Special Article

NUTRITION REVIEWS

Functional role and mechanisms of sialyllactose and other sialylated milk oligosaccharides

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Human milk is a rich source of oligosaccharides. Acidic oligosaccharides, such as sialyllactose (SL), contain sialic acid (SA) residues. In human milk, approximately 73% of SA is bound to oligosaccharides, whereas only 3% is present in free form. Oligosaccharides are highly resistant to hydrolysis in the gastrointestinal tract. Only a small portion of the available oligosaccharides in breast milk is absorbed in the neonatal small intestine. SL and sialylated oligosaccharides are thought to have significant health benefits for the neonate, because of their roles in supporting resistance to pathogens, gut maturation, immune function, and cognitive development. The need for SA to allow proper development during the neonatal period is thought to exceed the endogenous synthesis. Therefore, these structures are important nutrients for the neonate. Based on the potential benefits, SL and sialylated oligosaccharides may be interesting components for application in infant nutrition. Once the hurdle of limited availability of these oligosaccharides has been overcome, their functionality can be explored in more detail, and supplementation of infant formula may become feasible.

INTRODUCTION

Human milk is often referred to as the optimal source of nutrition for the first few months of human life, as it provides all the necessary nutrients for normal growth and development. In addition, human milk also contains components that may provide benefits for the infant beyond traditional nutrients.[1] Human milk is associated with the reduction of incidence and severity of infections and related morbidity and mortality in infancy.[2] It contains growth factors and immunological components that are well balanced with respect to the specific needs of the neonate during its developmental stages. Other advantages of human milk include possible enhancement of cognitive development, prevention of obesity and hypertension, and support of immune maturation associated with prevention of allergies and insulin-dependent diabetes mellitus.[3,4]

Human milk oligosaccharides (HMOs) are an important fraction in human milk, consisting of neutral and acidic oligosaccharides. A broad range of functions have been attributed to HMOs, both locally in the gut lumen and systemically after absorption. Locally, HMOs can act as a component of human milk, supporting innate immunity by preventing attachment of potential pathogens to the intestinal lining. Furthermore, they can function as a prebiotic, which is defined as selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.[5–8]

Neutral oligosaccharides, which include galacto-oligosaccharides and fructo-oligosaccharides, are already used in fortified infant formula. Studies to investigate their effects on health showed, among other things, modulation of microbial composition, with stimulation of bifidobacteria and lactobacilli, and a reduction in the

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incidence of atopic dermatitis in infants.[9,10] Acidic oligosaccharides, such as sialyllactose (SL), have not been added to infant formula to date. Since they, too, may contribute important health benefits, they have recently received increased attention. Because of their potential beneficial effects, it is relevant to gain an overview of the functional role of the different types of oligosaccharides. Several papers have reviewed the benefits of HMOs in general (including, but not limited to, those cited here[11–14]). This review specifically focuses on the current state of knowledge on the functional role and underlying mechanisms of the acidic oligosaccharides SL and other dietary sialylated milk oligosaccharides. These oligosaccharides are important nutrients for, e.g., supporting resistance against pathogens, gut maturation, and cognitive development in humans, and in particular in infants.

COMPOSITION OF HUMAN MILK OLIGOSACCHARIDES

Unique among mammalian milks, human milk contains a tremendous diversity of oligosaccharide structures.[15] More than 100 different HMOs have been identified, which can be separated into neutral and acidic fractions, mainly depending on the presence of one or more residues of, respectively, fucose or sialic acid (SA).[11,13,16] After lactose and lipids, HMOs are the third most predominant component in human milk.[17,18] HMO molecules are synthesized in the mammary glands, starting with lactose at the reducing end.[11] The core molecule is generally characterized by repetitive attachment of galactose (Gal) and *N*-acetylglucosamine (GlcNAc) via β-glycosidic linkage to lactose. Whereas the structure of the core molecule already gives rise to a wide range of different molecules, the structural and chemical variety becomes even greater because of α-glycosidic linkages of fucose (neutral oligosaccharides) and/or SA (acidic oligosaccharides) to the respective core molecules.[12] As a result, approximately 50–70% and 10–30% of HMOs are fucosylated or sialylated, respectively, and less than 10% are neither fucosylated nor sialylated.[19] The exact composition of fucosylated and sialylated oligosaccharides depends, among other things, on the secretor status of the mother, which is closely associated with the blood group status.[20] Although the relative quantities of oligosaccharides differ, both fucosylated and sialylated oligosaccharides can be present in milk from a variety of mammals.[15] Until recently, it was thought that bovine milk mainly contains sialylated oligosaccharides but lacks fucosylated oligosaccharides.[21] Recent studies using sensitive and advanced mass spectrometric technology showed that fucosylated oligosaccharides, such as 2′- and 3′-fucosyllactose, can be detected in bovine milk.[22,23] However, from the total pool of oligosaccharides, less than 1% were fucosylated, indicating that the levels of fucosylated oligosaccharides are very low, whereas almost 70% were sialylated.[21,23] This may explain why less advanced methodologies sometimes fail to detect bovine fucosylated oligosaccharides.

