REVIEW

Fructooligosaccharides: From Breast Milk Components to Potential Supplements. A Systematic Review

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ABSTRACT

Breast milk is the optimal food choice for infant growth and development. Among breast milk components, fructooligosaccharides (FOSs) are being actively studied because of their role in microbiota development. In particular, 2′-fucosyllactose is being proposed as a potential supplement/nutraceutical or component of infant formula. In this systematic review, we critically summarize the available information on FOSs and we discuss their future use in infant nutrition. We searched the main electronic databases (PubMed, Embase, and Scopus), with a final check in May 2021. Search terms were inserted individually and using the Boolean tools AND and OR. Relevant articles were identified using the following words: ("fructooligosaccharides" OR "FOS") AND ("human milk" OR "breast milk" OR "donor milk" OR "bank milk"). The search retrieved 1814 articles. After removal of duplicates, we screened 1591 articles based on title, abstract, and exclusive use of the English language. We included articles describing the concentration of FOSs in human milk and assessed the relevant ones. We excluded reviews, studies on animals, and studies exclusively carried out on adults. Also, we excluded studies that have not reported evidence either on FOSs or on galactooligosaccharides from human milk. The resulting publications were reviewed, and 10 studies were included in the systematic review. We conclude that human milk FOSs are, indeed, crucial to infant gut development and their addition to infant formula is safe, well-tolerated, and might provide immune benefits to newborns. However, we would like to underscore the scantiness of human data and the need to avoid the immediate translation of infant research to the commercialization of supplements marketed to adults. *Adv Nutr* 2022;13:318–327.

Statement of Significance: Given that human milk oligosaccharides are entering the market, it is timely to review their role in infant health. Because of the scantiness of human data, we need to avoid the immediate translation of infant research to the commercialization of supplements marketed to adults.

Keywords: breast milk, fructooligosaccharides, 2′-fucosyllactose, supplements, microbiota, infant development

Introduction

Breast milk is the optimal food choice for infant growth and development (1). This statement is supported by the totality of learned nutrition and clinical societies and is backed by a plethora of human studies (2). Breast milk is, of course, mostly composed of water. Yet, its lipid profile is quite peculiar. In addition to the mere provision of energy, i.e., 40%–55% of total energy, breast milk provides essential fatty acids, namely those of the omega-3 series, i.e., EPA (20:5n–3) and DHA (22:6n–3). Even though the biological actions of ω-3 fatty acids in infant development have not been proven beyond doubt by randomized clinical trials (3) [although women with low ω-3 fatty acid status might require supplementary ω-3 (4)], their importance as components of

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Abbreviations used: EFSA, European Food Safety Authority; FG, fructooligosaccharides + galactooligosaccharides mixture; FOS, fructooligosaccharide; GOS, galactooligosaccharide; GRAS, Generally Recognized as Safe; HMO, human milk oligosaccharide; LNT, lacto-N-tetraose; sn-2 + OF, formula containing increased *sn*-2 palmitate and oligofructose.

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breast milk appears to be pre-eminent. Other micronutrients, e.g., vitamins and folates, depend on maternal diet (5).

The proteic composition of breast milk is also noteworthy. Breast milk contains >400 different proteins which, in addition to providing calories, are endowed with antimicrobial and immunomodulatory activities and facilitate the absorption of nutrients (6, 7).

Simple and complex carbohydrates also provide energy and are crucial to the development of the intestinal microbiome (8). Indeed, of all the breast milk components, fructooligosaccharides (FOSs) are being actively studied because of their role in microbiota development (1). Notably, FOSs are the third largest component in breast milk (8) and their concentrations fluctuate from ∼13 to ∼21 g/L (8, 9). The biological importance of FOSs resides in the fact that they are not digested by humans and, therefore, act as prebiotics, facilitating the growth of a healthy microbiota (10).

