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COMMENTARY

Infant Formula Oligosaccharides Opening the Gates (for Speculation)

Commentary on the article by Barrat *et al*. on page 34

MILADY R. NINONUEVO, AND LARS BODE

Burnham Institute for Medical Research, La Jolla, California 92037

Human milk, in contrast to infant formula, contains a high amount of complex oligosaccharides that are thought to benefit the breast-fed infant. In an attempt to provide formula-fed infants with similar benefits, some companies started to supplement their formulas with oligosaccharides that are structurally different, but show similar prebiotic and immunomodulatory effects. Now, a study on neonatal rats reports that infant formula oligosaccharides increase bacterial translocation. This may not necessarily pose a threat for formula-fed infants, but the study raises several questions and reminds us once again that infant formula oligosaccharides are not the same as human milk oligosaccharides (HMO).

HMO are, after lactose and lipids, the third most abundant component of breast milk. High concentration as well as structural diversity and complexity (Fig. 1, top) are unique to human milk. Other natural resources are unavailable and chemical or enzymatic synthesis is far too tedious and expensive for commercial use in infant formula. HMO are considered beneficial for the breast-fed infant, promoting health and preventing disease, reviewed in Ref. (1). Because infant formulas lack HMO along with their potential benefits, some infant formula-producing companies set out to search for inexpensive alternatives, and developed mixtures of galactooligosaccharides (GOS, Fig. 1, bottom left) and fructooligosaccharides (FOS, Fig. 1, bottom right) or inulin that mimic the prebiotic effects of human milk and promote a bacterial microflora that closely resembles that of breast-fed infants (2,3). GOS/FOS supplemented formulae have also been reported to modulate the immune system in mice (4) and to reduce the incidence for infectious episodes (5) and atopic dermatitis in at risk infants (6).

In this issue of *Pediatric Research*, Barrat *et al*. report that a formula supplemented with GOS/inulin increases bacterial translocation in artificially reared newborn rats (7). Is there a potential risk for human infants that receive formula supplemented with these oligosaccharides?

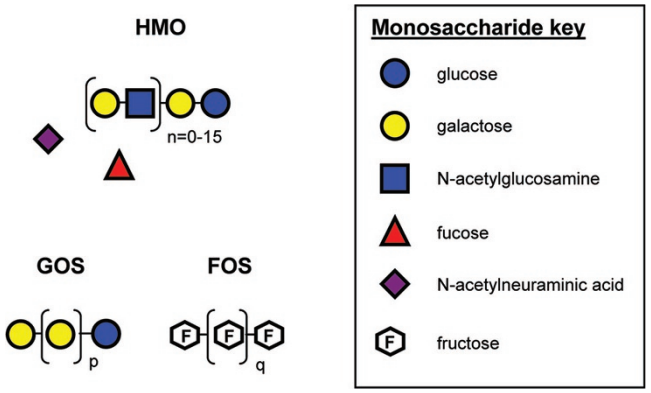

Figure 1. GOS or FOS are structurally different from oligosaccharides naturally occurring in human milk. Human milk oligosaccharides (HMO) contain lactose at their reducing end. They can be elongated with $n = 0–15$ lactosamine units and be modified with one or more fucose residues in α1–2, α1–3, and/or α1–4 linkage and/or carry *N*-acetylneuraminic acid in α2–3 and/or α2–6 linkage. Galactooligosaccharides (GOS) and Fructooligosaccharides (FOS) are polymers of galactose and fructose, respectively. Fructose is not naturally found as a building block for human milk oligosaccharides. *N*-acetylneuraminic acid and fucose, which are abundant constituents of HMO and may be important for some HMO functions, are not part of GOS or FOS.

A 2003 study on adult rats infected with *Salmonella enteritidis* showed that a diet supplemented with FOS increased *Salmonella* counts and infection-induced diarrhea, and enhanced *Salmonella* translocation (8). In parallel, cecal lactic acid concentration increased significantly, similar to the current study with GOS/inulin. Although FOS impaired the intestinal epithelial barrier function in the original study (9), the present study did not find that colonic permeability was affected (7). The method, however, may not have been sensitive enough to detect a significant difference. Although the original FOS study assessed *in vivo* chromium ethylenediaminetetraacetate uptake (9), the present study used Ussing chambers and measured the flux of fluorescein isothiocyanate-dextran through nonstripped colon explants (7). The labeled dextran had to cross not only the mucosal barrier, but also submucosa, muscularis externa, and serosa or adventitia. Nevertheless, the original FOS study results could not be repeated in adult healthy human men (10).