SA is the generic term for the family of *N*- and *O*-substituted derivatives of neuraminic acid, a 9-carbon acidic sugar molecule. More than 50 naturally occurring derivatives of SA have been described.[24] *N*-acetylneuraminic acid (2-keto-5-acetamido-3,5dideoxy-D-glycero-D-galactononulosonic acid, abbreviated as Neu5Ac or NANA) is the most widespread form and usually the only form found in humans (Figure 1). SA rarely occurs free in nature but terminates oligosaccharide side chains at the nonreducing end of Gal or *N*-acetylgalactosamine (GalNAc) of, for example, mucins, glycoproteins, and glycolipids and therefore mainly functions in cell membranes.[25] The trisaccharide SL consists of lactose at the reducing terminus and an SA residue at the nonreducing end via an α-2,3 binding or α-2,6 binding, resulting in, respectively, 3′-SL and 6′-SL (Figure 1).[24]

HUMAN MILK VERSUS BOVINE MILK

Human milk and bovine milk differ in composition with respect to the amount and type of glycoproteins and oligosaccharides. Human κ-casein, the major glycoprotein in human milk, contains more carbohydrate by weight (40–60%) than bovine κ-casein (10%).[26] Furthermore, the oligosaccharide moiety of human κ-casein is rich in SA compared with that of bovine κ-casein. Human milk is also a rich source of a large variety of free oligosaccharides, not only in colostrum (13.3–23 g/L) but also in mature milk (3.5–14 g/L).[13,18,27–29] Since the exact composition of the oligosaccharide fraction in human milk is variable, data reported in the literature vary strongly; however, the oligosaccharide content of bovine milk is reported to be lower (Table 1).[40] The most abundant sialylated oligosaccharides in human milk are 6′-SL and 3′-SL, disialyllactose-*N*-tetraose (DSLNT), and sialyllacto-*N*-tetraose (LNT). Whereas 6′-SL is the major SL in human milk, 3′-SL is predominant in bovine milk.[32]

Since concentrations of oligosaccharides in bovine milk are much lower than those in human milk, unsupplemented infant formulas derived from bovine milk have a much lower content of glycoproteins and oligosaccharides. Most (69–82%) of the SA in human milk is bound to oligosaccharides. A smaller fraction (15–28%) is bound to glycoproteins, whereas only 2–3% is present in free form.[33,34] Commercial infant formulas have been shown to contain a much lower SA content, and the SA is bound primarily to glycoproteins.[33]

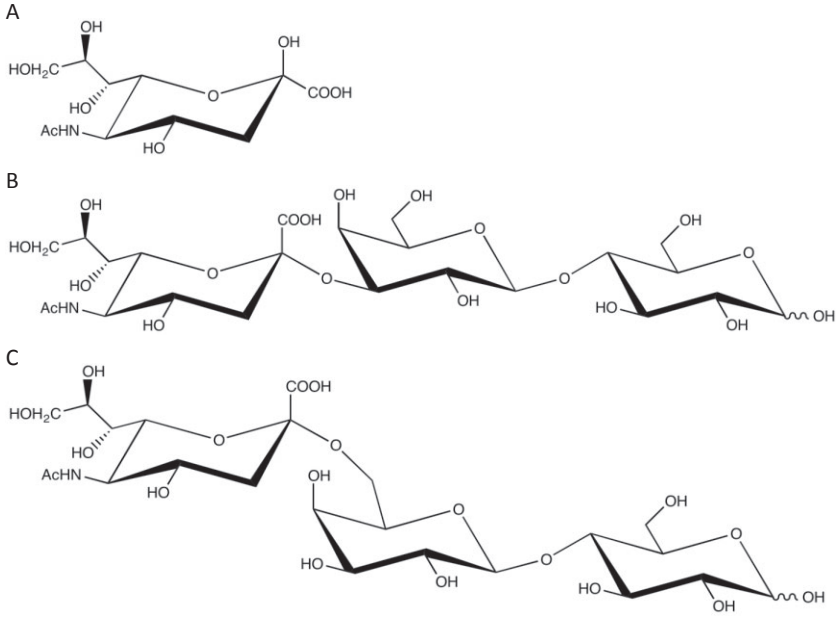

Figure 1 **Structure of (A) *N*-acetylneuraminic acid (Neu5Ac or NANA), the predominant sialic acid found in mammalian cells, and (B) 3′-sialyllactose and (C) 6′-sialyllactose, the most abundant sialylated oligosaccharides in human milk.**

SIALIC ACID AND SIALYLATED MILK OLIGOSACCHARIDES DURING PREGNANCY AND LACTATION

All mammals, including humans, can synthesize SA *de novo*. However, it is speculated that, during the neonatal suckling period, *de novo* SA production is insufficient to meet the needs of all tissues in the rapidly developing newborn and that SA is a conditionally essential nutrient for the suckling neonate.[24,33]

Pregnancy is associated with an increase in concentration of SA in maternal saliva and plasma. SA in saliva increases from 50 mg/L at 10 weeks of gestation to up to 150 mg/L at 20–40 weeks of gestation, corresponding to a period of rapid accumulation of SA in the fetal brain.[24] This suggests that the mother synthesizes much of the SA, which crosses the placenta to contribute to fetal brain growth in the third trimester.[24,46] This fits with observations in mothers of preterm infants. Breast milk from these mothers has 13–23% more SA than milk from mothers of full-term infants at most lactation stages.[33,47] This may reflect the increased plasma and saliva SA levels observed in mothers during pregnancy, which, under normal conditions, contribute to growth and development of the unborn child. Maternal dietary intake may influence the concentrations of sialylated glycoconjugates in human plasma, breast milk, and other secretions and tissues, but to date it is unknown how these are related.[24]

