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Review

# Biological Relevance of Goat Milk Oligosaccharides to Infant Health

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**ABSTRACT:** Milk is often regarded as the gold standard for the nourishment of all mammalian offspring. The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of the life of the infant, followed by a slow introduction of complementary foods to the breastfeeding routine for a period of approximately 2 years, whenever this is possible (*Global Strategy for Infant and Young Child Feeding*; WHO, 2003). One of the most abundant components in all mammals' milk, which is associated with important health benefits, is the oligosaccharides. The milk oligosaccharides (MOS) of humans and other mammals differ in terms of their concentration and diversity. Among those, goat milk contains more oligosaccharides (*g*MOS) than other domesticated dairy animals, as well as a greater range of structures. This review summarizes the biological functions of MOS found in both human and goat milk to identify the possible biological relevance of *g*MOS in human health and development. Based on the existing literature, seven biological functions of *g*MOS were identified, namely, MOS action as prebiotics, immune modulators, and pathogen traps; their modulation of intestinal cells; protective effect against necrotizing enterocolitis; improved brain development; and positive effects on stressor exposure. Overall, goat milk is a viable alternate supply of functional MOS that could be employed in a newborn formula.

**KEYWORDS:** *goat milk, oligosaccharides, human milk, biological functions, human health*



## INTRODUCTION

Milk oligosaccharides (MOS) are complex nondigestible carbohydrates that have been studied most in the milk of human (*h*MOS), bovine (*b*MOS), goat (*g*MOS) and sheep (*s*MOS). MOS are the third most abundant solid component in human milk, with levels over 20 g/L in colostrum and ranging from 5 to 12 g/L in mature milk.[1] Goat milk contains larger quantities of MOS compared to other domesticated dairy animals. The average reported concentrations in mature milk are 60−350 mg/L, while for colostrum, concentrations have been reported up to 2.4 g/L.[2−6] The breed of the studied animals may significantly affect these oligosaccharide concentration ranges.[2,4,7−9] Overall, the *g*MOS levels are 100 times lower than *h*MOS, but they are 4−10 times higher than the *b*MOS levels,[8] making *g*MOS interesting for MOS studies.

Over the years, 169 structures of *h*MOS have been structurally characterized,[10,11] compared to up to 40 for *b*MOS[12] and about 55 for *g*MOS.[13−17] The difference in the identified structures can partially be explained by the fact that human milk is studied more intensely than other milk types with multiple techniques, leading to a higher number of identified oligosaccharides.

With regard to MOS, the biggest difference between human and goat milk, apart from the concentration of the MOS, is the distribution of neutral and acidic oligosaccharides. All *h*MOS consist of glucose and galactose as core oligosaccharides. *h*MOS can be divided into three categories: 1) neutral nonfucosyl oligosaccharides; core oligosaccharides only/with the addition of *N*-acetylglucosamine (GlcNAc) (e.g., 3′-galactosyl-lactose (3′-GL), Lacto-*N*-neotetraose (LNnT)), 2) neutral fucosyloligosaccharides; core oligosaccharides with the addition of fucose (e.g., 2′-Fucosyllactose (2′-FL), 3-Fucosyllactose (3-FL)), and 3) acidic sialyloligosaccharides; core oligosaccharides with the addition of sialic acid (3′-Sialyllactose (3′-SL), 6′-Sialyllactose (6′-SL)).[1,18,19] Oligosaccharides can also be divided into type 1 and 2 molecules. Type 1 oligosaccharides contain Gal$\beta$1−3GlcNAc, whereas type 2 oligosaccharides contain Gal$\beta$1−4GlcNAc.[10] *h*MOS have a predominance of type 1 over type 2 oligosaccharides, whereas milk of other species either contains only type 2 oligosaccharides (as in the milk of cow, goat, sheep, pig and horse), or a predominance of type 2 oligosaccharides over type 1 oligosaccharides (as in some apes).[10,20]

Around 70% of *h*MOS are neutral, fucosylated, whereas milk from domesticated animals contain 80−90% acidic oligosaccharides, containing only minor levels of fucosylated neutral structures.[21] Van Leeuwen et al.[16] recently provided a comparison of structures and relative quantities based on the study by Albrecht et al. for goat milk[21] to term and preterm human milk on the basis of the study by Austin et al.[22] (Figure 5[16]). In addition, Figure 4 of the same work provides a comparison of the acidic and neutral MOS concentrations of milk of human and other mammalian origin.[16] Although the composition of *h*MOS is significantly different from *g*MOS,

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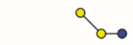
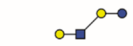
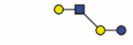
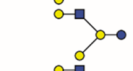
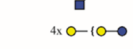
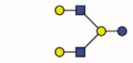
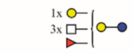
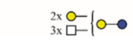
**Table 1a. Overview of All Neutral *g*MOS Structures Reported in Caprine Colostrum and Milk[16],[a]**

| RN ○ ▲ □ ◆ ◇ | Structure | Name (Abbrev.) | *h*MOS | Int (%) | Type oligosaccharide |
|---|---|---|---|---|---|
| 2 1 0 0 0 |  | 2`-fucosyllactose (2`-FL) | Y | 3 | - |
| 2 1 0 0 0 |  | 3-fucosyllactose (3-FL) | Y | 2 | - |
| 3 0 0 0 0 |  | α-3`-galactosyllactose (α3`-GL) | N | 10 | - |
| 3 0 0 0 0 |  | 3`-galactosyllactose (3`-GL) | Y | 27 | - |
| 3 0 0 0 0 |  | 6`-galactosyllactose (6`-GL) | Y | 15 | - |
| 1 1 1 0 0 |  | 3-fucosyllactosamine (3-FLN) | N | - | - |
| 2 0 1 0 0 |  | 6`-*N*-acetylglucosaminyllactose (NAL) | N | - | - |
| 2 0 1 0 0 |  | Lacto-N-triose II (LNT-II) | Y | 4 | - |
| 2 0 1 0 0 |  | α-3`-*N*-acetylglucosaminyllactose | N | <1 | - |
| 4 0 0 0 0 |  | DP4-GOS | N | <1 | - |
| 3 0 1 0 0 |  | Lacto-N-neotetraose (LNnT) | Y | 5 | Type II |
| 3 0 1 0 0 |  | *iso*-Lacto-N-neotetraose (*i*LNT) | N | 2 | - |
| 2 0 2 0 0 |  | di-*N*-acetyl-hexosaminyllactose | N | - | - |
| 5 0 0 0 0 |  | DP5-GOS | N | - | - |
| 3 1 1 0 0 |  | *iso*-Lacto-N-fucopentaose II (*i*LNFP II) | N | - | - |
| 3 1 1 0 0 |  | *iso*-Lacto-N-fucopentaose V (*i*LNFP V) | N | - | - |
| 4 0 1 0 0 |  | *N*-acetylglucosaminyl-dihexosyl-lactose (NADHL) | N | - | Type II |
| 4 0 1 0 0 |  | *novo*-lacto-N-pentaose (*n*LNP) | N | 2 | - |
| 3 0 2 0 0 |  | Lacto-N-*iso*-pentaose (LN*i*P) | Y | <1 | - |
| 6 0 0 0 0 |  | DP6-GOS | N | - | - |
| 4 0 2 0 0 |  | Lacto-N-neohexaose (LNnH) | Y | 6 | Type II |
| 4 0 2 0 0 |  | - | Y | <1 | Type II |
| 3 0 3 0 0 |  | - | Y | 2 | - |
| 2 0 4 0 0 |  | - | N | - | - |
| 4 1 2 0 0 |  | - | N | - | - |
| 3 1 3 0 0 |  | - | N | - | - |
| 4 0 3 0 0 |  | - | N | - | - |


[a]Int (%) = Relative peak intensities in the neutral and acidic pool respectively, based on Albrecht et al.[21]. The *h*MOS column indicates the presence or absence of the structure in human milk. Structures are drawn in the Consortium for Functional Glycomics graphical notation.[108]

there are sialylated, neutral nonfucosylated, and neutral fucosylated structures in common between the two. Moreover, both *g*MOS and *h*MOS are abundant in galactosyllactoses, with the predominant forms in *g*MOS being 3′-GL and 6′-galactosyllactose (6′-GL), while in *h*MOS, the predominant form is LNnT, all neutral nonfucosylated MOS. Neutral fucosylated MOS in common are 2′-FL and 3-FL, while sialylated MOS present both in *h*MOS and *g*MOS are 3′-SL and 6′-SL. There are also differences. Whereas most mammals have the capacity to synthesize *N*-Glycolylneuraminic acid (Neu5Gc) from *N*-Acetylneuraminic acid (Neu5Ac), only a few mammals (e.g., platypus, ferret, new world monkeys) and


