

Human milk oligosaccharides: Role in lactation and infant formulas

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ABSTRACT

Human milk oligosaccharides (HMO) are the specific and selective growth substrate for bifidobacteria, preventing pathogen adhesion, modulating the immune system, and impacting neurodevelopment. Human milk is the best food for the neonate; infant formulas enriched with HMOs are indicated when human milk is not possible or sufficient. HMOs developed and added to available infant formulas are 2'-FL (2'-fucosyl lactose), 3-FL (3-fucosyl lactose), 3'-SL (3'-sialyl lactose), 6'-SL (6'-sialyl lactose), LNT (lacto-N-tetraose), and 3'-GL (3'-galactosyl lactose), the latter being produced in situ by microbial fermentation. These HMOs are safe and contribute to satisfactory infant development. In addition, they were shown to promote the development of the intestinal microbiota in a way that is more similar to that of infants fed human milk than that of infants fed formula without HMOs.

Keywords: *microbiota; oligosaccharides; human milk; prebiotics; infant formulas.*

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INTRODUCTION

Human milk oligosaccharides (HMO), formerly known as “bifidogenic factors”, are a complex group of oligosaccharides (3 to 9 molecular units) and dynamic throughout lactation. HMOs are formed by combining and chaining five molecules, of which lactose is a common denominator. HMOs are the specific and selective substrate for the growth of bifidobacteria, the main microbial group in the intestine of the neonate and until the onset of complementary feeding, and also have other biological activities in the intestine (prevention of pathogen adhesion and modulators of the immune system associated with the intestine), but also in a systemic way, with a recognized impact on cognitive development.¹ In cases where breastfeeding is unfortunately not possible or sufficient, the indication is infant formulas enriched with some of these HMOs.

This narrative review aims to summarize the scientific evidence regarding the beneficial effects of HMOs naturally present in human milk and analyze the available evidence regarding those added to infant formulas.

HUMAN MILK OLIGOSACCHARIDES

Since the beginning of the 20th century, thanks to the pioneering work of Moro and Tissier, it has been known that the intestinal bacteria of infants fed with human milk (HM) are different from those of bottle-fed infants.² In 1930, specific carbohydrates were recognized in HM, then called “bifidogenic factors” due to their capacity to stimulate the development of certain bacteria.^{1,3}

At the beginning of the 21st century, the study of these carbohydrates was deepened, and their functional effects as fundamental bioactive molecules began to be considered.

HMOs are non-digestible oligosaccharides with no direct nutritional effects, consisting of variable combinations of five monosaccharides: glucose, galactose, fucose, *N*-acetylglucosamine, and sialic acid. These oligosaccharides can be short or long-chain (3 to 9 units) and are present in a 9:1 ratio. Intestinal hydrolysis of lactose into its two constituent molecules (glucose and galactose) provides part of the lactose content of the substrates necessary for HMO synthesis in the mammary gland. From lactose, the addition of specific monosaccharides gives rise to different groups of HMOs: beta HMOs by the addition of galactose and *N*-acetylglucosamine, neutral HMOs by the addition of fucose and acidic HMOs by the addition of sialic acid.⁴ More than 200

different HMOs have been described so far in HM. HMOs constitute the third solid component of HM, after lactose and lipids, and their concentration is from 100 to 300 times higher than in cow’s milk. Their amount is variable throughout lactation: in colostrum, it is 20-25 grams/liter, and in mature milk, approximately 15 grams/liter. The concentration in the HM of mothers of premature infants is higher.⁵ HMOs are resistant to cold and heat, pasteurization, and freezing.⁶

The variety and concentration of HMOs differ from that of other milk (bovine, caprine), standing out in HM the high concentration of compounds with fucose (70%) compared to the higher proportion of derivatives with sialic acid in cattle milk. Fucose derivatives selectively stimulate bifidobacteria.⁵ Its production by the mammary gland depends on factors such as maternal nutritional status, feeding, environment, type and gestational age at birth,⁷ and especially on maternal genetics due to the concentration of an enzyme, fucosyltransferase, whose production is encoded in the *FUT2* gene located on chromosome 19.⁸ Because of this, the HM content can be variable; there is an approximate percentage of 5% of mothers in whom the production of HM is less than 5 grams/liter.⁹