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Correspondence: Lars Bode, Ph.D., Burnham Institute for Medical Research, Glycobiology Unit, 10901 N Torrey Pines Road, La Jolla, CA 92037; e-mail: lbode@burnham.org

The studies raise several questions and open the gates for speculation: Does the rat model translate to human infants? Is bacterial translocation a risk for human neonates? How comparable are the different studies with respect to the structural composition of GOS, FOS, or inulin? How structure-dependent are the observed effects? How do they compare with the effects postulated for oligosaccharides that naturally occur in human milk?

Intestinal permeability in human infants is indeed higher during the first few days of life, but decreases faster in breast-fed infants than in formula-fed infants (without FOS/GOS) (11). Intestinal permeability measured in term infants during the first 4 mo of life is slightly higher in infants fed a GOS/FOS supplemented formula compared with formula without GOS/FOS (12), but that could have been due to lack of nucleotides and long-chain polyunsaturated fatty acids in the study's formula. Whether intestinal permeability, especially during the important first days of life, is lower in breast-fed infants than in GOS/FOS supplemented formula still needs to be addressed as well as the consequences that are associated with it, including bacterial translocation.

Barrat *et al*. speculate that bacterial translocation may not necessarily have adverse consequences, but instead contribute to postnatal maturation of the immune system. Several other researchers come to the conclusion that the amount of translocated bacteria is usually insufficient to reach a quorum sensing threshold needed to trigger virulence and inflammation (13–15). Preterm infants may be at higher risk because both their gut and their immune system are less mature. Further research in term and preterm infants is needed to clarify, whether increased intestinal permeability during the first few days of life is desired or a risk factor.

The exact structures of GOS, FOS, or inulin are often not very well defined, which makes it difficult to compare the different study results. Commercially available supplements are generally used without further purification. These products contain mixtures of different carbohydrate compositions that vary in chain lengths contingent upon the source. The difference could also arise from the amount of hydrolysates (*i.e.*, monomer residues), and monosaccharide constituents (Fig. 1). Inulin is a linear β2–1 fructose polymer with degree of polymerization (DP) ranging from 2 to 60 (average DP of 12). Glucose or fructose can be found in the terminal position. FOS are also β2–1 fructose polymers, but the average DP is less than 10. In some cases, FOS contain glucose in the terminal position. GOS are composed of one to seven galactose moieties with glucose in the terminal position (16).

Some may think that GOS, FOS, inulin, or HMO simply pass the upper parts of the gastrointestinal tract without any effects until they reach more distal parts of the ileum and the colon where they are metabolized by certain (desired) bacteria, producing short chain fatty acids (SCFA) that then affect the host through several different mechanisms. Because all of these oligosaccharides are degraded to SCFA in the end, their effects may very well be the same—It is most likely not as simple as that (Fig. 2). The intact oligosaccharides may have direct effects that highly depend on their distinct structures; and GOS, FOS, or inulin are structurally very different from the oligosaccharides that naturally occur in human milk (Fig. 1). Many bacteria, for example, express lectins that bind to specific glycans on the host's epithelial cell surface. This initial adhesion is essential for the virulence of most pathogenic microorganisms. Some HMO structurally resemble epithelial cell surface glycans and block pathogen adhesion,