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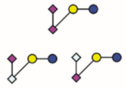
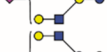
**Table 1b. Overview of All Acidic *g*MOS Structures Reported in Caprine Colostrum and Milk[16],[a]**

| RN ○ ▲ □ ◆ ◇ | Structure | Name (Abbrev.) | *h*MOS | Int (%) | Type oligosaccharide |
|---|---|---|---|---|---|
| 2 0 0 1 0 |  | 3`-sialyllactose (3`-SL) | Y | 13 | - |
| 2 0 0 1 0 |  | 6`-sialyllactose (6`-SL) | Y | 18 | - |
| 2 0 0 0 1 |  | 3`-*N*-glycolylneuraminyllactose (3`-NGL) | N | 30 | - |
| 2 0 0 0 1 |  | 6`-*N*-glycosylneuraminyllactose (6`-NGL) | N | 27 | - |
| 1 0 1 1 0 |  | 3`-sialyllactosamine (3`-SLN) | N | <0.1 | - |
| 1 0 1 1 0 |  | 6`-sialyllactosamine (6`-SLN) | N | <1 | - |
| 1 0 1 0 1 |  | 3`-*N*-glycolylneuraminyllactosamine (3`-NGLN) | N | <1 | - |
| 1 0 1 0 1 |  | 6`-*N*-glycolylneuraminyllactosamine (6`-NGLN) | N | 2 | - |
| 3 0 0 1 0 |  | 3`-Sialyl-6`-GL (3`-S6`-GL) | N | - | - |
| 3 0 0 1 0 |  | 6`-Sialyl-3`-GL (6`-S3`-GL) | N | - | - |
| 3 0 0 1 0 |  | 3`-sialyl-3`-GL (3`-S-3`-GL) | N | <1 | - |
| 3 0 0 0 1 |  | 3`-*N*-glycolylneuraminyl-3`GL) (3`-NG-3`-GL) | N | <1 | - |
| 3 0 0 0 1 |  | 6`-*N*-glycolylneuraminyl-3`GL (6'-NG-3'-GL) | N | <1 | - |
| 2 0 1 1 0 |  | 3`-sialyl-Lacto-N-triose II (3`-SLNT II) | N | <1 | Type I |
| 2 0 0 2 0 |  | Di-sialyllactose (DSL) | N | <1 | - |
| 2 0 0 1 1 |  | *N*-glycolyl-*N*-acetyl-dineuraminyllactose (NGSL) | N | <1 | - |
| 2 0 0 0 2 |  | di-*N*-glycoylneuraminyllactose (DNGL) | N | 2 | - |
| 4 0 0 1 0 |  | Sialyl-DP4-GOS | N | - | - |
| 3 0 1 0 1 |  | *N*-glycolylneuraminyl-LNnT (NGLNnT) | N | <1 | Type II |
| 3 0 0 2 0 |  | di-sialyl-DP3-GOS | N | - | - |
| 3 0 0 1 1 |  | *N*-aceyl-*N*-glycolyl-di-neuraminyl-DP3-GOS | N | - | - |
| 3 0 0 0 2 |  | di-*N*-glycolylneuraminyl-3`-GL (DN-3`-GL) | N | <0.1 | - |
| 4 0 1 0 1 |  | 3`-*N*-glycolylneuraminyl-*n*LNP (3`-NG*n*LNP) | N | <0.1 | - |
| 4 0 1 0 1 |  | 6`-*N*-glycolylneuraminyl-*n*LNP (6`-NG*n*LNP) | N | <0.1 | - |
| 4 0 2 1 0 |  | sialyl-Lacto-N-neohexaose (S-LNnH) | Y | - | Type II |
| 4 0 2 0 1 |  | 3`-N-glycolyneuraminyl-LNnH (3`-NG-LNnH) | N | <0.1 | Type II |
| 4 0 2 0 1 |  | 6`-N-glycolyneuraminyl-LNnH (6`-NG-LNnH) | N | <0.1 | Type II |


[a]Int (%) = Relative peak intensities in the neutral and acidic pool respectively, based on Albrecht et al.[21] The *h*MOS column indicates the presence or absence of the structure in human milk.

humans have lost this capacity during evolution.[23] This is the result of a genetic mutation in the sialic acid biosynthetic pathway, not allowing further addition of an oxygen atom to the sialic acid due to an inactivating mutation of the cytidine monophosphate *N*-acetylneuraminic acid hydrolase (CMAH). Therefore, milk acidic MOS are decorated either with Neu5Ac (>98%, in the milk of cows, pigs, horses and camels) or with Neu5Gc (64 and 94% of the total sialic acid content in the milk of goats (mature milk) and sheep (colostrum), respectively[16]). In humans, the sialic acid is mostly bound to



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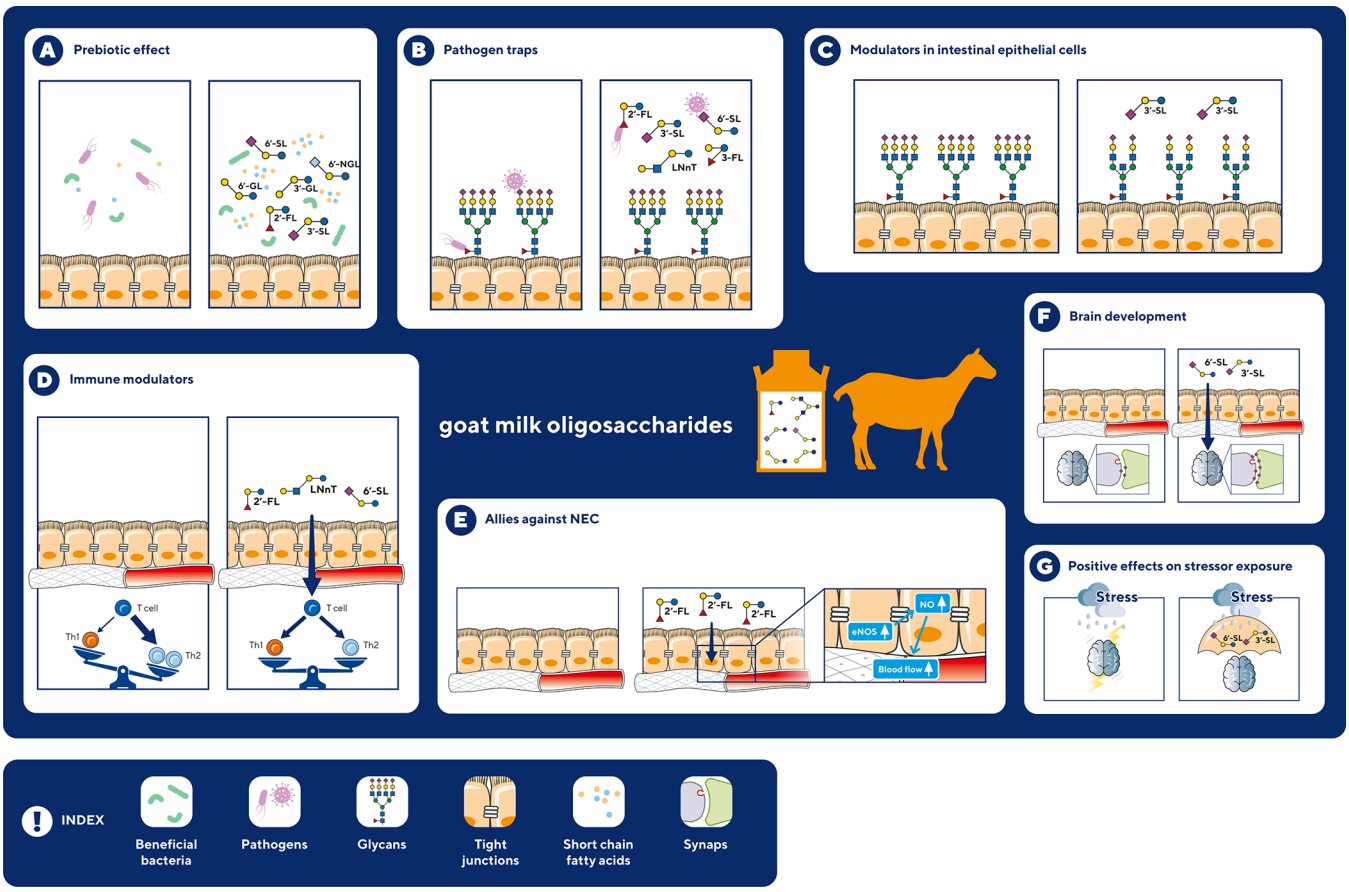