Both the amount and the composition of oligosaccharides in human milk vary with the infant's gestation, the genetic makeup of the mother, the duration of lactation, and even diurnally. Total HMO content and the concentrations of SA (bound to oligosaccharides, proteins, and fat) are both high in milk during early lactation but decrease toward weaning.[48] SA content of human milk decreases from 4.85 mmol/L on day 3–5 postpartum to 1 mmol/L at 4–8 months (corresponding to 1,500 mg/L and 300 mg/L, respectively).[33]

The natural oligosaccharide profile in humans is related to the individual's ABH and Lewis blood group and secretor status and, in women, is reflected in the SA content of breast milk. Milk of women with blood group (H+)Le(a+b+) contains the highest levels of sialylated oligosaccharide, whereas milk of women with blood group ABH(−)Le(a+b−) contains the lowest levels.[49] Whether this blood-group-associated variation in the levels of HMOs affects a child's predisposition to certain infectious diseases or brain development and function in vivo is largely unknown at present,[14,50] but there are studies indicating that it is relevant and should be considered when dietary intervention studies are designed.[7] SA appears to be one of the most variable fractions of human milk, as this acidic component can vary by a factor 3 between mothers at the same stage of lactation.[14,46] After delivery, total NANA and 6′-SL concentrations in human milk decrease with time, whereas the concentration of 3′-SL remains fairly stable.[17,28,51]

Table 1 **Total and sialylated oligosaccharides in human milk, bovine milk, and infant formulas based on cow's milk.**

Oligosaccharides	Human colostrum (<7 days)	Mature human milk (30–120 days)	Bovine colostrum	Mature bovine milk	Infant formulas
Total oligosaccharides[27–31]	13,300–23,000 mg/L	3,500–14,000 mg/L	~1,000 mg/L	300–500 mg/L	400–8,000 mg/L[a]
Sialylated (acidic) oligosaccharides[6,9,14,18,30,32–39]	1,000–3,300 mg/L	135–2,150 mg/L	230–1,500 mg/L	10–195 mg/L	14–288 mg/L
6′-sialyllactose[18,32,39–43]	250–1,300 mg/L	170–500 mg/L	30–243 mg/L	17–88 mg/L	3.8–4.6 mg/L
3′-sialyllactose[18,20,32,39–45]	90–350 mg/L	76–300 mg/L	354–1,250 mg/L	30–119 mg/L	17–19 mg/L

[a] Products include formulas supplemented with galacto-oligosaccharides and/or fructo-oligosaccharides.

DIGESTION OF SIALYLATED OLIGOSACCHARIDES IN THE UPPER GASTROINTESTINAL TRACT

It is generally accepted that most of the oligosaccharides resist the pH of the stomach in infants; they are resistant to enzymatic hydrolysis in the small intestine and are thus largely undigested and unabsorbed.[52] Therefore, it is likely that most oligosaccharides will pass through the intestinal tract and enter the colon intact.[52,53]

Indeed, in a model mimicking the physiological pH of the gastric fluid of the infant's stomach, Gnoth et al.[54] demonstrated that acidic oligosaccharides, including SL, show minor changes in their structure. Furthermore, it has been demonstrated that a mixture of SL and sialyllactitol is not hydrolyzed during retention in the stomach.[55]

In vitro experiments have shown that milk oligosaccharides, including their acidic fraction, are also highly resistant to hydrolysis by human salivary, pig pancreatic, and intestinal brush border enzymes.[54,56] This is because most enzymes present in the gastrointestinal tract are not capable of cleaving fucose, *N*-acetylneuraminic acid (Neu5Ac), or *N*-acetylglucosamine (GlcNAc) from oligosaccharides and glycoconjugates.[54] Although SL is highly resistant to hydrolysis by most enzymes in the intestinal mucosa of humans and rats, SL is hydrolyzed to some extent by mucosal sialidases. The intestinal mucosal sialidase activity seems to correlate with milk SA content, at least in the suckling period of rats, mice, rabbits, cats, and guinea pigs.[57] It has been shown in infant rats that sialidases are not located in the brush border, and are not secreted, but are probably of lysosomal origin.[31] Therefore, the SL of rat milk can be hydrolyzed only if absorbed by the enterocytes. Upon hydrolysis of SL, SA may be released as a nutrient for neonatal tissue and organ development. Whether SL hydrolysis occurs in a similar way in human infants is not known.