This genetic component in the HMO content of HM is essential for the proportion of fucose conjugates. The HMO content of HM has a decisive influence on the infant’s intestinal microbiota composition and function.¹⁰

BENEFICIAL EFFECTS OF HUMAN MILK OLIGOSACCHARIDES AND MECHANISMS OF ACTION

Prebiotics are substrates used selectively by host microorganisms that confer a health benefit.¹¹ In this context, HMOs can be considered prebiotics naturally present in HM. HMOs stimulate the proliferation of bacteria of the genus *Bifidobacterium*,¹² beneficial microorganisms dominant in the gut of healthy infants in the first years of life.¹³ These bacteria use HMOs as an energy source and produce short-chain fatty acids (SCFA), such as butyrate, propionate, and acetate, by colonic fermentation. Butyrate promotes the thickening of the mucous layer lining the intestine, preventing potentially pathogenic microorganisms’ adhesion and their entry into the bloodstream. Scientific evidence also suggests that HMO modulates the response of epithelial and immune cells, reducing the infiltration and excessive activation of leukocytes in the mucosa,

thus reducing the risk of necrotizing enterocolitis.¹

The type of birth (vaginal or cesarean) determines the profile of microorganisms that will colonize the infant's intestine. In infants born vaginally, the microbial composition is similar to that found in the birth canal and in the maternal intestine, but in those born by cesarean section, a microbiota composition similar to the mother's skin and the hospital environment is observed.¹⁴

In the case of those born by cesarean section, the microbiota has a lower diversity and quantity of beneficial bacteria than those born by vaginal delivery. In the intestinal microbiota of babies born by cesarean section, the predominant bacteria are *Staphylococcus*, *Corynebacterium*, *Propionibacterium*, and a lower concentration of *Bifidobacterium*. If these infants are fed with HM, the microbiota will progressively and in direct proportion to the duration of lactation resemble that of vaginally born infants, regarding the stability and diversity of the microbiota, since bacteria from HM contribute to the colonization of the intestinal mucosa, supported by the HMOs.¹⁵ Although different methods have been proposed to promote intestinal colonization of babies born by cesarean section, HM administration is the method that is the most effective.¹⁴

HMOs may benefit neurodevelopment by influencing the composition of the gut microbiota and the production of metabolites that could positively impact the brain. Although research in this area is in development, it is suggested that HMOs could have positive effects on neurodevelopment, both motor and cognitive function, especially in preterm infants who are more susceptible to neurodevelopmental deficits due to brain injury at birth and compromised brain maturation while in the neonatal intensive care unit. It has been observed that HM-fed preterm infants show better outcomes than formula-fed infants, especially those born prior to 30 weeks of gestation. In addition, MRI studies in HM-fed infants have demonstrated more mature white brain matter, fewer lesions and larger regional volumes.¹⁶ *Figure 1* summarizes the mechanisms of action that mediate the beneficial effects of HMOs.¹⁷

ADDITION OF HUMAN MILK OLIGOSACCHARIDES TO INFANT FORMULAS

Adding HMO analogs, structurally and functionally identical to those found naturally in HM, represents infant formula technology's most important innovation of the last decade. The