**Figure 1.** Summary of the biological relevance of oligosaccharides derived from goat milk.

free oligosaccharides, while in other domesticated animals, sialic acid is mostly protein-bound.[24]

Human and goat milks have several MOS structures in common, which are summarized in Tables 1a and 1b. The biological relevance of those structures in neonates' health and development will be the focus of the present review. It appears of interest to evaluate the MOS functional properties, with an emphasis on the biological effects of the MOS in infants that have been detected in both human and goat milk. The information presented is based on the, at present, still limited number of studies published on the prebiotic effects of *g*MOS, and on effects that have been reported for individual *h*MOS having identical structures to *g*MOS. Those effects refer to the prebiotics effects of MOS, their action as pathogen traps, as modulators in intestinal epithelial cells, protection against necrotizing enterocolitis (NEC) and human immunodeficiency virus (HIV), and nongut effects. An overview of those established health benefits of *g*MOS is graphically presented in Figure 1, and a summary is provided in Table S1.

## RESULTS AND DISCUSSION

**Prebiotic Functions of MOS.** One of the first positive effects reported for *h*MOS was that it serves as a growth substrate for beneficial bacteria and intestinal microbiota.[18,25] The composition, diversity and metabolic activity of the microbiota in the gastrointestinal tract has a strong influence on the well-being and health of humans.[26] The dominant bacterial genus in the intestines of breast-fed infants is *Bifidobacterium*. *Bifidobacterium* improves the microflora in the intestine and may reduce the chances of acute diarrhea and the risk of *Escherichia coli* (*E. coli*) infections.[27] Infants with bifidobacterial-dominated microbiota are more resistant to pathogen colonization and possess better functioning gut barriers.[28] The strains typically found in infants are *B. infantis* (JCM1222) and *Bifidobacterium bifidum* (JCM1255). When *h*MOS-grown Bifidobacteria were added to Caco-2 and HT-29 cell lines (both human colorectal adenocarcinoma cell lines), it was shown that both these bifidobacterial strains bind to intestinal epithelial cells, which is important for transient intestinal colonization.[29] Ward et al. reported that *B. infantis* consumed *h*MOS faster than *B. bifidum*.[30] This resulted in substantial degradation of oligosaccharides by *B. infantis* compared to moderate degradation by *B. bifidum*. Promotion of the growth of *Bifidobacterium* is beneficial because it may keep harmful bacteria away by competing for a limited nutrient supply. *B. infantis* also produced metabolites, including short-chain fatty acids (SCFA), that create an environment favoring the growth of beneficial (usually commensal) bacteria.[29,31,32] For instance, lowering the pH facilitates the growth of *Bacteroides* and lowers the number of Enterobacteria, of which some are pH sensitive. This may indicate that a low pH protects against infectious diseases.[32]

Lewis et al. tested the influence of milk of mothers with a 2′-FL secretor status on the infant's gut microbiota, using a subset with 44 infant/mother pairs.[32] The infants fed with milk of a 2′-FL *h*MOS secretor established Bifidobacteria in their gut microbiota earlier and more frequently. These infants also showed higher numbers of *Bifidobacterium* and *Bacteroides* (which plays a role in processing complex molecules) compared to infants fed with milk of a nonsecretor.


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Furthermore, the study showed that 2′-FL, produced by secretors, was responsible for a broader range of bifidobacterial species. *B. longum infantis* dominated in secretor-fed infants but not in nonsecretor fed infants. *B. breve* was dominant in infants, independent of the mother's secretor status.[32]

The beneficial prebiotic effects of *h*MOS are contributed to the total mixture, but also to 2′-FL alone. As recently reported, 2′-FL is also present in goat milk (Table 1a), in 73.7% of goat milk samples analyzed in this study.[20] Some studies of prebiotic effects have been conducted with *g*MOS, goat milk, or goat milk infant formula. Also a relatively small number of randomized controlled trials have been performed with goat milk infant formula.[33−36] The fecal microbiota analysis of 2 months old infants receiving goat milk infant formula revealed growth of Bifidobacteria and Lachnospiraceae.[34] More specifically *B. longum* subspecies *longum* were found in their stools, but not *B. longum* subspecies *infantis*.[37] Bifidobacteria dominate the stool microbiota regardless of whether the infants are fed human milk or formula based on ruminant milk (cow or goat). However, Bifidobacteria have about 20% higher relative abundances in human milk-fed babies compared to formula-fed babies.[38]

Paturi et al.[39] studied biomarkers of gut health in newly weaned rats, as a model for mammalian digestion. The 21 days old male rates (n = 12/diet) were fed ad libitum the diet and water during 21 days. The rats on goat and cow milk diets gained more weight than those on the control diet ($P < 0.001$), and animals with prebiotics, such as inulin and fructo-oligosaccharides (FOS), in their diet gained significantly less weight than those on the same diet without prebiotics (whole milk and cellulose). Total bacteria and *Lactobacillus* spp. were significantly higher in rats fed goat milk diets than those fed control diets ($P < 0.05$). *Clostridium perfringens*, an organism associated with gastrointestinal illnesses, was significantly lower in rats fed goat milk diets ($P < 0.05$). Lactic and succinic acid concentrations were significantly higher ($P < 0.001$) in goat milk diets compared to control diets.

Leong et al.[40] isolated *g*MOS from stage 1 and stage 2 goat milk infant formula. Major oligosaccharides were 3′-SL, 6′-SL, 2′-FL, 6′-sialyllactosamine (6′-SLN, Neu5Ac$\alpha$2−6Gal$\beta$1−4GlcNAc), disialylated lactose (DSL) and lacto-*N*-hexaose (LNH), and the minor MOS were *N*-acetyl-glucosaminyl-lactose (NAL), neoglycolipid (NGL), Lacto-*N*-Tetraose (LNT), 3′-sialyl hexosyl lactose (3′-SHL), 3′-*N*-glycolylneuraminyllactose (3′-NGL), *N*-glycolyl-neuraminyl-hexosyl-lactose (SNGHL) and sialyl-*N*-glycolyl-neuraminyl-lactose (SNGL). The isolated *g*MOS of both stages 1 and 2 were found to be effective in promoting the growth of both *Bifidobacterium* and *Lactobacillus* species. They appeared to be more efficient at promoting the growth of *B. longum* BB536 and *L. casei* 2607 than a galacto-oligosaccharide (GOS) mixture.

In the *in vitro* fermentation model SHIME with the setting of 3-month-old infants, goat milk infant formula decreased colonic pH by boosting the SCFA acetate, lactate, and propionate production, which related to increased abundances of acetate/lactate-producing Bifidobacteriaceae as observed with human milk.[41]

Bifidobacterial strains are able to utilize *g*MOS as a fermentable substrate, as shown by Thum et al.[13] In their study, strains of *B. bifidum, B. longum*, and *B. breve* were isolated from the feces of 4 breast-fed infants. The growth medium was enriched with 5 g/L *g*MOS plus 1 g/L GOS, 2 g/L lactose, 1 g/L glucose, and 1 g/L galactose. The presence of *g*MOS enhanced growth of *Bifidobacterium* and stimulated the *in vitro* production of lactate and SCFA, compared to addition of glucose, lactose, GOS, inulin, FOS, 3′-SL and 6′-SL separately. Especially *B. bifidum* was able to ferment *g*MOS. Bifidobacterial strains isolated from feces of exclusively breast-fed infants thus are able to ferment *g*MOS, stimulating bifidobacterial growth and metabolism.[13]