Of all the FOSs, 2′-fucosyllactose is being actively investigated as a potential supplement/nutraceutical or component of infant formula (11). Indeed, 2′-fucosyllactose comprises ≤30% of total breast milk oligosaccharides (12, 13), with great variation among lactating women (10, 13). Notably, 2′-fucosyllactose has been granted Generally Recognized as Safe (GRAS) status by the US FDA, e.g., in GRAS Notices 924, 929, or 749, and is deemed safe by the European Food Safety Authority (EFSA) (14) when obtained by fermentation with a genetically modified strain of *Escherichia coli* K12. It must be underscored, however, that the mammary gland synthesizes a wide variety of oligosaccharides. Examples include non-fucosylated oligosaccharides such as lacto-N-tetraose (LNT) as well as sialyated and nonsialyated molecules (10), all of which vary in concentration depending on women's genetics and lactating stage. In brief, there is a need to summarize the available data and their limitations in light of the future commercialization of human milk FOSs.

In this systematic review, we critically summarize the available information on FOSs and we discuss their future use in infant nutrition.

Methods

In view of the aforementioned future use of human FOSs in the supplement arena, we aimed to systematically review the effects of FOSs in infant nutrition, with particular focus on growth, microbiota, stool consistency, and immune system development. We searched the main electronic databases (PubMed, Embase, and Scopus), with a final check in May 2021. Search terms were inserted individually and using the Boolean tools AND and OR. Relevant articles were identified using the following words: ("fructooligosaccharides" OR "FOS") AND ("human milk" OR "breast milk" OR "donor milk" OR "bank milk"). The search retrieved 1814 articles. After removal of duplicates, we screened 1591 articles based on title, abstract, and exclusive use of the English language. We included those articles describing the concentration of FOSs in human milk and assessed the relevant ones. We excluded reviews, studies on animals, and studies exclusively carried out on adults. Also, we excluded studies that have not reported evidence either on FOSs or on galactooligosaccharides (GOSs) from human milk. The resulting publications were reviewed, and 10 studies were included in the systematic review. Articles had to be primary studies or articles presenting data analyses from these studies and be published in a peer-reviewed journal or edited book. **Figure 1** shows the search strategy (15).

Results

Nijman et al. (16) quantified oligosaccharides in 20 breast milk samples from 10 women who delivered term infants and in 5 infant formula brands (**Table 1**). Fucosylated oligosaccharides comprised 58.2% ± 7.4% (means ± SD) of total oligosaccharides in human milk (day 42). LNT was the largest part (17.0% ± 6.6%) of total oligosaccharides. The sialylated fraction of FOSs of the day 42 milk samples constituted 8.3% ± 2.0% of total oligosaccharides based on abundance, and 3.4% ± 0.9% of these oligosaccharides contained both sialic acid and fucose residues. All the infant formulas were supplemented with hexose oligomers, and neutral nonfucosylated milk oligosaccharides and sialylated oligosaccharides derived from bovine milk used as a base for the formulation were identified only at a low abundance. Fucosylated oligosaccharides were absent from the formula assayed, and the prebiotic oligosaccharide LNT was also not detected. Moreover, the content of bound sialic acid was ∼8 times lower in infant formula than in human milk. From a potential supplement viewpoint, it is worth noting that human milk contains a complex mixture of oligosaccharides, whereas infant formula mainly contains individual components. The overall composition of the 5 different formulae of the brands tested by Nijman et al. (16) was similar and mainly consisted of hexose oligomers. Furthermore, the authors compared the amounts of oligosaccharides in early and mature milk. From day 3 to day 42, the total amount of oligosaccharides decreased significantly. FOS concentrations in milk were 6.38 ± 0.29 g/L at day 42 as opposed to 9.15 ± 0.25 g/L measured in milk sampled at day 3 (a 30.3% decrease) (16).