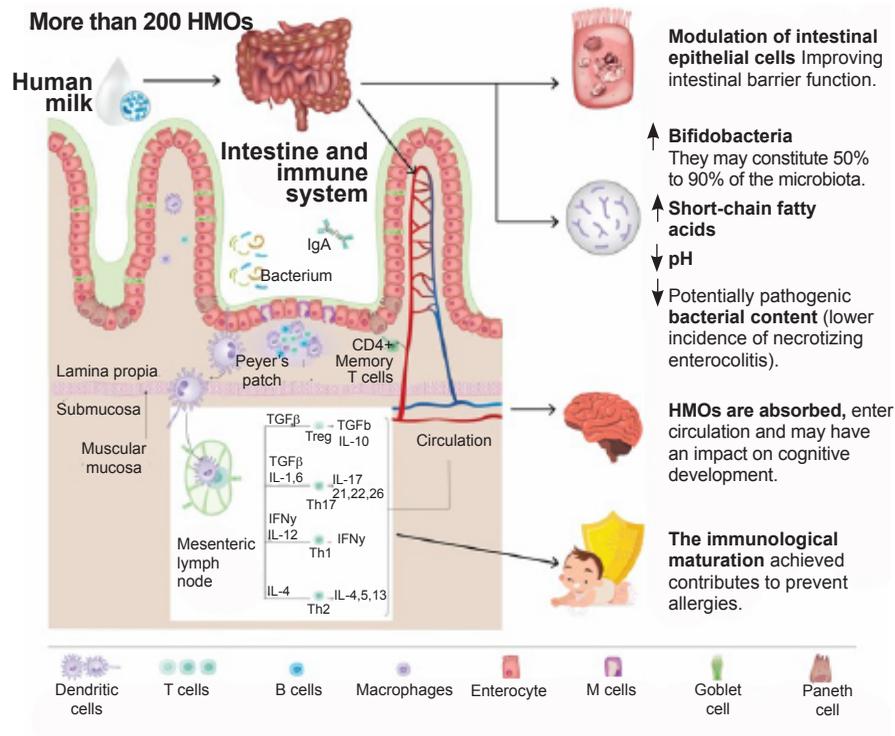
European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) will soon publish a position paper proposing the use of the term "HMO analogs" to refer to those obtained by chemical synthesis since those added to formulations are not isolated and purified from HM (Swajezka 2024, personal communication). 2'-fucosyl lactose (2'-FL) was the first HMO available for use in infant formulas. Currently, 2'-FL (2'-fucosyl lactose), 3-FL (3-fucosyl lactose), 3'-SL (3'-sialyl lactose), 6'-SL (6'-sialyl lactose), and LNT (lacto-N-tetraose) are available for addition to infant formulas (Hill et al.). 3'-GL (3'-galactosyl lactose) is the only one produced in situ by microbial fermentation by *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065 in the same milk used as a base for formula preparation.¹⁸

Most infant formulas are produced from cow's milk with virtually no HMO content and little variability.¹⁹ Advances in biotechnology now allow the synthesis of HMOs that are chemically and structurally identical to those containing HM. Organizations such as the European Food Safety Agency (EFSA) and the Food and Drug Administration (FDA) in the United States has approved its use. The first formula containing HMO was marketed in Spain and the United States in 2016. The approved use for 2'-FL is 1.2 g/L, and for LNT, 0.6 g/L, in a 2:1 ratio for infants under one year of age.^{17,20,21} The number of HMOs that can be used in infant formulas has increased in recent years: 2'-FL, 3'-FL, 3'-GL, LDFT, LNnT, LNT, 3'-SL, 6'-SL.¹⁸

Different clinical studies have been published, which include mostly term and healthy infants.²² In *Table 1*, some of the controlled clinical studies are summarized. These studies evaluated different HMOs, individually or in combination, at different doses. In general, most of them include aspects of safety and tolerance.²³⁻³³ Other aspects investigated are growth, stool characteristics, microbiota composition, and biomarkers.^{27,29} Some studies found that HMO supplementation is safe and well tolerated, and growth was adequate.³¹

Other results observed were that the feces were of better consistency and more frequent, similar to that of infants fed with HM.³⁴ Some of these studies analyzed the responses in children with cow's milk protein allergy^{26,31} and observed good tolerance, improvement of symptoms, and adequate growth in the period studied. Other authors found positive effects on the composition of the microbiota.²⁸ In particular, it was observed

FIGURE 1. Proposed mechanisms of action for the local and systemic beneficial effects described for human milk oligosaccharides



Source: adapted from Dinleyici et al., 2022.¹⁷

that the administration of a formula with GOS/FOS and HMO 3'-GL (produced *in situ* by microbial fermentation) was able to maintain a microbiome and metabolome closer to those of human milk-fed infants than the control formula without GOS/FOS nor 3'-GL.³² GOS/FOS are oligosaccharides of galactose and fructose (chains of between 3 and 9 units), where these sugars are chemically linked in such a way that they escape digestion and absorption in the small intestine, and reach the large intestine intact, where they are selective substrates for bifidobacterial growth. Despite

these findings, further studies are needed to demonstrate health effects related to the structure and dosage of HMOs in infant formulas.