**MOS as Pathogen Traps.** *h*MOS possess an antiadhesive microbial activity.[18,19] Similar structures are found between human milk (*h*MOS) and epithelial glycoproteins (glycans). Pathogens with lectin-like receptors may adhere to *h*MOS instead of binding to a glycan of the epithelium. As a result, the pathogens are not able to bind to epithelial cells. Fucosylated oligosaccharides protect against heat stable enterotoxin STa produced by some strains of *E. coli* and other enteric bacteria by functioning as antiadhesive microbials, as shown in a T84 cell line (human carcinoma).[42] STa is a known cause of diarrhea in infants.[42,43] Binding of STa to the extracellular domain of guanylate cyclase, in enteric bacteria responsible for transmitting signals from outside a cell to the interior of the cell, causes an inhibition of the chloride function (via cyclic guanosine monophosphate, cGMP), and hence infection.[44] The accumulation of intracellular GMP levels in the lumen of the intestine are part of a cascade of events that leads to increased chloride secretion, causing diarrhea.[45] The oligosaccharides used in the study were pooled from 20 to 30 human donors. After separating the milk into fucosylated and nonfucosylated oligosaccharides, only the fucosylated oligosaccharide fraction inhibited STa stimulated guanylate cyclase. This protective effect was larger on cGMP production than on STa binding. Also, the three-dimensional structure of STa shows no similarity with various fucosylated oligosaccharides from milk. Therefore, the authors speculate that fucosylated oligosaccharides bind to the extracellular portion of guanylate cyclase instead of competing for the STa binding site. By binding to the toxin receptor, the fucosylated oligosaccharides act as antiadhesive antimicrobials.[42]

Fucosylated oligosaccharides also serve as antiadhesive antimicrobials in an infection by *Campylobacter jejuni*. This effect was tested with the HEp-2 cell line (human epithelial type 2), *in vivo* in mice (breed BALB/c, an albino, immunodeficient inbred strain) by feeding them *h*MOS, and *in vitro* on human intestinal mucosa.[43] Only the neutral, fucosylated oligosaccharide fraction inhibited infection of HEp-2 cells with the used *Campylobacter* strains (84sp, 135ip, 166ip, 173ip, 180ip, 187ip, 193ip, 225sp, 268ip, 287ip, and 383ip). Inhibition of the fucosylated oligosaccharides was then tested in three models: 1) *in vivo*; mice receiving neutral, fucosylated *h*MOS showed significantly less *Campylobacter* colonization, 2) *ex vivo* using human intestinal mucosa (strain 287ip) which showed that administration of 0.2 mg/mL (half of the concentration in pooled human milk) neutral oligosaccharides caused a larger reduction (93%) than administration of 2′-FL alone (69%), and 3) in a model that tested inhibition in mice carrying the FUT1 human fucosyltransferase gene. Epithelial cell surface fucosyl epitopes serve as ligands to bind *Campylobacter* to the intestinal mucosa. This mechanism is inhibited by fucosylated oligosaccharides. The $\alpha$1,2-linked fucosyloligosaccharides appear to serve as ligands that compete with the intestinal epithelial cell surface receptor for binding to *Campylobacter* (and other pathogens) that target $\alpha$1,2-ligands.[43]


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*h*MOS also serve as antiadhesives in rotavirus infection. Rotavirus is a known cause of gastroenteritis. Neonatal rotavirus infections are caused by a different rotavirus strain in older children. The effects of pure 3′-SL, 6′-SL, 2′-FL, and of GOS, were tested on different rotavirus strains.[46] The study, conducted on African green monkey kidney epithelial cells (MA104 cells), used the globally dominant rotavirus genotypes strains G1P and G2P. With all tested oligosaccharides, a severe reduction in infectivity was observed at a concentration of 5 mg/mL (concentration of the individual oligosaccharides). At lower concentrations, a significant reduction was only observed with 3′-SL and 6′-SL. Treatment of the epithelial cells with *h*MOS before infection did not result in a reduction in infectivity, so *h*MOS act on the virus rather than on the cells.[46] The concentrations of oligosaccharides used in this study, however, were higher than the concentrations of the individual *h*MOS in human milk. Also, the rotavirus strains used are globally dominant genotypes, but not in neonates. Ramani et al. tested the effects of LNT, Lacto-*N*-fucopentaose I (LNFP I), Lacto-*N*-fucopentaose II (LNFP II), 2′-FL and 6′-SL on the G10P rotavirus strain.[47] This strain is associated with a high incidence of neonatal infections in India. Milk from more than 50 human donors was pooled and analyzed to determine the composition. The effects of *h*MOS on infection with rotavirus were again tested on MA104 cells. *h*MOS showed no significant impact on the infectivity of the G1P and G2P strains of the rotavirus, but enhanced infectivity of the G10P (especially 2′-FL and LNFP I).[47] These studies highlight the importance to use the right strain, but also the interplay between *h*MOS, milk microbiome and infant gut microbiome.[47]

Acute gastroenteritis is also caused by human noroviruses.[48] Two main groups of noroviruses exist: GI and GII. GI involves monomeric interactions, whereas GII involves dimeric interactions. The dominant type of outbreak worldwide is caused by type GII.4. Human noroviruses interact with histo-blood group antigens (HBGAs), and this interaction may be important for infection. *h*MOS are structurally similar to HBGA epitopes, so *h*MOS could compete with the HBGA binding sites on the noroviruses.[48] Weichert et al. tested whether 2′-FL and 3-FL block GII.10 norovirus virus-like particles (VLPs) from binding to HBGAs using X-ray crystallography.[49] 2′-FL and 3-FL structurally mimicked HBGAs. Fucose of *h*MOS and HBGAs were identically positioned on the P domain of the VLPs.[34] It is hypothesized that this will also occur with strain GII.4, since these strains are structurally comparable.[48,49] The *h*MOS may thus act as natural decoys in an infection by mimicking HBGAs and binding at the HBGA pocket and thereby preventing the virus to bind.[49]

LNFP II may also serve as a protective oligosaccharide in respiratory and gastrointestinal illness.[50] To test the relationship between *h*MOS consumption and disease in breastfed infants, 49 mother-infant pairs were investigated. Infant health was assessed by maternal report and the concentration of LNFP II was measured to represent levels of total oligosaccharides consumed. Higher LNFP II levels in milk 2 weeks postpartum were associated with fewer infant respiratory- and gastrointestinal problems by 6 and 12 weeks, respectively.[50] The study design was not strong enough to make a correlation, and it is not known whether this specific effect was caused by LNFP II or other *h*MOS. The mechanism behind the demonstrated effect remains to be studied.

*h*MOS also act as pathogen traps against influenza virus (IAV) and respiratory respiratory syncytial virus (RSV). RSV and IAV are common causes of infantile lower respiratory tract infection. The protective effect of 3′-SL, 6′-SL, 2′-FL and LNnT was analyzed by Duska-McEwen et al.[51] Human respiratory cells (Calu-3 and 16HBE) and peripheral blood mononuclear cells (PBMCs) were pretreated with the different *h*MOS for 24 h to determine the effect on virus load and cytokines. The effects differed between the tested *h*MOS and between the cells. 2′-FL decreased RSV load in both the 16HBE cells and Calu-3 cells. It also caused a decrease in the cytokines interleukin (IL)-6), IL-8, macrophage inflammatory protein-1 alpha (MIP-1$\alpha$), monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor $\alpha$ (TNF-$\alpha$). 3′-SL significantly decreased nonstructural protein 1 (NS1) of the RSV virus in 16HBE cells and decreased IL-6, MIP-1$\alpha$ and IL-8. Regarding infection with influenza virus, LNnT and 6′-SL caused a decrease in viral load in 16HBE cells, 6′-SL caused also a decrease in viral load in Calu-3 cells and a decrease in IL-6. Immune cells are necessary for the clearance of respiratory pathogens; therefore, infection with RSV was tested on PBMCs. None of the tested *h*MOS showed a significant decrease in viral load, but 6′-SL caused a decrease in interferon-gamma inducible protein 10 (IP-10) and TNF-$\alpha$. The concentrations used in the study are at and below the concentrations found in human breast milk. The *h*MOS 3′-SL, 6′-SL and 2′-FL thus enhance innate immunity to RSV and IAV (*in vitro*) whereas 6′-SL may also modulate the innate immune response.[51]

Several *h*MOS thus may act as a pathogen trap, 2′-FL, 3-FL, 3′-SL, 6′-SL, LNFPII and LNnT, all of which are present in goat milk (Tables 1a and 1b).

