

## Review Article



# The Role of Two Human Milk Oligosaccharides, 2'-Fucosyllactose and Lacto-N-Neotetraose, in Infant Nutrition

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### Conflict of Interest

YV has participated as a clinical investigator,  
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## ABSTRACT

Human breast milk contains numerous biomolecules. Human milk oligosaccharides (HMOs) are the third most abundant component of breast milk, after lactose and lipids. Amongst the synthesized HMOs, 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT) are widely studied and are considered safe for infant nutrition. Several studies have reported the health benefits of HMOs, which include modulation of the intestinal microbiota, anti-adhesive effect against pathogens, modulation of the intestinal epithelial cell response, and development of the immune system. The amount and diversity of HMOs are determined by the genetic background of the mothers (HMO secretors or non-secretors). The non-secretor mothers secrete lower HMOs than secretor mothers. The breastfed infants of secretor mothers gain more health benefit than those of non-secretor mothers. In conclusion, supplementation of infant formula with 2'-FL and LNnT is a promising innovation for infant nutrition.

**Keywords:** Human milk; Oligosaccharide; 2'-fucosyllactose; Lacto-N-neotetraose; Infant; Breast feeding

## INTRODUCTION

According to the recommendations of the World Health Organization, infants must be exclusively breastfed during the first six months of life. Human breast milk provides more than half of the child's nutritional needs during the second year of life [1]. The infants who are formula-fed are more prone to infectious diseases, such as gastroenteritis and acute otitis media, and immune-mediated diseases such as allergy, when compared to the infants who are exclusively breastfed [2].

The composition of breast milk is highly complex, as it contains numerous biomolecules. The human breast milk has 2 types of carbohydrates: lactose and oligosaccharides. Lactose, a major component of human breast milk, has a high nutrition value. Human milk oligosaccharides (HMOs) are the third most abundant component of breast milk, after lactose and lipids. HMOs in the human breast milk are a complex mixture of more than 200 non-digestible and non-nutritional carbohydrates [3]. Among the various compositional differences between human breast milk and cow milk, one of the major differences is the presence of HMOs in human milk, which are virtually absent in cow milk and infant formula [4].

This review discusses the composition of human milk and its biological benefit for infants. Additionally, we also discuss how these beneficial effects can be mimicked if breastfeeding is not possible.

## HMOs

The survival advantage of breastfed infants over non-breastfed infants is known since the 1900s. The stool bacterial composition of breastfed infants was reported to be different from that of the formula-fed infants. Additionally, the presence of an unidentified carbohydrate fraction was also reported in human breast milk.

The amount and composition of HMOs vary among women, and also during the lactation period. Generally, the total HMO concentration is higher during the early stages of lactation and decreases within the first three months [5-7]. The HMO content of breast milk after term delivery is higher than that after preterm delivery. The HMO fraction is the third most abundant component in human milk after lactose and lipids, excluding water. The HMO content usually varies between 10–15 grams per liter (g/L) of mature milk (or 1.5–2.3 g/100 kcal, assuming an energy density of human milk of 64 kcal/100 mL) and 20–25 g/L of colostrum [4,8,9]. The HMO content in the human breast milk is more abundant than the protein content, which is typically around 10 g/L or 1.5 g/100 kcal.

The human breast milk contains three major HMO types: fucosylated HMOs (35%–50%), sialylated HMOs (12%–14%), and nonfucosylated neutral HMOs (42%–55%) [10]. Fucosylated HMOs include 2'-fucosyllactose (2'-FL), while non-fucosylated neutral HMOs include lacto-N-neotetraose (LNnT). The neutral HMOs account for more than 75% of the total HMOs in the human breast milk. The most abundant HMO is the 2'-FL, which constitutes nearly 30% of the total HMOs among secretor mothers. The factors that account for the variability in the secretion of HMOs include the geographical origin and genetic background of the mothers. The HMO content in the breast milk varies among women. Furthermore, the amount and diversity of HMOs are determined by the genetic background of the mothers (**Table 1**). The Lewis antigen system is a human blood group system based upon the genes on chromosome 19p13.3 (fucosyltransferase [FUT] 3 or Lewis gene) and 19q13.3, (FUT2 or secretor gene). Both genes are expressed in the glandular epithelia. The dominant allele of these genes encodes enzymes with FUT activity, while the recessive alleles are non-functional. Globally, it is estimated that about 20% of mothers secrete lower amount of HMOs (non-secretor mothers) than the secretor mothers. An earlier study evaluated the HMO content during the lactation period based on the concentration of 2'-FL. The study suggested that the concentration of LNnT and lacto-N-tetraose (LNT) is co-regulated by the FUT2-dependent 2'-FL concentration. The concentration of 2'-FL was