Still, the majority of HMOs seem to reach the colon, where they are available for fermentation by the microbiota, and as much as 40–50% may pass unaltered into the feces.[17,58–60]

ABSORPTION OF SIALYLLACTOSE AND SIALYLATED MILK OLIGOSACCHARIDES

A small fraction of milk oligosaccharides, including SL, is absorbed (partly intact) by the paracellular route, transported via blood, and excreted in urine.[61] In vitro experiments using Caco-2 intestinal epithelial cell lines showed that acidic oligosaccharides, including 6′-SL, pass via the nonspecific paracellular route only, whereas neutral oligosaccharides can pass by either the paracellular or the (receptor-mediated) transcellular route.[61] Moreover, dietary SA in free form can be taken up by the distal small

intestine by plasma membrane endocytosis/pinocytosis. A recent study in rats showed that, from the HMOs fed to rat pups, only 3′-SL was absorbed and detected in serum and urine.[62] This is in contrast to findings in human studies, where the HMOs detected in urine of infants are more diverse.[63] This may be related to the fact that the oligosaccharides in human milk are more diverse than those in rat milk, which contains mainly 3′-SL.[15] In fasted mice, it was shown that 50% of orally administered (^{14}C-labelled) SL was excreted unchanged in urine within 24 hours.[48,55] Furthermore, after 24 hours, only 1% of the labelled SL was still detectable in the body, indicating that only a minor fraction is metabolized upon absorption.[55] In infant urine, HMOs are detectable in small amounts, in a range of 50–500 mg/day, which corresponds to less than 10% of the daily HMO intake.[63,64] This suggests that a larger fraction of the HMOs is absorbed in humans than in mice. HMOs are also detectable in the urine of the mother (500–800 mg/day).[65] The excretion of oligosaccharides in the mother's urine during lactation suggests that the oligosaccharides synthesized by the mother not only enter the breast milk but also become available systemically as well, as reflected by urinary excretion. This has been suggested to protect the mother against urinary tract infections.[64] In a human study, oral ingestion of 13C-labeled galactose by a lactating woman resulted in 7% of the label ending up in the milk in the form of lactose and neutral oligosaccharides after 16 hours. After breastfeeding, about 1% of the label derived from this labeled human milk was detectable in the urine of the breastfed child.[66]

SA is detectable in various organs, including the brain,[24] and is found in human milk, plasma, and urine. Moreover, SA is present in many other body fluids, including saliva, gastric juice, and tears, in the form of glycoproteins or as terminal sugars of oligosaccharide chains of mucins.[25] Both the bound and free SA content of saliva is higher in breastfed infants than in bottle-fed infants.[14,67] This suggests that sialylated oligosaccharides present in breast milk may act as a source of SA for the newborn.

POTENTIAL EFFECTS OF SIALYLLACTOSE AND SIALYLATED OLIGOSACCHARIDES

This section provides an overview of the potential health effects and mechanisms of SL and sialylated oligosaccharides. Figure 2 summarizes this overview.

Development of the microbiota

The gut microbiota during early infancy is relatively simple but highly dynamic. Incidental exposures (e.g., method of delivery, maternal microbiota, breastfeeding versus formula, environmental exposure) play a major role in colonization of the neonatal gut.[68] HMOs are able to shape the composition of neonatal gastrointestinal microbial communities.[48] Different HMOs are differently fermented by gut microbiota, as was recently shown for breastfed and formula-fed infants.[69] Among the main products resulting from fermentation of oligosaccharides by bacteria present in the gut are short-chain fatty acids (SCFA). These are produced in varying amounts, depending on the diet and on the composition of the intestinal microbiota.[70]

It is well accepted that the gut microbiota and SCFA play a key role in the maturation of the intestine and act as a barrier against pathogens. SCFA also influence nutrient absorption, host metabolism (amino acid, lipid, antioxidant and drug), and immune development and response.[71] In addition, they are considered antidiarrheal agents, since the uptake of SCFA coincides with the absorption of sodium and water.[72] Furthermore, SCFA have been associated with a reduced risk of diseases, including cardiovascular disease, cancer, and inflammatory bowel disease.[73]

In vitro, bifidobacteria and *Bacteroides* strains are able to grow on HMOs as the sole carbon source.[74,75] Recently, evaluation of the growth and metabolism of various microbiota species in the presence of fucosylated or sialylated HMOs showed that specific species preferentially digest specific HMOs.[76,77] This may subsequently protect the newborn against infection by pathogens[78,79] and has been associated with the development and maturation of the immune system.[80] Bifidobacteria and *Bifidobacterium longum* strains often predominate the colonic microbiota of exclusively breastfed infants.[81] Of the three subspecies of *B. longum* (*infantis*, *longum*, and *suis*), only *B. longum* subsp. *infantis* grows robustly on HMOs.[82] It has two sialidases that predominantly cleave α-2,6 linkages.[74,83] SL has been shown to support not only the growth of *B. longum* subsp. *infantis* but also its adhesion to intestinal epithelial cells, which may be involved in intestinal colonization.[84] Moreover, *Bifidobacterium infantis*, a bifidobacterium present mainly in infants, preferentially consumes small HMOs and possesses fucosidase and sialidase activities not present in several other bifidobacteria strains.[74,82] A recent in vitro study showed that *Bifidobacterium bifidum* expresses an *exo*-α-sialidase that is is capable of liberating SA from sialo-oligosaccharides, gangliosides, mucins, and glycoproteins.[85] In contrast, bifidobacteria that are present mainly in adults, such as *B. longum* subsp. *longum* and *Bifidobacterium adolescentis*, are not able to ferment SAs.[74]