Breast-milk FOSs are able to modify the intestinal microbiota by acting as prebiotics. In an in vitro study, fresh fecal samples were collected from 9 healthy infants and the fecal microbiota was cultured in the presence of breast-milk FOSs and individual fucosylated milk oligosaccharides. The in vitro data showed that supplementation with breast-milk FOSs significantly increased the number of bifidobacteria. In particular, *Bifidobacterium longum* ATCC15697 and *B. longum* JCM7007 exhibited growth increases even greater than that of the positive prebiotic control when added at equal concentrations of 5 g/L. In contrast, the proportion of *Escherichia* K12 and *Clostridium perfringens* declined. Furthermore, in this in vitro fermentation model the pH was drastically reduced by the total breast-milk FOSs in the *Bifidobacterium* spp. (17). Another study evaluated the probiotic properties of *Lactobacillus* spp. isolated from 5 human milk samples collected from healthy first-time

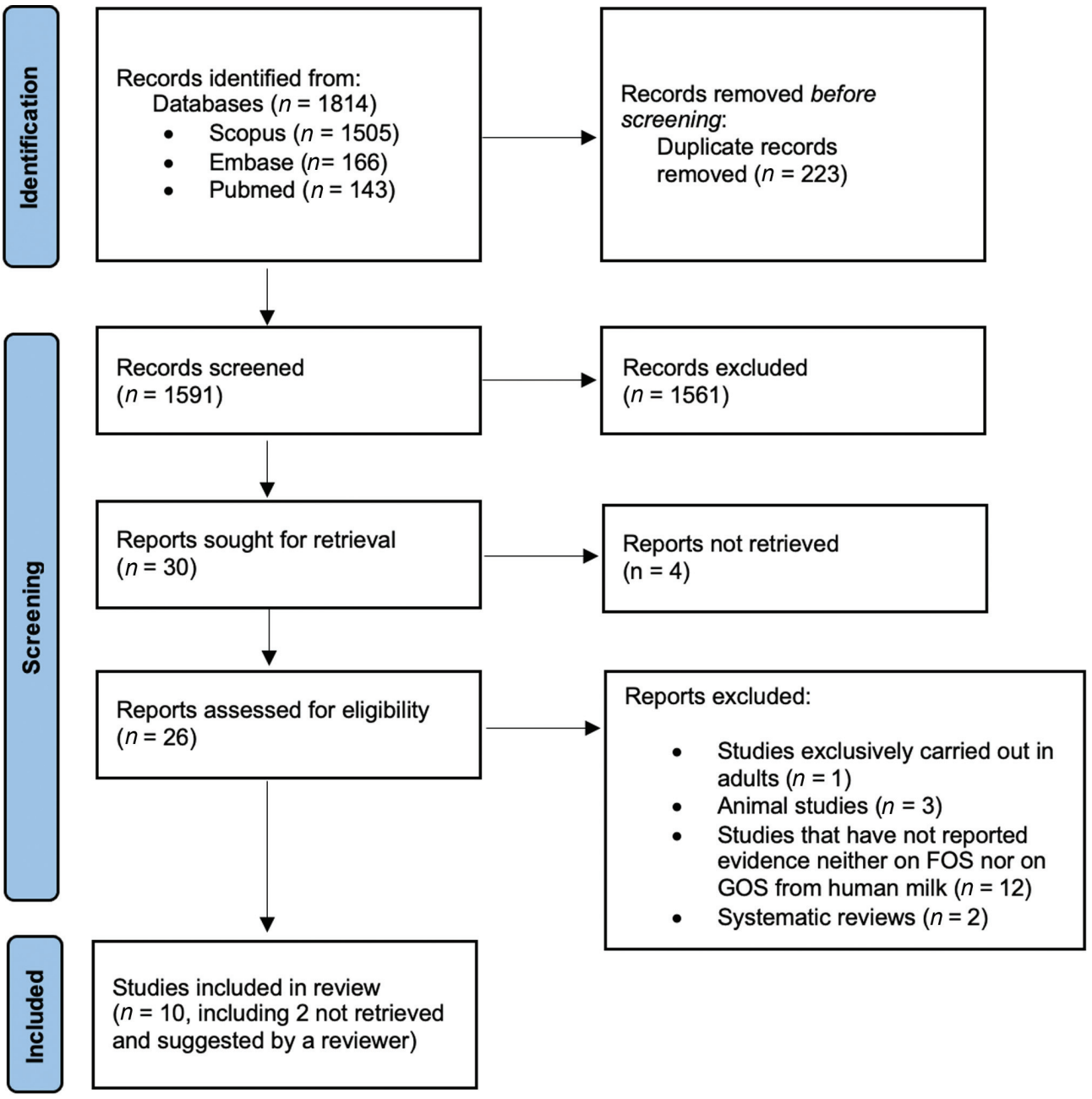

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram of human-milk FOS studies retrieved. FOS, fructooligosaccharide; GOS, galactooligosaccharide.

mothers in the early (within 80 d of delivery) lactation period in the presence of prebiotics such as inulin and FOSs. The results showed that *Lactobacillus casei* L1 was able to utilize these oligosaccharides with a high tolerance to acid and bile salt, powering an antagonistic effect against pathogens and cholesterol assimilation (18).

The aforementioned studies build upon an earlier one that, in contrast with those, reported that supplementation with 1.5 or 3.0 g FOSs/L had minimal effect on the fecal flora. Researchers studied healthy term infants and found that counts of fecal lactobacilli and bifidobacteria were similar to those seen in human milk–fed infants. Moreover, influence on *Clostridium difficile* toxin was minimal, whereas in human milk–fed infants the toxin was not even detected in the stools (19).

Randomized trials are scant. In 1 of them, the effect of maternal FOS ingestion on maternal and neonatal gut bifidobacteria was investigated in a sample of 64 pregnant women, where the amount of fecal *Bifidobacterium* spp. in the FOS group (8 g/d) at 36 weeks of gestation was significantly higher than that in the placebo group. However, in the analyses of neonatal feces, no differences in the amount of

TABLE 1 Summary of the human studies addressing the effects of FOSs on infant health, included in the systematic review[1]

Reference	Location	Participants, *n*	Study population	Study design	Study aims	Results
