechanisms: 1) by secreting compounds yet to be identified (136), independently or together with butyrate (144), or 2) by inducing regulatory T cells through specific Toll-like receptors or in some cases mediated by capsular polysaccharide A (145, 146). Colonization with SFB was correlated with increased expression of genes associated with inflammation and antimicrobial defenses. The proinflammatory properties of SFB seem to be mediated by Th17 cells, which have potent inflammatory potential, through the production of IL-17, IL-17F, and IL-22 (147–149). Whereas the precise mechanisms behind the beneficial effects of Lactobacillus and Bifidobacterium have not been clearly characterized, a significant amount of their activities can be attributed to cell surface–associated structures and extracellular protein interaction with mucosal immune cells (150–153). Such cell surfaces include exopolysaccharides, bacteriocins, lipoteichoic acids, and extracellular proteins (154, 155). Many of these proteins from both Bifidobacterium and Lactobacillus are primarily identified in vitro, and their role needs to be confirmed in vivo (152). Full characterization of secreted and surface proteins from these groups of bacteria could further advance therapeutic strategies in intestinal diseases and our knowledge of immune regulation by commensal bacteria.

The knowledge on commensal microbes that potentially contribute to immune maturation and homeostasis remains relatively limited to a few species, and their effect has been shown in rodent models. Even though the increase in Bifidobacterium and Lactobacillus may partly mediate the effect of NDOSs on the immune system, other commensal bacteria could act as key drivers for immune maturation and/or tolerance induction. As discussed above, the so-called omics technologies have the potential to generate an in-depth understanding of the microbial components in the gut and how these microbes influence the development of our immune system.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

Human milk plays a crucial role in the healthy development of infants. Through so-called programming effects, early-life events may lead to long-term physiologic outcomes that are important in health and disease. Recognizing the importance of the gut microbiota in human physiology, the introduction of NDOSs with similar impact to HMOs on the developing microbiota can be considered as a breakthrough in infant nutrition. The literature reviewed here shows the significant impact of these prebiotics on the microbial ecosystem, and the modulation of the composition and activity of the gut microbiota provides, in part, an explanation of the benefits of prebiotics shown in clinical studies. In the coming years, novel tools such as metabolomics, metaproteomics, and metagenomics will extend our knowledge on bacterial functionalities in the gut, which may allow for further identification of groups of bacteria, bacteria-derived components, or microbial metabolites that are particularly relevant for the optimal healthy development of infants. The likely challenge that lies ahead is to identify nutritional concepts that can bring health benefits by modulating the gut microbiota in early life. An important source of inspiration probably will continue to be the unique composition of human milk.

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Research Centre–Specialised Nutrition in Wageningen essentially focuses on infant nutrition and medical nutrition products. Several disciplines (eg, Milk Science, Immunology, Microbiology, Nutrition and Metabolism, Neurobiology) in the life sciences and technology are represented.

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