Gut maturation

In early life, maternal antibodies are important for protection of the infant against infections. Maternal immu-

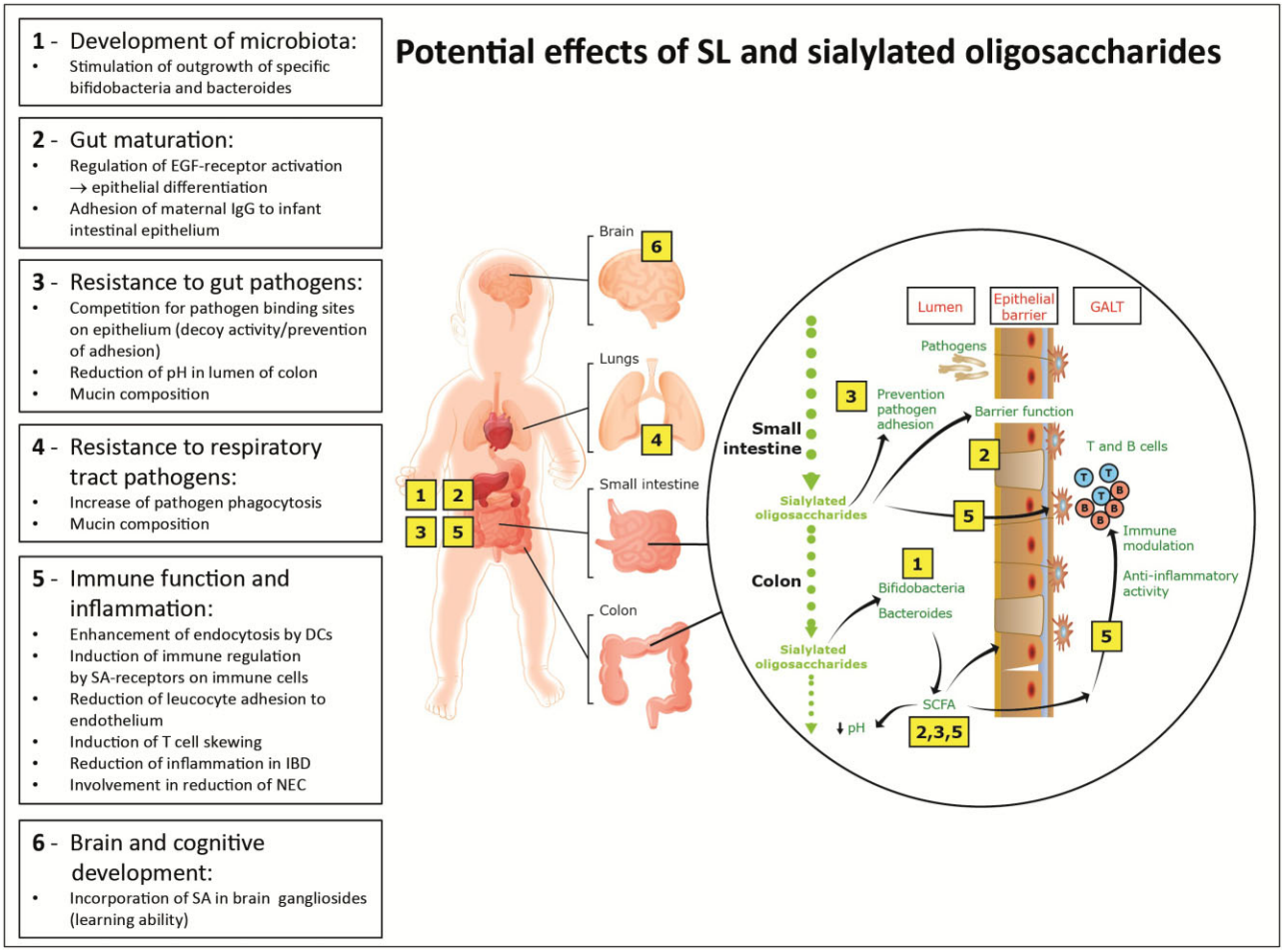

Figure 2 **Potential effects and mechanisms of SL and sialylated oligosaccharides.**
Abbreviations: DCs, dendritic cells; EGF, epidermal growth factor; GALT, gut-associated lymphoid tissue; IBD, inflammatory bowel disease; IgG, immunoglobulin G; NEC, necrotizing enterocolits; SA, sialic acid; SCFA, short-chain fatty acids; SL, sialyllactose.

noglobulin G (IgG) antibodies are taken up in the intestine via specific receptors, such as FcRn.[86] In rat neonates, SA on the surface of the intestinal microvilli has been shown to enhance the binding of IgG antibodies to the epithelium.[87] Towards the time of weaning, after which maternal IgG is no longer available, the expression of SA decreased, explained by a decrease in α-2,6-sialyltransferase activity.[88,89]

Recent in vitro studies suggest that acidic milk oligosaccharides may inhibit intestinal epithelial cell proliferation and induce cell differentiation.[90,91] This effect is mediated via interaction of the oligosaccharides with carbohydrate moieties on the epidermal growth factor (EGF) receptor, thereby regulating activation of this receptor.[91] In addition to oligosaccharides, breast milk is also an important source of EGF in neonates.[92] Via modulation of EGF receptor signaling, EGF and oligosaccharides from breast milk may promote intestinal maturation in early life.