Yu et al. (17)	USA	9	Fresh fecal samples from healthy infants who had not received antibiotics or pre-/probiotics since birth and had no recent history of gastrointestinal disorders	In vitro study	To investigate in vitro the physiology of consumption of the natural mixture of HMOs and of its principal individual fucosylated oligosaccharides by the entire infant fecal microbiota community. To select major individual bacteria from this community	Two of the bifidobacteria, *B. longum* (designated as *B. infantis*) ATCC15697 and *B. longum* JCM7007, exhibited strong growth stimulation in response to HMOs. In contrast, *Clostridium perfringens* and *Escherichia coli* K12 exhibited growth suppression when fecal slurry was supplemented with HMOs. Fecal microbiota cultures grown in the medium containing HMOs had significantly lower pH after 48 h of incubation than control cultures grown in the basal medium. HMOs decreased the pH even more than FOS-supplemented positive controls. Over 90% of 2′-FL and LDFT and 53% of 3-FL from the HMO supplement were consumed by cultured fecal microbiota
Shen et al. (24)	United Kingdom	Three healthy infants 7 mo old who had begun weaning	A batch culture inoculated with fecal microbiota from FF infants	In vitro study	To investigate the impact of HMOs from a single donor, HMOs from multiple donors PO, and an FG mixture on the composition of a batch culture inoculated with fecal microbiota from FF infants	All 3 substrates increased numbers of bifidobacteria, bacteroides, and those aligning with the clostridial cluster XIVa. Neither the FG nor the HMO substrates supported the growth of the *C. perfringens*—histolyticum group. SCFA production corresponded to changes observed in bacterial populations. A distinct profile of fecal bacteria was present in each infant
Euler et al. (19)	USA	Total: 72 FOS 1.5: $n = 28$ FOS 3.0: $n = 30$ HM: $n = 14$	Two- to 6-wk-old healthy infants of gestational age between 37 and 42 wk	Prospective, randomized, crossover, outpatient, single-site study of FOSs added to term infant formula with a nonrandomized comparison group of HM-fed infants	To determine if FOSs at either of 2 concentrations (1.5 or 3.0 g/L) in term infant formula had a beneficial prebiotic effect on the gastrointestinal tract flora of infants	The mean bifidobacteria counts were statistically greater ($P < 0.0450$) in the 1.5 g FOS/L formula group than in either the HM-fed or 3.0 g FOS/L formula groups after supplementation. Seven days after the conclusion of FOS supplementation: - There were no differences in *Lactobacillus* or *Bifidobacterium* counts between the treatment groups.