**Table 1.** Diversity of HMOs based on the genetic background of the mother

Gene	Lewis gene (+)	Lewis gene (-)
Secretor gene (+)	Lewis-positive secretors	Lewis-negative secretors
	Secrete all HMOs	Secrete 2'-FL, 3'-FL, LNFP-I, and LNFP-III
Secretor gene (-)	Lewis-positive non-secretors	Lewis-negative non-secretors
	Secrete 3'-FL, LNFP-II, and LNFP III	Secrete 3'-FL, LNFP-III, and LNFP-V

HMO: human milk oligosaccharide, 2'-FL: 2'-fucosyllactose, LNFP: lacto-N-fucopentaose.

positively correlated with the concentration of LNnT and was negatively correlated with the concentration of LNT [11].

One of the factors that affect the composition of primary gut microbiome in infants is feeding. The intestine of a neonate is already colonized at birth. A healthy gut microbiome protects the host against pathogens by various mechanisms such as enhancing the immune development and stimulating the digestive and metabolic functions. Other factors that affect the colonization of the gut include the gestation period, mode of delivery, environment, and medication. Dysbiosis during early life is a risk factor for immune-mediated diseases, such as allergy and asthma, and intestinal and metabolic diseases.

The gut microbiome of infants born through cesarean section (c-section) is different from that of infants born through vaginal birth. The number of bifidobacteria in the intestinal microbiome of infants born through c-section may take up to 6 months to reach the number of bifidobacteria observed in the intestinal microbiome of infants born through vaginal delivery [12]. The infants born through c-section have a higher risk to develop allergic disease (odds ratio [OR], 1.23;  $p=0.007$ ), obesity (58% higher) and type 1 diabetes mellitus (adjusted OR, 1.19; 95% confidence interval [CI], 1.04–1.36;  $p=0.01$ ) compared to the infants born through vaginal birth [13-15]. Additionally, infant feeding can influence the microbiome composition. The difference in the microbiome composition between the breastfed infants and formula-fed infants is mainly due to the absence of HMOs in cow milk. The consumption of infant formula not supplemented with HMOs will result in a microbiome composition that is poor in bifidobacteria, which can affect the immune development [16]. Additionally, the exposure to antibiotics during the first year of life can also affect the gut microbiome composition. The administration of antibiotics earlier in life and the frequency of antibiotic administration affect the gut microbiome composition [17]. Infants treated with antibiotics are at a higher risk to develop cow's milk protein allergy (OR, 1.26; 95% CI, 1.20–1.33) [18], obesity (32.4 vs. 18.2%,  $p=0.002$ ) [19], asthma (OR, 7.77; 95% CI, 6.25–9.65) [20], otitis (OR, 1.30; 95% CI, 1.040–1.63) [21], inflammatory bowel disease (OR, 5.51; 95% CI, 1.66–18.2) [22], and diabetes (OR, 1.08; 95% CI, 1.05–1.11) than infants not treated with antibiotics [23]. Antibiotics kill not only the pathogen but also the beneficial bacteria, which compromises immune homeostasis and deregulates metabolism. Additionally, antibiotic administration is also associated with the development of antibiotic resistance [24]. Exposure to proton pump inhibitors (PPIs) and H<sup>2</sup> receptor antagonists can also increase the risk to develop immune-mediated diseases, such as atopic dermatitis, asthma, and allergic rhinitis [25].