Resistance to gut pathogens

Diarrheal disease is the second leading cause of death in children under 5 years of age and is responsible for killing 1.4 million children every year, mainly in developing countries.[93] During the first years of life, most enteric infections and diarrheal diseases may be attributed to infection with enteric pathogens, including *Escherichia coli* (enterotoxigenic *E. coli* and enteropathogenic *E. coli*), rotavirus, *Campylobacter*, and *Salmonella*.[68,94] Rates of morbidity and mortality due to diarrhea are lower in breastfed infants than in formula-fed infants. This has been attributed primarily to the secretory antibodies and prebiotic factors in human milk.[7] The ability of HMOs to protect against infectious agents may result, in part, from the effects of HMOs on the gut microbiota, but it is thought to be due primarily to their inhibitory (decoy) effect on pathogen binding to host cells in the small intestine.[7]

SA, present as glycoconjugates on bacterial surfaces as well as on host cell membranes, has multiple substitutional binding sites. This enables specific binding to adjacent molecules through distinct glycosidic linkages.[95] By competing for these binding sites, SA components, including 3′-SL and 6′-SL, can prevent or reduce in vitro adhesion of pathogens such as *Salmonella*,[96–98] various *E. coli* strains (S-fimbriated strains in particular),[96,98–101] *Vibrio cholerae*, *Helicobacter pylori,*[98,102–105] *Campylobacter jejuni,*[98,106] and rotavirus.[107,108]

Infection studies in mice showed anti-infective effects of SA components against *H. pylori* (SA)[109] and rotavirus (sialyl lipids, SA).[7,53,108,110,111] SL was shown to prevent cholera toxin binding and its sequelae in rabbit ileal loops.[112,113] This was shown to be specific to SL and was not mediated by SA or lactose.[112] Furthermore, SL promoted eradication of *H. pylori* in rhesus monkeys.[114] Recently, a mixture of SA and SL was shown to reduce rotavirus binding and infection in vitro, whereas both acidic and neutral HMOs were able to reduce rotavirus replication in situ in pigs.[115]

A study in infants in Mexico showed that lactadherin levels in breast milk were associated with protection against rotavirus in infants.[110] Lactadherin contains an *N*-linked carbohydrate moiety that includes SA.[110] The inhibitory effect of lactadherin on rotavirus replication in vitro and in experimental rotaviral-induced gastroenteritis depended on the presence of SA.[111] In a study with a small number of adult human subjects infected with *H. pylori* (a pathogen occurring in the stomach), 1-day oral treatment with 3′-SL was ineffective in reducing the number of *H. pylori.*[116] Supplementation with 3′-SL for 4 weeks in a larger trial was also ineffective.[117] Furthermore, oral supplementation with 3′- SL (doses of 1–5 g per day) for several weeks did not change Lewis antigen expression of *H. pylori* strains isolated from human gastric mucosa.[118] The lack of a protective effect of SL against *H. pylori* infection may be due to the strong mucus layer in the stomach, which covers the bacteria and prevents access of SL to the pathogen. To date, the effect of SL on gut infections in humans has been studied only for *H. pylori* infection, and not for other gut infections.

So far, evidence of the protective effect of SL and sialylated oligosaccharides against gut infections has been obtained mainly from in vitro studies, since data from animal models and humans is limited.

Resistance to respiratory tract pathogens

Acute lower respiratory tract infection (pneumonia), which in children is due mostly to bacterial infection, is one of the leading causes of child mortality in developing countries.[119] *Streptococcus pneumoniae* is a leading cause of bacterial pneumonia, meningitis, and sepsis in children worldwide.[120] Viral lower respiratory tract infections are mild and self-limiting in most cases. Worldwide, the respiratory syncytial virus is by far the most common cause of viral lower respiratory tract infections in infants and young children, followed by influenza viruses.[121]

Exclusive breastfeeding for 3 or more months is associated with a lower risk of hospital admission for respiratory tract infections in the first 6 months of life.[122] This effect might be attributable to HMOs.[6] Absorption of milk oligosaccharides by breastfed infants may provide an adequate source of SA, which can be incorporated in, for example, mucins in the respiratory tract. The influenza virus initiates infection by attachment to host cells, which is followed by endocytosis and fusion of the viral and endosomal membranes. Attachment is mediated by the interaction of the viral surface glycoprotein hemagglutinin, with host cell surface receptors containing sialylated oligosaccharides.[123] An extracellular agent that resembles the host receptors may inhibit this binding. Indeed, intranasal inoculation of an SA bound to a polymeric compound (6-sialyl-*N*-acetyllactosamine) reduced disease symptoms and decreased mortality in influenza-infected mice.[123] In contrast, another study showed that intranasally administered soluble 3′-SL worsened influenza infection outcomes, as it decreased mouse survival and increased the lung inflammatory response.[124] This was shown to be due primarily to interference with phagocytosis of infected lung cells. To limit influenza infection, virus-infected cells become apoptotic and are phagocytosed by macrophages. Phagocytosis depends on desialylation of sugar moieties on the macrophage cell membrane by viral neuraminidase. The data suggested that SL interfered with this desialylation and thereby reduced phagocytosis, leading to reduced elimination of the virus.[125] The discrepancy between these studies may be explained by the fact that the effect of SA compounds on viral respiratory tract infection is dependent on the type of compound provided. The interaction between viral hemagglutinin and SA on host cells is a low-affinity binding. By involving multiple binding sites, the overall multivalent binding provides a high-avidity binding. Therefore, polymeric SA compounds may be more effective than monovalent compounds. Indeed, a large study in children aged 10–24 months who had been given intranasal monovalent 3′-sialyllacto-*N*-neotetraose found no effect on either nasopharyngeal carriage of *Haemophilus influenzae* or prevention of otitis media infections.[126] In contrast, polymeric SL was shown to inhibit adenovirus binding and infection of human corneal epithelial cells in vitro. This effect was stronger with increasing multivalency.[127] Furthermore, a recent study that used 3′-sialyllacto-*N*-neotetraose in liposomes, thereby providing polyvalent binding sites, showed that it