(*Continued*)

TABLE 1 (*Continued*)

Reference	Location	Participants, *n*	Study population	Study design	Study aims	Results
						- The FF groups had ~100-fold greater counts of *Bacteroides* ($P = 0.016$) and *Enterococcus* ($P = 0.0001$) than did the HM-fed group. - Clostridia colony counts were highest for the 1.5 g FOS/L formula group and were similar in the HM-fed and 3.0 g FOS/L formula group. Groups fed 1.5 g FOSs/L and 3.0 g FOSs/L experienced similar declines in the proportions of infants with *Clostridium difficile* toxin (from 14% to 4% and from 23% to 17%, respectively)
Jinno et al. (20)	Japan	Total: 64 FOS group: 35 Placebo group: 29	Women from the 26th week of gestation to 1 mo after delivery	Double-blind, randomized, placebo-controlled study	To investigate the effect of maternal FOS ingestion on the number of gut bifidobacteria in both the maternal gut and neonatal gut	Number of fecal *Bifidobacterium* spp. in the FOS group at 36 weeks of gestation was higher than that in the placebo group. The number of fecal *B. longum* in the FOS group at 36 weeks of gestation was higher than that in the placebo group. Neonatal feces: no correlation in the number of fecal *Bifidobacterium* spp. and no correlation in the number of fecal *B. longum*
Nijman et al. (16)	USA	Twenty HM samples from 10 women and 5 infant formulas	Women who delivered healthy term infants. HM collected at day 3 and day 42 postnatal	Cross-sectional	To compare the oligosaccharide profile of HM, including neutral and acidic oligosaccharides, at day 3 to that of day 42 of lactation. To compare the oligosaccharide profile of infant formulas	The oligosaccharide content decreased in HM from (means ± SD) 9.15 ± 0.25 g/L at day 3 to 6.38 ± 0.29 g/L at day 42 of lactation. All formulas resulted as fortified with GOSs, with 1 also fortified with polydextrose and another with long-chain FOSs. In the HM samples, 130 unique oligosaccharide structures were identified, whereas infant formula contained less diversity of structures
Mao et al. (22)	China	427	Healthy term infants. Feeding regimens: infants exclusively breastfed, exclusively consuming sn-2 + OF, or consuming both HM and the sn-2 + OF formula	Cohort study (48-d)	Amount of hard stools and watery stools and the gastrointestinal tolerance among infants receiving 1 of 3 different feeding regimens	Low incidences of hard stools in each group. Lower incidence of watery stools in the group exclusively receiving formula. IGSQ scores low in all groups

(*Continued*)

TABLE 1 (*Continued*)