The establishment of a bifidobacteria-predominant microbiota in the infants who are exclusively breastfed by non-secretor mothers is slower when compared to that in infants who are breastfed by secretor mothers [26]. The infants who are breastfed by secretor mothers are less likely to develop diarrhea, specifically *Campylobacter* gastroenteritis, when compared to those who are breastfed by non-secretor mothers [27]. Several studies have suggested that

the health outcome of infants who are exclusively breastfed by secretor mothers is better than that of infants who are breastfed by non-secretor mothers.

The HMO composition also represents the blood group characteristics. The presence of different neutral oligosaccharides in the human breast milk depends on the activity of specific enzymes (FUTs), which are related to the Lewis blood group [8,28].

## HEALTH BENEFIT OF HUMAN MILK OLIGOSACCHARIDES

Several studies have reported the beneficial effects of HMOs that include modification of the intestinal microbiota, anti-adhesive effect against pathogens, modulation of the intestinal epithelial cell response, and development of the immune system. We will discuss each of these effects further.

### Modulation of intestinal microbiota

HMOs are intrinsic components that affect the gut microbiota by providing an energy source for the beneficial intestinal bacteria. Additionally, HMOs affect the health of the host by serving as a decoy receptor for the opportunistic pathogens in the mucosal surface [29]. One study reported that none of the selected Enterobacteriaceae strains exhibited growth on a medium containing 2'-FL, 6'-sialyllactose or LNnT as a carbohydrate source. However, several strains were capable of utilizing galacto-oligosaccharides (GOS), maltodextrin, and monosaccharide and disaccharide components of HMOs for their growth [30]. The enriched fecal consortia also did not exhibit growth on a medium containing 2'-FL or 6'-sialyllactose, but exhibited limited growth on a medium containing LNnT [30]. Several *in vitro* studies have demonstrated that HMOs promote the growth of certain but not all *Bifidobacterium* [31]. *Bifidobacterium longum* subsp. *Bifidobacterium infantis* exhibit good growth on medium supplemented with HMOs, including 2'-FL, as the sole source of carbohydrate [32-35]. Over time, *B. infantis* consumes all HMOs including its monosaccharide and disaccharide metabolites [34].

The growth of *Bifidobacterium bifidum* is slower than that of *B. infantis* in the presence of HMOs. Additionally, certain *B. longum* strains metabolize fucosylated HMOs [4,34,36]. The *Bifidobacterium kashiwanohense* strain exhibits growth in the presence of 2'-FL and 3'-FL [35]. HMOs are a preferred substrate for *B. infantis*. Other bifidobacteria may reduce the nutrients available for potentially harmful bacteria and limit their growth. Additionally, *B. infantis* produces short-chain fatty acids (SCFAs), which favor the growth of commensal bacteria and not pathogenic bacteria [37]. A study reported that among the 24 probiotic strains, only *B. longum* subsp. *B. infantis* ATCC 15697 and *B. infantis* M-63 were able to ferment 3'-sialyllactose, 6'-sialyllactose, 2'-FL, and 3'-FL [38].

When infants are fed with a formula supplemented with 2'-FL and LNnT, they develop a distinctive stool bacterial profile that is more similar to that of the breastfed infants compared to the infants that are fed with a formula not supplemented with prebiotics. The bacterial diversity of infants at the age of 3 months exhibited increased colonization with beneficial bifidobacteria and decreased colonization with pathogenic bacteria [39].

### Antiadhesive properties

HMOs improve the host defense mechanism by strengthening the gut barrier function [40]. The HMO, 2'-FL inhibits *Campylobacter jejuni* infection and *C. jejuni*-associated mucosal

inflammation [41]. An *in vitro* study demonstrated that 2'-FL attenuates *C. jejuni* invasion by 80% and inhibits the release of mucosal pro-inflammatory signals. A study on mouse model revealed that the ingestion of 2'-FL inhibits the *C. jejuni* colonization by 80%, weight loss by 5%, intestinal inflammation, and induction of inflammatory signaling molecules [42]. A prospective study on infants suggested that the beneficial effect of 2'-FL includes a reduction in the number of episodes of *C. jejuni*-associated diarrhea [43]. LNnT was reported to reduce the abundance of *Streptococcus pneumoniae* in the lungs of an animal model [44]. HMOs may function as a decoy receptor for group B *Streptococcus* [45].