is efficient in preventing infection in vitro in cell lines and in vivo in a mouse model.[128]

In human milk, most SA-containing compounds are monomeric (such as 3′-SL and 6′-SL). Some dimeric (oligosaccharides such as disialyllacto-*N*-tetraose or disialyllacto-*N*-fucopentaose, or glycolipids such as disialogangliosides) or trimeric (e.g., trisialyllacto-*N*-hexaose) compounds have also been identified.[13,129] However, little is known about the difference in the potential of these mono-, di- or trimeric compounds to prevent infection. Glycoproteins with up to 18 SA residues have been detected in human milk. One of these was further characterized as CD36. CD36 as present in breast milk was shown to contain at least 18 SA residues per molecule. In contrast, CD36, when present in blood platelets, was not polysialylated, suggesting that the multiple SA residues in CD36 are specific for CD36 in milk.[130] This glycoprotein was suggested to be important for reduction of pathogen invasion, but supporting data are missing.

Overall, in vivo human evidence of the positive effect of oral SL and sialylated oligosaccharides on resistance against respiratory infections is limited. The data suggest that polyvalent compounds containing SA are more effective than monovalent structures, which may explain discrepancies between studies. Only intranasal administration has been studied, which is a completely different approach from the potential application of these compounds in infant formulas. So far, no studies have been carried out in young infants. Furthermore, viral receptor binding is highly dependent on the virus strain, host species, and tissue, making extrapolation of research findings difficult.[131–133]

Immune function and inflammation

SA also plays an important role in immune function and regulation. Inflammatory diseases can develop in early infancy. Prevention or reduction of early inflammation may prevent the subsequent development of disease. 3′-SL has anti-inflammatory properties, shown by the reduced mRNA levels of proinflammatory cytokines, such as IL-8 and TNF-a, in Caco-2 cells. This effect was mediated via enhancement of the expression of peptidoglycan reco gnition protein 3 (PGlyRP3).[134] PGlyRP3 is a pathogen recognition receptor, shown previously to regulate inflammatory responses in vitro.[135]

Prebiotic oligosaccharides can be transported across the epithelium, as shown in vitro, which suggests that they can reach the immune cells that are present in the epithelial layer.[136] Direct in vitro exposure of peripheral blood mononuclear cells from pigs to various HMOs was shown to alter proliferation and to increase IL-10 production.[137] Direct exposure of human naïve cord blood mononuclear cells to acidic oligosaccharides was shown to skew the cytokine response towards production of IL-10 and interferon-γ (IFN-γ), without induction of interleukin (IL)-13, which is considered a Th1-polarized regulatory immune response that may suppress the induction of Th2 responses associated with allergy.[136] Indeed, when peripheral blood mononuclear cells from peanut-allergic patients were stimulated with peanut allergen in the presence of these oligosaccharides, IFN-γ increased and IL-4 decreased, which is a more Th1-skewed response.[136] These cytokine profiles suggest that oligosaccharides may be able to reverse an imbalanced immune response towards a balanced response, at least in vitro.

SA is important for the dampening of immune responses via IgG. IgG can have either pro- or anti-inflammatory effects. The anti-inflammatory effects have been shown to be mediated via sialylation of IgG. Sialylated IgG binds to inhibitory rather than activating IgG receptors on dendritic cells (DCs), which results in downregulation of the immune response.[138]

SA is also present on the surface of monocytes and DCs as part of glycan structures and seems to be involved in the regulation of endocytosis and immune activation. During DC maturation, the SA composition on the cell surface changes, resulting in an enhanced capacity for bacterial endocytosis and induction of a proinflammatory T-cell response.[139,140] Early T-cell activation has been shown to result in increased SA content on the T-cell surface.[141] This surface SA is involved in the interaction of T cells with antigen-presenting cells (e.g., DCs, B cells), which express specific receptors for SA, such as SA-binding immunoglobulin-like lectins (Siglecs).[142] Many Siglecs have inhibitory effects on immune cells and are therefore believed to be important for immune regulation.[143]

Together, these data show that SA is an important building block for adequate immune function. This underlines the importance of adequate SA availability in infancy, when proper development of the immune system is important for the prevention of diseases or to combat infections.