Reference	Location	Participants, *n*	Study population	Study design	Study aims	Results
Tulumoğlu et al. (18)	Turkey	5	HM samples were collected from healthy first-time mothers who were in the early lactation period (within 80 d of delivery)	Cohort study	To investigate the effects of the prebiotics inulin and FOSs on the probiotic properties of *Lactobacillus* spp. isolated from HM	*Lactobacillus casei* L1 was able to utilize inulin and FOSs as a carbon source as well as glucose which other strains were also able to use, including *Lactobacillus rhamnosus* GG. This strain also showed high tolerance to acid and bile salt, even at pH 2.5 and 0.5% bile salts, respectively. Inulin and FOSs promoted the antimicrobial activity of *L. casei* L1 against pathogenic bacteria. Cholesterol assimilation was higher than in the other and control probiotic strains in the presence of inulin and FOSs, which were measured as 14 and 25 mg/dL, respectively
Kongnum et al. (21)	Thailand	Six healthy term infants	Two infants exclusively breastfed, 2 fed formula supplemented with either FOSs-GOSs or inulin-GOSs, and 2 fed a combination between HM and the 2 formulas. HM and infant fecal samples were taken from the first week until 10 mo after birth	Longitudinal study	To understand the effect of infant diets on the population, diversity, and composition of fecal lactobacilli at the species level in breastfed, FF, and combination-fed infants	Higher fecal lactobacilli total cell counts in infants fed by combining HM and formula containing either FOSs-GOSs or inulin-GOSs and the exclusively FF ones. Greatest relative abundance of fecal lactobacilli species was observed in all infants receiving prebiotic-containing diets, whereas bifidobacteria appeared predominantly in exclusively breastfed infants
Alderete et al. (41)	USA	25	Healthy breastfed infants. Relations between breast-milk FOSs and infant growth and body composition were examined by using multiple linear regression	Longitudinal study	To ascertain whether differences in FOS composition in mother's milk are associated with infant growth and body composition	Differences in FOS composition in mother's milk were associated with infant growth and body composition. Notably, higher HMO diversity and evenness at 1 mo were associated with lower total and percentage fat mass at 1 mo. At 6 mo, each 1-mg/mL increase in fucosyl-disialyllacto-N-hexaose and LNnT was associated with 0.04% higher ($P = 0.03$) and 0.03% lower ($P = 0.01$) body fat, respectively

(*Continued*)

TABLE 1 (*Continued*)

Reference	Location	Participants, *n*	Study population	Study design	Study aims	Results
Lagström et al. (42)	Finland	812	Healthy mothers and breastfed infants	Cohort study	To determine the association between maternal FOS composition and child growth during the first 5 y of life	Maternal FOS composition 3 mo after delivery was associated with height and weight during the first 5 y of life in children of secretor mothers. Specifically, FOS diversity and the concentration of LNnT were inversely associated and concentration of 2′-FL was directly associated with child height and weight *z* scores in a model adjusted for maternal prepregnancy BMI, mode of delivery, birth weight *z* score, sex, and time. Maternal prepregnancy BMI was associated with FOS composition

[1]FF, formula-fed; FG, fructooligosaccharides + galactooligosaccharides mixture; FOS, fructooligosaccharide; GOS, galactooligosaccharide; HM, human milk; HMO, human milk oligosaccharide; IGSQ, Infant Gastrointestinal Symptom Questionnaire; LDFT, lactodifucotetraose; PO, pooled from multiple donors; LNnT, lacto-N-neotetraose; sn-2 + OF, formula containing both increased *sn*-2 palmitate and oligofructose; 2′-FL, 2′-fucosyllactose; 3-FL, 3-fucosyllactose.

Bifidobacterium spp. and *B. longum* in neonates at 1 mo of age between groups were observed, showing no evidence for a bifidogenic effect on infants by maternal FOS ingestion (20).

Recently, Kongnum et al. (21) tried to understand the effect of healthy term infants' diets on the composition of fecal lactobacilli. Two infants were exclusively breastfed, 2 infants were given formula supplemented with either FOSs-GOSs or inulin-GOSs, and 2 infants received a combination of breast milk and the 2 formulae. Breast milk and infant fecal samples were taken from the first week until 10 mo after birth. At the first week after birth, the 2 infants fed a combination between breast milk and a formula supplemented with either FOSs-GOSs or inulin-GOSs had significantly higher counts of fecal lactobacilli than the ones fed exclusively breast milk and a formula brand.