In young mammals, the Neu5Gc containing oligosaccharides are important in protection against infections. The trisaccharide Neu5Gc($\alpha$2−3)Gal($\beta$1−4)Glc as part of N-glycolyl-GM3 glycolipids acts as a decoy for pathogenic *E. coli* K99 in goat kids, piglets, lambs, and calves, which causes severe diarrhea.[52,53] Potentially, the free trisaccharide (3′NGL) could promote such binding of similar or other pathogens, preventing their pathogenic action. Leong et al.[40] isolated *g*MOS from stage 1 and stage 2 goat milk infant formula. The isolated *g*MOS were effective in reducing the adherence of *E. coli* and *S. typhimurium* to Caco-2 cells. Both neutral and acidic oligosaccharides mixtures isolated from goat milk inhibited the adhesion of *Salmonella enterica* to Caco-2 cells analogous to oligosaccharides levels from human milk.[54] *g*MOS (LNnT, lacto-N-neohexaose (LNnH), 3′-SL, 6′-SL, LS-tetrasaccharide c and DSL were able to stimulate the growth of *B. longum* subsp. *infantis* ATCC 15697. This *g*MOS-treated *B. infantis* showed a 42% decrease in the attachment of a highly invasive strain of *Campylobacter jejuni* to intestinal HT-29 cells.[55] Isolated *g*MOS reduces the adhesion of *Staphylococcus aureus* to Caco-2 similar to *h*MOS, indicating that *g*MOS may act as a pathogen trap.[56]

**MOS as Modulators in Intestinal Epithelial Cells.** During postnatal intestinal development, the surface glycosylation pattern of enterocytes changes; it shifts from a high sialic acid phenotype to a high fucose phenotype.[57] Intestinal development is an important contributor to the protection against bacterial adherence. *h*MOS are able to directly modulate intestinal epithelial cells. This was studied on Caco-2 cells, cultured for 7 and 21 days.[58] Following spontaneous differentiation or on pretreatment with 3′-SL,


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Caco-2 cells showed a reduction of specific glycan epitopes. Over time, the α2,3-linked sialic acids and α2,6-linked sialic acids decreased. Cells cultured for 7 days were treated with 3′-SL and showed a decrease in α2,3-linked sialic acids, α2,6-linked sialic acids, and fucose and galactose moieties to a level similar to that of cells cultured for 21 days without 3′-SL treatment (control). 3′-SL treatment reduced the sialyltransferase gene expression of *ST3Gal-I* (2.5-fold), *ST3Gal-II* (2-fold) and *ST3Ga* (5-fold), which are responsible for the formation of α2,3-linked sialic acids. Exposure of Caco-2 cells to *E. coli* showed a 10-fold increase in attachment of bacteria to the cells cultured for 7 days compared to the 21 days controls. Pretreatment of the cells cultured for 7 days with 3′-SL showed a reduction in total adherence of 50%.[58] The addition of 3′-SL to milk thus may stimulate intestinal development, which helps to protect against bacterial attachment.

Hypoxia-induced injury is a known factor that damages the intestinal barrier and is a known cause for NEC. *h*MOS may act as modulators by mediating activation of the epidermal growth factor receptor (EFGR) to protect intestinal epithelial cells against hypoxia-induced injury. This was tested on Caco-2 cells and in 7-days old C57BL/C mice.[59] *h*MOS were purified from pooled milk of 10 secretor-mothers. Different experimental conditions were tested; 1) *h*MOS, 2) FOS and 3) GOS separately were added to infant formula. Supplementing *h*MOS at 10−20 mg/mL into the formula protected against hypoxia-induced epithelial cell injuries. This was accompanied by the upregulation of phosphorylated EFGR and the downregulation of phosphorylated P38. GOS and FOS showed no protective effects.[59]

*h*MOS have a role in modulation of intestinal epithelial cells; until now, only 3′-SL has been studied separately. In rats, however, the standard diet supplemented with goat milk powder seemed to be as effective as supplementation with bovine colostrum in reducing the effect of heat induced gastrointestinal hyperpermeability.[60] A possible mechanism of this protective action that supplementation with both goat and bovine powders has is through modulation of the tight junction in epithelial cells. Metabolic pathways of the citrate cycle (TCA cycle) and pyruvate metabolism were abundant in the goat milk fed mice showing that the metabolism of nutrients was more extensive in the goat milk group.[61]

**MOS Protection against NEC and HIV.** NEC is the most common inflammatory intestinal disorder, affecting (mostly) preterm neonates. It leads to a severe and often fatal destruction of the infant's intestine.[62,63] About 7% of very-low-birth-weight (VLBW, < 1500 g) infants develop NEC, and their mortality rate goes up to 30%.[64] Most common treatments for NEC are broad-spectrum antibiotics, discontinuation of enteral feeding and surgical interventions, usually accompanied by long-term complications.[65] NEC is accompanied by suppressed proliferation of crypt cells and increased cell death, what leads to intestinal barrier dysfunction.[59] Strategies for treatment and prevention of NEC are required, and gaining a better understanding of the pathogenesis and progression of the disease is critical.

Breastfed infants have a 6-fold to 10-fold lower risk of developing NEC than formula-fed infants.[66] In the above, we described various effects of *h*MOS (prebiotic, antiadhesive, etc.) resulting in a "healthier" microbiome. In the following, we evaluate studies of the effects of *h*MOS on NEC specifically in animal and human studies.[62,65]

*NEC Animal Studies.* Jantscher-Krenn et al., based on a rat NEC model of Nadler et al.,[67] confirmed that the risk of developing NEC was highest for formula-fed Sprague−Dawley rats compared to mother's milk-fed pups.[65] The *h*MOS pool derived from milk collected from 12 healthy human volunteers that gave birth to preterm infants. The study showed that the addition of *h*MOS to infant formula improved survival rate from 73.1% to 95% and reduced NEC-related pathology score. No effect in survival of pathology score was observed with the addition of GOS in the diet of the rats.[65] Macroscopic and microscopic observation of the intestines of the formula-fed pups, with and without the addition of GOS, indicated that they had more pathological signs, when compared with the formula-fed that were supplemented with *h*MOS. Chromatographic separation of *h*MOS by charge with HPLC-FL and identification with MALDI-TOF showed that the compound causing the protective effects is disialyllacto-*N*-tetraose (DSLNT).[65] The sialic acids from DSLNT are necessary for the protective properties; when both sialic acids were removed from DSLNT it also lost its protective power against NEC. Adding DSLNT to infant formula showed again the hereabove described effects.[65]

Another beneficial *h*MOS in the protection against NEC is 2′-FL. It was tested in mice (C57BL/6) by Good et al.[68] The 2′-FL was produced through a proprietary fermentation process, and its addition to infant formula showed a protection against NEC, based on gross images of the intestine. 2′-FL restored mesenteric perfusion in NEC, as shown by fluorescent tracer tomato lectin, quantitative real-time PCR (qT-PCR) and Western Blot analysis. The restoration of the mesenteric perfusion was shown to be dependent on the expression and function of endothelial nitric oxidase synthase (eNOS). eNOS has an important function in the central cardiovascular system; it produces nitric oxide (NO) that activates guanylate cyclase. Administration of 2′-FL to *eNOS*-deficient mice and to mice that received eNOS inhibitors did not have a protective effect against the development of NEC. 2′-FL thus helps to protect against NEC by regulating the blood flow to the newborn intestine via eNOS.[68] The data suggest that the 2′-FL found in human and goat milk may be mediating some of the protective benefits of breast milk in the clinical setting against NEC.

Contrary to the above-mentioned beneficial effect of 2′-FL toward NEC, Rasmussen et al. reported limited effects of this structure in a preterm pig NEC model.[69] In this case, the tests were performed providing a mixture of 4-*h*MOS (the most abundant *h*MOS structures) and 25-*h*MOS (a more representative composition of *h*MOS), with 2′-FL present in both mixtures. Although DSLNT was included in the 25-*h*MOS, no preventive effect toward NEC was reported for the 5-day intervention performed with this *h*MOS composition.[69] It is worth noting that the control pigs in this study were fed formula with maltodextrin added at the same concentration as the 4-*h*MOS (5.0 g/L) or 25-*h*MOS mixtures (7.0 g/L).

*NEC Human Studies.* The protective effect of DSLNT against the development of NEC has also been proven in infants.[62] In an observational study including 200 mother-VLWB infant dyads, the *h*MOS composition of breast milk fed to infants was analyzed. Each of the 8 neonates that developed NEC was matched with five controls. Generalized estimating equation (GEE) models showed that the most protective effect against NEC was caused by DSLNT. Moreover, the lower abundance of DSLNT in the mother's milk of neonates that develop NEC could be predictive prior to onset of NEC.