CONCLUSION

HM is the best food for all newborns, particularly because of its HMO content. HMO is a complex and dynamic group of oligosaccharides selectively utilized by bifidobacteria, which positively impacts the intestinal microbiota and the immunological, metabolic, and neurological development of newborns.

TABLE 1. Controlled clinical studies of infant formulas' developmental and health effects with added human milk oligosaccharides

Reference	Inclusion criterion	Intervention	Control group	Duration	Results
Marriage et al. 2015 ²³	Healthy infants >2500 g > 5 days	2'-FL and GOS in 3 concentrations	Infants Exclusive HM	4 months	No significant differences in weight, length, and head circumference. Good tolerance.
Puccio et al. 2017 ²⁴	Healthy infants 0-14 days	2'-FL (1.0 g/L) and LNnT (0.5 g/L)	Starter formula without HMO.	12 months	Safe, well-tolerated, adequate growth. Secondary outcomes: lower morbidity, lower antibiotic and antipyretic use.
Alliet et al. 2022 ²⁵	Healthy infants <14 days	2'-FL	Formula without 2'-FL	6 months	Significantly lower content of <i>Costridioides difficile</i> and a higher content of <i>Bifidobacterium</i>
Ramírez Farías et al. 2021 ²⁶	Infants 0-6 months, CMPA suspected	Hydrolized extensive formula with 0,2 g/L of 2'-FL	Hydrolized extensive formula	2 months	Adequate tolerance, improvement of symptoms. Adequate growth in the period studied.
Parshat et al. 2021 ²⁷	Healthy infants <14 days	HMO: total 5.75 g/L (52% 2'-FL, 13% 3'-FL, 26% LNT, 4% 3'-SL, and 5% 6'-SL)	Starter formula without HMO HM exclusive	4 months	No difference in weight gain with both formulas. Both HMO formula group and HM group presented softer and more frequent stools than the control group.
Bosheva et al. 2022 ²⁸	Healthy infants BW >2500 g 7-21 days	2'-FL, LNT, 3'SL, 6'-SL	Starter formula without HMO HM exclusive	15 months	The microbiota was significantly different in HMO and HM groups vs. formula without HMO: higher concentration of <i>Bifidobacterium longum</i> subsp. <i>infantis</i> and lower concentration of <i>Clostridium difficile</i> .
Goehring et al. 2022 ²⁹	Healthy infants 5 days of life	2'-FL, with or without GOS	Starter formula without HMO HM exclusive	4 months	29-83% lower concentration of plasma cytokines than those fed GOS-only formulas.
Vandeplass et al. 2020 ³⁰	Infants ≥ 14 days fed with formula	Formula including 3'-GL, scGOS/lc FOS (9:1) 2'-FL	Formula with scGOS/lcFO	17 weeks	Maintained adequate growth, good tolerance, no differences in adverse events.
Vandenplas et al. 2022 ³¹	Infants 0-6 months diagnosed with CMPA	Hydrolized extensive formula 2'-FL, LNnt	Hydrolized extensive formula without HMO	4 months	No significant differences in anthropometry. Significant decrease in the frequency of infections in the upper airway and otitis media.
Rodríguez Herrera 2022 ³²	NB	3' -GL, 25 mg/100 mL	Infants with exclusive HM	17 weeks	Improves the composition of the microbiota and profile of the intestinal metabolome was more similar to those fed with HM.

HMO: human milk oligosaccharides, BW: birth weight, NB: newborn, HM: human milk, CMPA: cow's milk protein allergy.

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