HMOs reduce preterm mortality and morbidity by modulating the gut microbiome to protect against necrotizing enterocolitis, candidiasis, and several immune-related diseases [46]. LNnT reduces the risk of developing necrotizing enterocolitis in preterm infants [47]. Similarly, 2'-FL has also been reported to exhibit beneficial effect against necrotizing enterocolitis [48].

### Modulators of intestinal cell response

HMOs are able to directly affect the intestinal cell response by reducing the cell growth and by inducing differentiation and apoptosis [49]. Intestinal health and barrier function are considered to be the first line of defense in innate immunity [50]. HMOs have been reported to increase the intestinal cell maturation [50].

### Immune modulators

One of the important properties of HMOs is the immunomodulation. HMOs directly modulate the gene expression of intestinal cells, leading to changes in the expression of cell surface glycans and other cell responses [51]. HMOs modulate lymphocyte cytokine production and enable a more balanced TH1/TH2 response. An increasing number of *in vitro* studies suggest that HMOs exert microbiota-independent effects by directly modulating the immune response and by regulating the immune cell population and cytokine secretion [52]. HMOs may either act locally on the mucosa-associated lymphoid tissue or act at a systemic level [4]. The plasma concentration of inflammatory cytokines in the breastfed infants and infants fed with experimental formula supplemented with 2'-FL was markedly lower than that in the infants fed with control formula supplemented with galacto-oligosaccharides [53]. These data indicate that infants fed with a formula supplemented with 2'-FL exhibit lower plasma inflammatory cytokine profiles, which is similar to those of a breastfed reference group [53]. HMOs were more effective than non-human prebiotic oligosaccharides in modulating the systemic and gastrointestinal immune cell responses in pigs [54]. These altered immune cell populations may mediate the rotavirus infection susceptibility [54]. The symptoms of food allergy are reduced by 2'-FL through induction of interleukin-10<sup>+</sup> T-regulatory cells and through indirect stabilization of mast cells [55].

HMOs, especially 2'-FL, directly inhibit the lipopolysaccharide-mediated inflammation during enterotoxigenic *Escherichia coli* invasion of T84 and H4 intestinal epithelial cells through attenuation of CD14 induction [56]. CD14 expression mediates the lipopolysaccharide-Toll-like receptor 4 stimulation of a part of the macrophage migration inhibitory factors inflammatory pathway by suppressing the cytokine signaling 2/signal transducer and by activating the transcription factor 3/nuclear factor- $\kappa$ B. The direct inhibition of inflammation supports the role of HMOs as a stimulator of the innate immune system [56]. Two-year-old children who were born through c-section and fed on an infant formula supplemented with 2'-FL had a lower risk of developing immunoglobulin E-associated allergies compared to those fed unsupplemented formula [11].

## BRAIN DEVELOPMENT

HMOs and their metabolic products, such as sialic acid, have a role in brain development, neuronal transmission, and synaptogenesis. HMOs are a source of sialic acid, which is an essential nutrient for optimal brain development and cognition [57,58]. L-fucose and 2'-FL stimulate brain development of [59]. Dietary 2'-FL affects cognitive domains and improves learning and memory in rodents [60]. The HMOs 3'-sialyllactose and 6'-sialyllactose support normal microbial communities and behavioral responses during stress by modulating the gut-brain axis [61].

## SAFETY AND CLINICAL OUTCOMES

Oligosaccharides were identified as the bifidogenic factor in human milk in the 1930s. The most abundant oligosaccharides in the human breast milk were discovered and characterized in 1954. However, the industrial production of some of the HMOs was recently achieved. The molecular structure of industrially produced 2'-FL and LNnT is identical to that of the oligosaccharides present in the human breast milk. Unlike probiotics, HMOs are resistant to pasteurization and freeze-drying [62,63].