The total cell counts of fecal lactobacilli from infants with the combination diet and the exclusive formula supplemented with FOSs-GOSs were greatly enhanced and rapidly reached a plateau at 10^9 cells/g feces within the first week after birth. Total cell counts of lactobacilli from the exclusively breastfed infants and those exclusively formula-supplemented with inulin-GOSs slowly developed to reach the maximum number of 10^9 cells/g feces and remained constant throughout 5 mo (21).

The *Lactobacillus* profile of the maternal breast milk clearly corresponded to the one detected in the feces of her infant. Breast milk was the most important source of indigenous lactobacilli, which established in the infant's gut, suggesting mother–infant transfer of lactobacilli (21).

Another cohort study lasting 48 d and involving 427 healthy term (35–49 d of life) infants explored the proportion of hard stools and watery stools and the gastrointestinal tolerance among infants receiving 1 of 3 different feeding regimens (22). The feeding regimens included infants exclusively breastfed, exclusively consuming sn-2 + OF (formula containing increased *sn*-2 palmitate and oligofructose), or consuming both breast milk and the sn-2 + OF formula. Infants from all 3 groups had similar low proportions of hard stools, including the infants who were exclusively fed the sn-2 + OF formula; incidence of watery stools was consistently lower in the group exclusively receiving formula than in the other groups. The Infant Gastrointestinal Symptom Questionnaire scores were low (indicating good tolerance) in all groups. These findings confirmed that a strategy to soften stools in term infants is to supplement them with FOSs (in this case a proprietary formulation) (22). Breast-milk FOSs have been proposed to be one of the "bifidogenic factors" in human milk (23).

Some studies addressed the mechanisms of action of human FOSs. Shen et al. (24) investigated the impact of pure human milk oligosaccharides (HMOs) from 6 donors and a fructooligosaccharides + galactooligosaccharides mixture (FG) on the composition of a batch culture inoculated with fecal microbiota from formula-fed infants ($n = 3$). HMOs induced the growth of several saccharolytic bacterial groups. The authors studied the fermentation profile of HMOs

using this system inoculated with infant fecal microbiota. Most bacterial groups increased in number after 5 h of the fermentation in both HMO preparations [single donor (SO) and pooled from multiple donors (PO)] and FG mixture. In particular, beneficial bifidobacteria and eubacteria/clostridia increased in larger quantities, which was in accordance with the significant increase in acetate production. The increase in *Bacteroides* numbers may also correlate with the increase in propionate production. However, the FG mixture induced more acetate and i-valerate production as well as a higher Lab158 concentration at 10 h of the fermentation than did the HMO substrates. HMOs were less suited to supporting the growth of *Lactobacillus* spp. than was the FG mixture (24).

Lactobacilli appeared to be dominant over bifidobacteria, clostridia, and bacteroides present in feces from combination-fed and exclusively supplementing formula–fed infants (20%–64% and 21%–80%, respectively). Bifidobacteria were detected at concentrations of 32%–70% and lactobacilli at 7%–45% in the feces of exclusively breastfed infants' group. However, the relative abundance of lactobacilli rather fluctuated at certain time points at 4 wk postpartum in exclusively formula-fed infants. The prebiotic (FOSs-GOSs and inulin-GOSs)-containing formulae seemed to significantly enhance the early establishment of beneficial lactobacilli and reduced the relative abundance of the detrimental genera of *Clostridium* and *Bacteroides*, whereas breast milk seemed to promote the abundance of *Bifidobacterium*.