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LNFP I and difucosyllacto-*N*-tetraose (DFLNT) also contributed to the effects of *h*MOS on NEC.[62] After removal of the sialic acid(s) of DSLNT, the beneficial effects were lost. This showed that the effects of DSLNT are highly likely to be structure-specific.[62,65]

Van Niekerk et al., in a substudy of 82 mother-infant pairs, which was part of larger randomized clinical trial, observed that infants that were diagnosed with NEC received mother's milk containing lower concentrations of DSLNT.[70] No other individual *h*MOS was associated with NEC. It is important to note that the study was not designed to determine whether individual *h*MOS in the mother's milk are linked to infant NEC risks.[70]

*HIV*. *h*MOS may also protect against the transmission of HIV from the mother to infant. HIV is an enveloped RNA virus causing acquired immunodeficiency syndrome (AIDS) in late stages. Without effective antiretroviral interventions, around 20% of children born to HIV-infected mothers will acquire HIV. An additional 15% of these children who did not acquire infection, will so during the postnatal period. Children born to HIV-infected mothers that are not affected are called HIV-exposed uninfected (HEU) children. The mortality rate of HEU children is higher than that of children born to uninfected mothers.[70,71] A study of the effects of *h*MOS on mortality in children born to HIV-infected mothers was conducted in a population in Zambia in an early weaning trial.[71] Breastfeeding proved to be important for HIV and HEU children: cessation of breastfeeding was associated with an increased risk of death among HIV and HEU children compared with healthy individuals. Another observation was the higher concentration of 3′-SL and LNnT in the milk of HIV infected women. A difference in survival rates showed that the *h*MOS LNFP I, LNFP II/III, 3-FL, 2′-FL and LNT were lower in mother's milk of HEU children who died during breastfeeding than mothers of survivors, and this difference disappeared after breastfeeding.[71] 3′-SL was increased in HIV-infected women compared to uninfected women, whereas higher concentrations of nonsialylated fucosylated *h*MOS protected against mortality during breastfeeding.[71] Van Niekerk et al. studied 184 mother/infant dyads and confirmed that HIV-infected mothers have a higher relative abundance of 3′-SL compared to HIV-uninfected mothers. Low concentrations of DSLNT in the 4-day milk samples increased the risk of NEC.[70]

Examples of *h*MOS that can protect against NEC and HIV are 3′-SL, DSLNT, 2′-FL and LNnT. Since 3′-SL, 2′-FL and LNnT are present in goat milk (Tables 1a and 1b), one could expect that *g*MOS could also act against NEC or HIV, but no studies have been reported with *g*MOS and NEC or HIV.

**MOS as Immune Modulators.** *h*MOS may also affect the infant's immune system.[18] T cells are important in the immune response. There are two different types of T helper (Th) cells: Th1 and Th2. Th1 cells drive the type-1 pathway involved in cellular immunity. This pathway is used to fight viruses and other intracellular pathogens. Th2 cells drive the type-2 pathway, which is involved in humoral immunity. This pathway upregulates antibody production to fight extracellular organisms, such as parasites. Th1 cells secrete cytokines that inhibit Th2 proliferation; Th2 cells secrete cytokines that inhibit Th1 proliferation. In a mature immune system, a homeostasis is reached between Th1 and Th2.[72,73] In an immature immune system, there is no homeostasis yet. The immature immune reaction has a biased Th2 response, which makes the infant more susceptible to pathogens.[74] This response is important during the pregnancy because it prevents adverse immunological reaction between mother and the fetus.[72,73] Pregnancy is chiefly a Th2 situation; babies tend to be born with Th2 biased immune responses. The influence of *h*MOS on the immature T cell response was studied in human umbilical cord blood obtained by venipuncture immediately after delivery by Eiwegger et al.[75] This study showed that only the acidic *h*MOS stimulated cord blood cells to produce more of the Th1 type cytokines, interferon-$\gamma$ (IFN-$\gamma$) and IL-10. Acidic *h*MOS thus play a direct role in the postnatal maturation of the immune system by directing the neonatal Th2 response to a more balanced Th1/Th2 response.[75]

He et al. tested the effect of *h*MOS from colostrum on the immune response in intact immature human intestinal mucosa.[76] These colostrum *h*MOS again stimulated cytokines associated with the Th1 response and suppressed Th2 cytokine production. The positive effect of *h*MOS toward the Th1/Th2 response could not be reproduced with *b*MOS.[75]

LNnT coupled to dextran had beneficial effects regarding innate response, as analyzed by Terrazas et al.[77] Tested in BALB/c mice and C57B2/6 mice, administration of injected LNnT-Dex caused an expansion of peritoneal exudate cells (PEC), which harbor a number of immune cells including macrophages, B cells, and T cells. The PECs from LNnT-Dex injected mice suppressed proliferation of stimulated $CD4^+$ cells in a dose-dependent way (Th2 response). The suppression was mediated by cell-to-cell interaction and soluble factors; fixed cells still caused inhibition of $CD4^+$ cells, but the inhibition was lower than that caused by living cells. After blocking cell-to-cell interaction by using transwells, there was still a significant inhibition in the proliferation of $CD4^+$ cells, caused by soluble factors (contributors were IL-10, IFN-$\gamma$ and NO). LNnT coupled to dextran thus also contributes the shift of the neonatal Th2 response to a more balanced Th1/Th2 response.[77]

3′-GL, 4′-galactosyllactose (4′-GL), 6′-GL, and GOS reduce inflammatory signaling pathways in the immature gut mucosa. This was tested by Newburg et al. on human metastatic colonic epithelial cells (T84), human normal colon mucosal epithelial cell lines (NCM-460), on human normal fetal intestinal epithelial cells (H4) and *ex vivo* immature human intestinal tissue.[78] The inflammatory response was measured as induction of IL-8, MCP-1 and macrophage inflammatory protein-3$\alpha$ (MIP-3$\alpha$). GOS and *h*MOS reduced TNF-$\alpha$ induced expression of IL-8, MCP-1 and MIP-3$\alpha$. Pro-inflammatory signaling by TNF-$\alpha$ was mediated by nuclear transcription factor $\kappa$B (NF-$\kappa$B). *h*MOS and GOS inhibited the NF-$\kappa$B signaling pathway and attenuated the intestinal epithelial response of inflammatory stimuli and, thereby, have a positive effect on inflammation.[78]

In an allergic individual, stimulation by an allergen causes mast cell degranulation, which produces responses characteristic of an allergic reaction.[79] A mast cell primed by an antibody (IgE) activates enteric nerves, which results in pain and diarrhea.[80] *h*MOS are able to reduce symptoms of food allergy as shown by Castillo-Courtade et al.[81] BALB/c mice were used to test the influence of 2′-FL and 6′-SL on food allergy symptoms. These symptoms were induced by an oral ovalbumin (OVA) challenge in sensitized mice. This is a nontoxic T cell dependent antigen. Successive oral feeding of OVA led to diarrhea of increasing severity, hypothermia, and an increase in MCP-1. Treatment with 2′-FL or 6′-SL showed


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a significant reduction of intestinal allergy, measured by diarrhea- and hypothermia scores and a reduction of MCP-1 cells. Also, the cytokine profiles changed; mice treated with 6′-SL or 2′-FL showed a decrease in IFN-$\gamma$ production, whereas 6′-SL also caused a 3-fold increase in IL-10. Besides other effects, there was a reduction of the passive cutaneous anaphylaxis (PCA) response, which is an animal model for inflammatory reactions in Type I allergy. IgE dependent T cell activation was caused by the $CD4^+CD25^+$ population from 2′-FL and 6′-SL treated mice. At last, 2′-FL and 6′-SL caused an increase in $CD11^+CD103^+$ (mucosal classical dendritic cells) in Peyer's patches.[81] Antigen presentation by intestinal $CD11^+CD103^+$ dendritic cells is thought to play a role in inducing regulatory T cells and establishing oral tolerance.[82]

The effects of feeding formulas supplemented with 2′-FL alone on immune function was tested by Goehring et al. in 424 infants.[83] This was done by comparing breastfeeding (1.9−4.9 g/L 2′-FL) with formula-feeding without 2′-FL (2.4 g/L GOS) and with the addition of 2′-FL in two concentrations (condition 1:0.2 g/L 2′-FL + 2.2 g/L GOS, condition 2:1 g/L 2′-FL + 1.4 g/L GOS). The inflammatory cytokine levels of IL-1$\alpha$, IL-1$\beta$, IL-6 and TNF-$\alpha$ were shown to be higher in infants fed formula without the addition of 2′-FL. Breastfed infants and infants fed formula supplemented with 2-′FL were not different. RSV-stimulated PBMC cultures, breastfed infants, and infants fed 2′-FL supplemented formula had lower concentrations TNF-$\alpha$ and IFN-$\gamma$. 2′-FL lowered the percentages of circulating T-lymphocytes for total cell and CD4+ cells and increased the percentage of apoptotic cells. Apoptosis might remove activated T cells in order to down-regulate inflammatory cytokines. Formulas supplemented with 2′-FL thus modify the immune profiles to be more like breastfed infants.[83]

gMOS mixture has anti-inflammatory effects in rats with chemically induced colitis.[84] More specifically, the oligosaccharides isolated from goat milk mainly consisted of 6′-SL, 3′-SL, DSL, NGL, 3′-GL, LNH and NAL. The pretreatment of rats with the above-mentioned mix of gMOS was associated with lower levels of colon IL-1$\beta$, one of the predominant cytokines in rat trinitrobenzenesulfonic acid (TNBS) colitis.