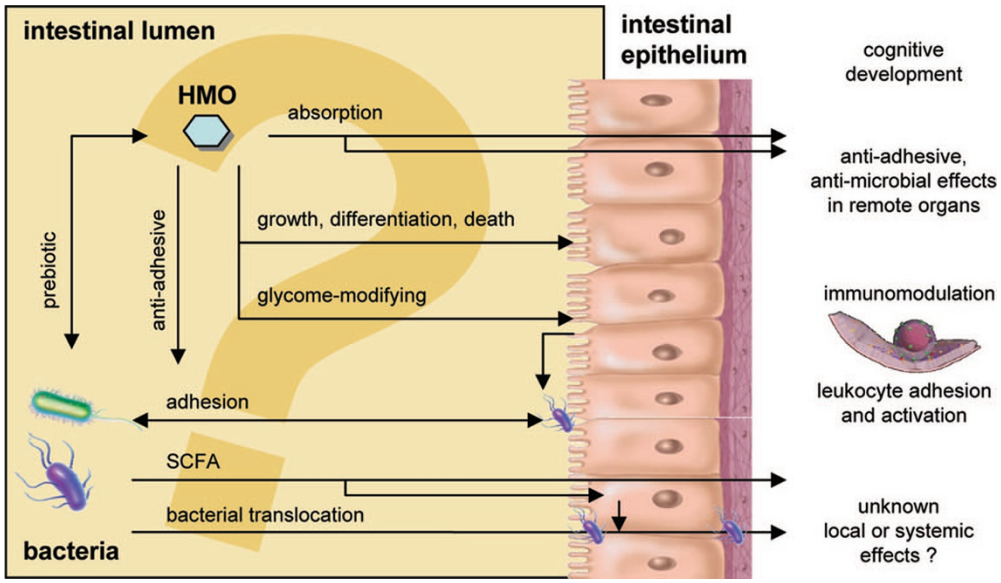

Figure 2. Postulated effects of human milk oligosaccharides. Human milk oligosaccharides (HMO) are postulated to play an essential role in modulating bacterial-host interactions in the infant's intestine. HMO are prebiotics, serving as "fuel" for certain desired bacteria, but not for others. Bacterial exo- or endoglycosidases digest or cleave off some or all of the HMO building blocks, leaving behind either SCFA or truncated and modified HMO that may have different effects than the intact HMO. SCFA and HMO may have several effects either on the intestinal epithelium itself or, after passing the intestinal epithelial barrier, on other cells and tissues, *e.g.*, the immune system. Although structurally different, GOS, FOS, or inulin may have similar prebiotic effects as HMO. Now, Barrat *et al*. report that SCFA production from GOS or inulin may increase bacterial translocation. Whether increased bacterial translocation poses a threat for formula-fed infants and whether HMO have similar or adverse effects remains to be seen. Nonetheless, HMO may have other, structure-specific effects on a local and systemic level that can most likely not be mimicked by the structurally unrelated GOS, FOS or inulin.

which protects the breast-fed infant from certain infections and diarrhea (17). GOS, FOS, or inulin may not be able to mimic these protective effects because most of them do not resemble epithelial cell surface glycans.

There is also first *in vitro* evidence that HMO may alter intestinal epithelial gene expression and cell surface glycome composition (18), as well as cell differentiation, proliferation, and apoptosis—at least at very high concentrations (19). Although the underlying mechanisms, receptors and signaling pathways are currently unknown, it can be speculated that these effects are also mediated through epithelial cell surface lectins. Again, GOS, FOS, or inulin may not be recognized by these lectins or bind to other lectins that trigger a different cell response.

HMO are partially absorbed intact and later excreted with the urine (20,21), which is indirect proof that some HMO are systemically available. *In vitro* and *ex vivo* data show that HMO reduce selectin-mediated leukocyte adhesion and activation at sites of inflammation, which is highly structure-dependent (22,23). If intact HMO can cross the intestinal epithelial barrier, GOS, FOS, or inulin may also be partially absorbed—with yet unknown consequences.

SCFA modulate the gut-associated lymphoid tissue, but intact oligosaccharides, either HMO or GOS, FOS, or inulin, could also have direct effects *via* structure-specific cell surface lectins on different gut-associated lymphoid tissue cells (24).

Even prebiotic effects may, to a certain extent, be structure-dependent: LoCascio *et al*. reported recently that *Bifidobacterium longum* biovar *infantis*, an isolate from the infant gut, prefers certain short chain HMO over high molecular weight HMO, which correlates with the expression profile of bacterial enzymes capable of cleaving oligosaccharides (25).

In conclusion, infant formula oligosaccharides have several beneficial effects for the infant. Although animal studies have shown that these oligosaccharides can enhance bacterial translocation, it needs to be further elucidated how these results translate to human infants and to what extent an increase in bacterial translocation poses a threat for formula-fed infants. Additional research is needed to uncover the entire spectrum of effects of infant formula oligosaccharides and, even more so, of HMO. Oligosaccharides currently added to infant formula are structurally different from those naturally occurring in human milk, and it is likely that their effects differ as well. To date, GOS, FOS, or inulin represent a reasonable and inexpensive way to supplement infant formula with prebiotic oligosaccharides. In the future, extensive research is needed to be able to provide formula-fed infants with structurally and quantitatively the same oligosaccharides that breast-fed infants receive with their mother's milk.

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