Two intervention studies tested the safety of an infant formula supplemented with approximately 0.2 g/L 2'-FL [64,65]. When combined with GOS, 2'-FL (0.2 or 1.0 g/L) did not affect the growth parameters, was well tolerated, and did not influence the stool frequency or consistency [64]. There was no difference in the frequency of adverse events between the infants who were fed with a formula supplemented with 2'-FL and those fed with a control infant formula supplemented with GOS. Similarly, when infants were fed with an infant formula supplemented with fructo-oligosaccharides (FOS) and 2'-FL (0.2 g/L) for a period of approximately 1 month, the formula was well tolerated [65].

Janas et al. [66] fed the infants with a formula supplemented with 2'-FL or LNnT from less than 14 days of life to the age of 6 months (4 months exclusively). The formula was reported to be safe and well-tolerated, and supported an age-appropriate growth [66]. Additionally, there was no difference in the stool consistency and stool frequency between the 2'-FL-fed group and the LNnT-fed group, except at 2 months when stools were softer in the 2'-FL-fed group [66]. In infants aged less than 8 days of life at inclusion, Kajzer et al. [65] showed excellent tolerance fed of a formula supplemented with 2'-FL and FOS for 1 month. Prieto [67] demonstrated that administration of LNnT to older infants and young children, aged 6–24 months, for a period of 4 months resulted in normal growth. This was the only study that used a follow-up formula.

Puccio et al. [39] analyzed the incidence of different health outcomes as secondary outcomes, and observed a reduced incidence of bronchitis at 4, 6 and 12 months, reduced incidence of lower respiratory tract infections through 12 months, reduced use of antipyretics through 4 months, reduced use of antibiotics through 6 and 12 months with protective effects that continued after 6 months post-intervention period of an infant formula supplemented with 2'-FL and LNnT.

The US Food and Drug Administration categorized three HMOs as generally regarded as safe (GRAS notice n° 650): 2'-O-fucosyllactose, 2'-FL, and LNnT. In the European Union, HMOs are considered as novel foods and the oligosaccharides, 2'-FL and LNnT, and their

combination passed the safety assessment [29]. The European Food Safety Authority (EFSA) positively assessed 2'-FL on June 29, 2015, based on the scientific and technical information and reported that [68]: "2'-FL is safe for infants (up to one year of age) when added to infant and follow-on formulae, in combination with LNnT, at concentrations up to 1.2 g/L of 2'-FL and up to 0.6 g/L of LNnT, at a ratio of 2:1 in the reconstituted formulae. 2'-FL is safe for young children (older than 1 year of age) when added to follow-on and young-child formulae, at concentrations up to 1.2 g/L of 2'-FL (alone or in combination with LNnT, at concentrations up to 0.6 g/L, at a ratio of 2:1)." The HMO, 2'-FL is reported to contribute to the infant's and child's health. Amongst the HMOs, 2'-FL and LNnT are widely studied and have a chemically simple structure. These two HMOs are more abundant in the human breast milk compared to other HMOs. Additionally, these 2 HMOs can be produced on an industrial scale. Hence, these HMOs can be used as supplements to infant formula.

## CONCLUSION

HMOs can serve as soluble decoy receptors that block the attachment of viral, bacterial, or protozoan parasitic pathogens to the epithelial cell surface receptors, which may aid in preventing infectious diseases. HMOs are also antimicrobials that act as bacteriostatic or bactericidal agents. Additionally, HMOs enhance host epithelial and immune cell responses in the neonate.

Although the functions of HMOs were known previously, a strategy for industrial production was not available. Hence, non-human milk oligosaccharides, mainly FOS and GOS, were used as an alternative supplement for the infant formula. Currently, 2'-FL is added to the infant formula as the industrial production capacity has increased. The industrial production of other HMOs, such as LNnT is still limited. Hence, LNnT is not routinely used as a supplement to the infant formula.

The gastrointestinal microbiome of infants fed with a formula supplemented with 2'-FL is similar to that of the infants who are exclusively breastfed. There have been no adverse effects reported till date for 2'-FL. Clinical studies have demonstrated that infants fed on a formula supplemented with 2'-FL exhibit a normal growth pattern and normal defecation. Therefore, it can be concluded that 2'-FL is a safe supplementation for infant formula.

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