*h*MOS thus seemed to have a favorable effect on immune modulation of infants, especially 2′-FL, 6′-SL and LDFT. Of these, 2′-FL and 6′-SL both are present in goat milk. Goat milk intake modulates the immunological function in mice. Goat milk-fed mice had an increasing proportion of natural killer (NK) cell activity and phagocytosis activity in the spleen. Goat milk feeding during pregnancy and lactation periods in mice can confer protective activity onto offspring by alleviating the airway inflammation of allergic asthma induced by mite allergens.[85] IEC18, HT29, Caco-2 and colonic explants of TLR4 KO mice exposed to *g*MOS showed that the *g*MOS acts as TLR4 ligands stimulating cytokine response in the intestine models.[86]

**Non-Gut Effects of MOS.** At 18 months, breastfed infants show a superior cognitive development score compared to that of formula fed infants. Breastfed infants also have higher intelligence quotients at 7 years. Brain development and cognition are in part dependent on gangliosides and glycoproteins, both containing sialic acids.[87,88] They play a role in the functional establishment of synaptic pathways. Sialic acids create a negative charge on the cell membrane that facilitates the binding of positively charged neurotransmitters. Sialylated proteins are involved in cell migration, neurite outgrowth, regeneration, sprouting, and synaptic plasticity. An important sialylated protein is neuron cell adhesion molecule (NCAM), involved in learning and memory in rodents.[89] All mammals have the capacity to synthesize sialic acid from sugar precursors; however, infants present only limited capacity of synthesizing endogenously sialic acid.[90] Piglet models in nutrition studies are very useful. The piglet digestive tract is physiologically and anatomically similar to the human digestive tract. Also, both require similar nutrients, and birth occurs in the midst of the growth developmental of the brain.[87] However, the gut colonization differs significantly between humans and pigs.[91]

The effect of the specific sialic acid structures 3′-SL and 6′-SL on the brain was studied by Jacobi et al.[92] The dietary concentration of 3′-SL and 6′-SL administrated to the crossbred pigs ($n$ = 54) resembled that in human colostrum (1−3.3 g/L). Pigs either received 2 or 4 g/L 3′-SL or 6′-SL, fed a standard laboratory control diet, or received 2 g of polydextrose +2 g/L GOS. After 21 days the piglets were sacrificed, and the left side of the brain was studied. The addition of 4 g/L 3′-SL caused an increase in ganglioside-bound sialic acid in the cerebellum compared to the control group. The highest increase in the sialic acid concentration in the corpus callosum (15%) was seen in the groups that received either 2 g/L 3′-SL or 2 g/L 6′-SL. This value also corresponds to that in colostrum.[92] Mudd et al. also showed the effects of sialyllactose on the brain using a piglet model ($n$ = 38).[93] The pigs received either a diet consisting of bovine-derived modified whey, a diet enriched with 65 mg SL/100 g milk replacer powder, a diet with 190 mg SL/100 g milk replacer powder, or a diet with 380 mg SL/100 g milk replacer powder. Magnetic resonance imaging (MRI) and tract-based spatial statistics (TBSS) showed differences in measurements of myelin within the corpus callosum; mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) between the experimental groups. The group that received the moderate amount of SL (190 mg SL/100 g milk replacer powder) showed the highest rates of diffusion.[93] Diffusion is an important feature of development. These studies highlight the importance of sialic acids in brain development. Regarding the limited capacity to biosynthesize sialic acids in the early life, it is well-understood that sialic acids need to be delivered by an exogenous source in that demanding period for the infant's brain development.[90]

*h*MOS may also play a role in long-term information storage. Long-term potentiation is a model for long-term information storage in the brain. It is an experimentally evoked process where brain synaptic strength is rapidly increased by means of pre- and postsynaptic structures. An important presynaptic feature is the population spike amplitude (POP). Movement of ions causes a shift in electrical potential. A postsynaptic feature is the field excitatory postsynaptic potential (fEPSP). fEPSP makes synaptic neurons more likely to fire an action potential.[94] Matthies et al. used 8 weeks old male mice (Wistar) in their study.[94] Fucose and 2′-FL enhanced POP and fEPSP in the hippocampus, whereas 3-FL did not show a difference. These results showed that fucose and 2′-FL play a role in long-term information storage.[94] This effect of 2′-FL was also observed by Vázquez et al. when testing 2′-FL and fucose on mice (C57BL/6) and on rats (Sprague−Dawley).[88] The same enhanced POP and fEPSP in the hippocampus was found. The positive effect of fucose, however, was not replicated. The effects of 2′-FL on spatial learning, working


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memory, and operant conditioning using an IntelliCage system for mice was also tested. Rats were submitted to a Skinner Box, where 2′-FL treated animals performed significantly better. Finally, it is shown that the postsynaptic density protein 95 (PSD-95), phosphorylated calcium/calmodulin-dependent kinase II (pCaMKII) and brain-derived neurotrophic factor (BDNF) increased in the 2′-FL group, all of which are involved in learning and memory.[88] These studies highlight the importance of 2′-FL in brain development.

There seems to be a positive association between brain development and *h*MOS consumption. This has been studied for 2′-FL, but especially for 3′-SL and 6′-SL, due to the high sialic acid content of the brain. *g*MOS is rich in such acidic oligosaccharide structures, e.g., 3′-SL and 6′-SL (Table 1b).

**Effects of MOS on Stressor Exposure.** Stressors induce anxiety-like behavior, but also significantly change the structure of the gut microbiota, as shown by Tarr et al.[95] In their study, mice were fed a standard diet, a diet containing 3′-SL or a diet containing 6′-SL. A male intruder was introduced in the cage as a stressor, and the levels of stress were evaluated in an open field test (to measure the tendency to avoid open spaces), light/dark test (anxious animals will spend a longer time in the dark), measuring corticosterone levels, and by detecting proliferating cells. After stressor induction, mice fed a standard diet showed changes in composition of the colonic microbiota, which was not observed after a diet with 3′-SL or 6′-SL. In mice fed the standard diet, the stressor resulted in anxiety-like behavior due to a reduction in immature neurons in the dentate gyrus. Mice fed the diet with either 3′-SL or 6′-SL showed normal behavior in the anxiety tests. No difference was observed in spleen mass, levels of IL-6 or corticosterone levels. 3′-SL and 6′-SL are thus able to diminish stressor-induced changes in colonic microbiota structure, anxiety-like behavior and immature neuron levels.[95] 3′-SL and 6′-SL, two structures that are among the most abundant *g*MOS structures, thus have a positive effect on stressor exposure.

**Final Remarks on gMOS.** Despite the overlap in MOS between goat milk and human milk, there are also differences caused by the absence of Neu5Gc in humans.[21] Human milk does not contain intrinsically synthesized *h*MOS with Neu5Gc,[96] while *g*MOS does contain Neu5Gc (e.g., 3′-NGL, 6′-NGL). However, in human milk the dietary Neu5Gc can be metabolically incorporated.[97] Neu5Gc has been reported in relatively low levels in a pooled human milk sample of a single volunteer, which is hypothesized to occur due to the maternal diet (meat and cheese intake).[98] Additionally, Quin et al. showed that in 16 mother-infant dyads, all human milk samples consist of Neu5Gc-containing human milk oligosaccharides (Neu5Gc-*h*MOS).[99] These Neu5Gc-*h*MOS levels were positively correlated to Bacteroides spp. and not correlated with immune markers in the infants stool. Both *Akkermansia muciniphila* and *Bacteroides* contain sialidases that are capable of degradation of Neu5Gc.[100] Neu5Gc from glycans can be hydrolyzed by bacterial sialidases *in vitro*[101] and by the gut microbiome of ancestral hunter-gatherers Hadza people in Tanzania *in vivo*.[102] This sialidase activity thus will result in Neu5Gc degradation or Neu5Gc excretion into the urine. *Bifidobacterium bifidum* AGR2166 is also able to breakdown Neu5Gc, as showed by depletion of 6′-NGL.[13] Studies have linked Neu5Gc incorporation to potential link diseases such as atherosclerosis with dietary incorporated Neu5Gc,[103] while small levels of Neu5Gc have been found in human malignancies. However, no causality is established for Neu5Gc and certain disease outcomes. Mainly the research attempting to link Neu5Gc to the generation of different diseases focuses on red meat, which is the most concentrated in Neu5Gc among common food items.[103,104] The fate and effect of Neu5Gc in infants and on infants long-term health is not known, though one might speculate that there is a possibility that infant's microbiota can profit from presence of Neu5Gc containing *g*MOS. Currently existing epidemiological studies are limited regarding the role of Neu5Gc in human health.[105] Further epidemiological studies, apart from mechanistic studies, would provide valuable insight on the role of Neu5Gc in human health and disease[90] and decipher the origin of the Neu5Gc from specific food items or other—currently unknown—sources.