Discussion

Research on the microbiome has been gaining traction of late, following multiple publications that have associated dysbiosis with increased incidence of several disorders (25). It follows that several companies are marketing supplements aimed at optimizing individuals' microbiotas (26). Among the most innovative ingredients, FOSs and, particularly, 2′-fucosyllactose appear to be the most interesting ones because they play an important role in infant gut development and, by syllogism, they are supposed to positively influence human health at large (27). Currently, there is no EFSA-approved health claim pertaining to prebiotics. It is worth underscoring that, under European Union law, it is illegal to state to consumers that a food can prevent, treat, or cure a disease (28). Indeed, there appears to be a contradiction, in that European Union regulations demand that a claim is a statement about the food ingredient and its "beneficial physiological effect" on the body (29). Yet, it is difficult to have a physiological effect if the molecules do not cure a disease or reduce the risk of becoming ill (30). This epistemological mismatch between experimental pharmacology and pharma-nutrition has been pointed out earlier and still needs to be sorted out (31–33).

In 2018, the FDA published draft guidance to help producers determine what is a fiber and what is not (34). Their definition is: "non-digestible soluble and insoluble carbohydrates (with 3 or more monomeric units), and lignin that are intrinsic and intact in plants; isolated or synthetic non-digestible carbohydrates (with 3 or more monomeric units) determined by FDA to have physiological effects that are beneficial to human health" (34). As compared with the EFSA, this definition leaves room for the more rapid development of human FOS-based supplements, although the FDA is not authorized to review dietary supplement products for safety and effectiveness before they are marketed: the manufacturers and distributors of dietary supplements are the ones responsible for making sure their products are safe before they go to market (35). This approach is stirring some controversy (36, 37) and will likely be addressed in the near future by political bodies and stakeholders (38).

One potential area in which breast-milk FOSs (either as natural components of milk or—potentially—as supplements) could play interesting roles is that of cognitive development. There is evidence linking FOSs to cognitive development in animals, but we lack human trials (39). One notable exception is a recent study by Berger et al. (40), who reported that exposure to a higher concentration of 2′-fucosyllactose at 1 mo predicted higher cognitive development scores in infants at 24 mo, but not at 6 mo (40). We must acknowledge that other as yet unknown components of breast milk might be responsible for this effect and that only opportune randomized trials will eventually clarify this issue.

In keeping with the aforementioned, FOSs and, notably, 2′-fucosyllactose are also being proposed as potential supplements aimed at improving child growth, with putative long-term preventive actions on obesity. Indeed, some reports have been published [e.g., (41–43)] that associate breast-milk FOSs with child growth programming (42), conceivably yet elusively mediated by the microbiota (42). It should be underscored that most data available to date are observational in nature and do not—as yet—prove causality. Only well-performed short-, mid-, and long-term randomized trials will eventually elucidate the active roles of FOSs in infant development and, in turn, allow for science-backed health claims.

Some investigators have addressed the mechanisms of action of breast-milk FOSs, which appear to selectively enrich the intestinal proportion of putatively eubiotic bacteria such as bifidobacteria and lactobacilli. However, the extent and precise nature of this selectivity remain elusive and require further molecular investigations. It is worth reminding ourselves that the definition of a "normal" or "healthy" microbiota as opposed to a dysbiotic one is still elusive (44) and that arbitrarily altering such a highly personal environment might not be devoid of untoward consequences (45, 46).

Some upper limits of natural molecules that—because of their nature—are perceived as safe must be carefully established (47, 48). As outlined in this review, no safety concerns have been raised over breast-milk FOSs and, in particular, 2′-fucosyllactose. However, long-term postmarketing studies are warranted and will provide important information on potential adverse effects.

In conclusion, human-milk FOSs are crucial to infant gut development (49, 50) and their addition to infant formula is safe, well-tolerated, and likely provides immune benefits to newborns (51). However, we would like to underscore the current scantiness of human data and the need to avoid the immediate translation of infant research to the commercialization of supplements marketed to adults. Future pertinent research, including high-quality randomized controlled trials, is much needed to back the potential use of FOSs as human supplements.

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