Overall, goat milk oligosaccharides may exert a wide range of beneficial effects. A summary of the reported beneficial effects of *h*MOS is shown in Table S1 for those structures reported to be present in *g*MOS and in *h*MOS. The health benefits reported in Table S1 are, prebiotic effects, antiadhesive properties (pathogen traps), modulation of intestinal epithelial cells, and protection against various (severe) diseases, as visualized in the infographic Figure 1.

The beneficial effects are attributed to *h*MOS as a pool, but there is also experimental evidence of the effects of individual *h*MOS structures. The most-reported individual *h*MOS studied are 2′-FL, 3-FL, 3′-SL, 6′-SL, 3′-GL, 4′-GL, 6′-GL, LNFP I, LNFP II, DSLNT, LNT, LNnT, DFLNT and LDTF. These *h*MOS also occur in goat milk, except DSLNT, LNFP I, 4′-GL and LDFT (Tables 1a and 1b). Goat milk oligosaccharides do contain LFNP II and LNT, but as an iso-form. As mentioned above, the overall concentration of *g*MOS is lower compared to that of *h*MOS; however, it is 4−5 times higher compared to the MOS found in cow milk, which is widely used for infant formula. Considering the relatively high diversity of MOS structures in goat milk compared to cow milk, goat milk appears to be an attractive alternative source of MOS for infant formula. It is worth noting here, that the yield of goat milk is around 2 L per milking, with goats remaining persistent milkers throughout the year.[106] However, from the perspective of *g*MOS-based industrial applications, no appropriate method appears to be in place for the purification of *g*MOS from goat milk to allow their use in other applications. The current methods of purification require large amounts of goat milk, setting a limitation for the large-scale purification of *g*MOS.

Since *g*MOS are abundant in acidic oligosaccharides (80−90%), it would be most interesting to focus especially on these compounds. Besides other effects, the acidic *h*MOS play a direct role in brain development and in the postnatal maturation of the immune system by directing the Th2 response to a more balanced Th1/Th2 response.[75,76] However, this effect could not be reproduced with cow milk oligosaccharides.[75] It is therefore of interest to investigate whether this effect can be reproduced with the acidic *g*MOS. If positive, this would give goat milk a significant advantage in this respect. In a recent consensus paper, it was stated that the addition of one simple, short-chain oligosaccharide to infant formula does not lead to a similarity with the complex composition of hundreds of short- and long-chain oligosaccharides in human milk.[107] Importantly, the use of single isolated or synthetic MOS could lead to stronger and more straightforward conclusions regarding the functional role in human health. Overall, the use of well-defined MOS extracts could lead to multiple health benefits for the infant.


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## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c02194.

Summary of the reported beneficial effects of MOS found both in human and goat milk (Table S1) (PDF)

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## ■ ABBREVIATIONS USED

| | |
|---|---|
| 2′-FL | 2′-fucosyllactose |
| 3-FL | 3-fucosyllactose |
| 3′-GL | 3′-galactosyllactose |
| 3′-NGL | 3′-*N*-glycolylneuraminyllactose |
| 3′-SHL | 3′-sialylhexosyllactose |
| 3′-SL | 3′-sialyllactose |
| 4′-GL | 4′-galactosyllactose |
| 6′-GL | 6′-galactosyllactose |
| 6′-NGL | 6′-*N*-glycolylneuraminyllactose |
| 6′-SHL | 6′-sialylhexosyllactose |
| 6′-SL | 6′-sialyllactose |
| 6′-SLN | 6′-sialyllactosamine |
| AIDS | acquired immunodeficiency syndrome |
| BDNF | brain-derived neurotrophic factor |
| *b*MOS | bovine milk oligosaccharide(s) |
| cGMP | cyclic guanosine monophosphate |
| CMAH | cytidine monophosphate *N*-acetylneuraminic acid hydrolase |
| DSL | disialyllactose |
| DSLNT | disialyllacto-N-tetraose |
| *E. coli* | *Escherichia coli* |
| EFGR | epidermal growth factor receptor |
| eNOS | endothelial nitric oxidase synthase |
| fEPSP | field excitatory postsynaptic potential |
| FOS | fructooligosaccharide(s) |
| Fuc | fucose |
| FUT1 | Fucosyltransferase 1 (H Blood Group) |
| Gal | galactose |
| GalNAc | N-acetylgalactosamine |
| Glc | glucose |
| GlcNAc | *N*-acetylglucosamine |
| *g*MOS | goat milk oligosaccharide(s) |
| GOS | galactooligosaccharide(s) |
| HBGAs | histo-blood group antigens |
| HEU | HIV-1-exposed uninfected |
| Hex | hexose |
| HexNAc | *N*-acetylhexosamine |
| HIV | human immunodeficiency virus |
| *h*MOS | human milk oligosaccharide(s) |
| HPLC-FL | high-pressure liquid chromatography coupled to fluorescence detector |
| IAV | influenza A virus |
| IFN-$\gamma$ | interferon gamma |
| IL | interleukin(s) |
| iLNT | *iso*-Lacto-*N*-tetraose |
| LNFP | lacto-*N*-fucopentaose |
| LNH | lacto-*N*-hexaose |
| LNnH | lacto-*N*-neohexaose |
| LNnT | lacto-*N*-neotetraose |
| LNT | lacto-*N*-tetraose |
| MALDI-TOF | matrix assisted laser desorption ionization−time-of-flight |
| MCP-1 | monocyte chemoattractant protein 1 |
| MIP-1$\alpha$ | macrophage inflammatory protein-1 alpha |
| MOS | milk oligosaccharide(s) |
| MRI | magnetic resonance imaging |
| Muc2 | mucin 2 |
| NAL | *N*-acetyl-glucosaminyl-lactose |
| NCAM | neuron cell adhesion molecule |
| NEC | necrotizing enterocolitis |
| Neu5Ac | *N*-acetylneuraminic acid |
| Neu5Gc | *N*-glycolylneuraminic acid |
| NGL | neoglycolipid |
| NK | natural killer |
| NS1 | nonstructural protein 1 |
| OVA | oral tolerance to ovalbumin |
| PBMCc | peripheral blood mononuclear cells |
| qT-PCR | quantitative real-time polymerase chain reaction |
| pCaMKII | phosphorylated calcium/calmodulin-dependent kinase II |
| PEC | peritoneal exudate cells |
| POP | population spike amplitude |
| PSD-95 | postsynaptic density protein 95 |

| | |
|---|---|
| RSV | respiratory syncytial virus |
| SCFA | short-chain fatty acid |
| SHIME model | Simulator of Human Intestinal Microbial Ecosystem model |
| *s*MOS | sheep milk oligosaccharide(s) |
| SNGHL | *N*-glycolyl-neuraminyl-hexosyl-lactose |
| SNGL | sialyl-*N*-glycolyl-neuraminyl-lactose |
| ST3Gal-I and II | $\beta$-galactoside $\alpha$2,3-sialyltranferase |
| STs | heat-stable enterotoxins |
| TBSS | tract-based spatial statistics |
| TCA | tricarboxylic acid cycle |
| Th | T helper |
| TLR | toll-like receptor |
| TNBS | trinitrobenzenesulfonic acid |
| TNF-$\alpha$ | tumor nectrosis factor $\alpha$ |
| VLBW | very-low-birth-weight neonates |
| VLPs | virus-like particles |
| $\alpha$-3′-GL | 3′$\alpha$-galactosyllactose |

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