In humans, inflammatory bowel disease (Crohn's disease and ulcerative colitis) develops mainly in adulthood (75% of the patients) but can also begin during childhood or adolescence (up to 25%).[144] Fuhrer et al.[145] showed that exposure of infant mice to 3′-SL increased susceptibility to colitis at adult age, whereas exposure to 6′-SL did not. This was associated with a change in intestinal bacterial colonization and not with altered immune maturation.[145,146] The proinflammatory effect of 3′-SL in this colitis model involved direct stimulation of DCs in the mesenteric lymph nodes via Toll-like receptor 4, resulting in the expansion of Th1 and Th17 cells and production of proinflammatory cytokines.[147] In another study, however, breast milk was suggested to have a pro-

tective effect against the development of early-onset pediatric inflammatory bowel disease.[148] Although the mechanism of this effect is not clear, it may suggest that either different compounds in breast milk are involved, or that early exposure to SL has different effects on inflammatory processes in infancy and adulthood. Furthermore, differences in the relative amounts of 3′-SL and 6′-SL in breast milk of different species (humans or animals) may play a role.

Preterm delivery is known to increase the risk of the development of necrotizing enterocolitis (NEC) in infants. It has been shown that the level of various milk oligosaccharides is more diverse in mothers who delivered preterm and that these levels do not normalize during lactation. This may be related to the enhanced susceptibility of the preterm neonate to NEC.[149] In a rat model of NEC, sialylated oligosaccharides were able to prevent NEC and reduce the pathology. SA was required for this effect.[150] Remarkably, only one specific dimeric SA structure, disialyllacto-*N*-tetraose, and not sialylated oligosaccharides containing one, three, or four SA groups, was effective, indicating that the exact structure is important for the protective effect.[150]

Together, these studies show that in vivo evidence of the ability of sialylated oligosaccharides to intervene in inflammatory diseases or to actively modulate immune responses is still limited, and mechanisms underlying the regulatory action of oligosaccharides remain largely unknown.

Brain and cognitive development

The highest amount of SA in the body is found in the brain's grey matter, where SA is present in gangliosides and glycoproteins. Sialoglycopeptides are highly concentrated in the synaptosomal fraction and may have a role in cell-to-cell communication. It has even been postulated that SA is the actual receptor for neurotransmitters in the central nervous system. All human brain gangliosides contain NANA as SA.[46]

SA seems to be able to pass the blood-brain barrier.[46,151,152] Breastfed babies have 32% more ganglioside-bound SA and 22% more protein-bound SA in brain tissue when compared with formula-fed babies.[46] Intraperitoneally administered SA in rats resulted in accumulation in brain gangliosides, especially in the synaptosomal fraction.[46,153–157]

Many studies, including meta-analyses, have shown that breastfed infants have biologically significantly higher intelligence scores and better learning abilities, also when results are corrected for socioeconomic confounders.[158] This effect of breastfeeding has not been directly attributed to SA or sialylated oligosaccharides. However, strikingly, several diseases of the brain such as retardation, psychosis in schizophrenia, brain dysfunction in phenylketonuria, and Alzheimer's disease have been shown to be associated with lower SA content of the brain and/or brain gangliosides.[46] Evidence of potential causal mechanisms related to these observations to date comes from animal studies.

A recent study in rats showed that feeding dietary SA to pregnant and lactating rats but not to their litters did not affect total SA content in the cortex of the rat pups.[159] Another study showed that, when SA was fed to both the mothers and the pups, cortical ganglioside SA content did increase.[160] In addition, SA feeding in aged rats normalized brain SA levels displayed on gangliosides to the levels measured in young rats.[161] Furthermore, in a swimming learning test, rats that had been fed SL or galactosylated SA showed improved learning ability, which was associated with increased SA and ganglioside content of the brain.[162] Dietary SA supplementation in developing piglets exposed to active learning tests resulted in increased learning and memory function, increased sialylated brain proteins, and increased levels of mRNA expression of uridine-diphospho-*N*-acetylglucosamine-2-epimerase, a key enzyme in the biosynthetic pathway of SA, in brain and liver.[24,163] In addition, in rats, feeding SA during pregnancy and lactation improved the recognition index of rat pups.[159] Together, these animal studies suggest that oral SL or SA administration increases brain SA content and can improve learning function. Whether brain dysfunction in later life is related to a shortage of SA or sialylated oligosaccharides in early life remains a matter of speculation. No behavioral studies in humans are yet available to resolve the question.[164]

APPLICATION OF OLIGOSACCHARIDES TO FORTIFY INFANT FORMULAS

Because of the known beneficial effects of HMOs, many attempts have been made to develop infant formulas that beneficially stimulate gut microbial colonization. One of the strategies used to achieve this is the supplementation of prebiotics to infant formula.[165] Examplesk of prebiotic oligosaccharides that are currently used in infant formulas are galacto-oligosaccharides and fructo-oligosaccharides, which are both examples of neutral oligosaccharides. Several studies in infants have shown that consumption of these structures can exert beneficial effects such as the promotion of a bacterial microbiota dominated by bifidobacteria. However, galacto-oligosaccharides and fructo-oligosaccharides are not sialylated or fucosylated, while the carboxyl group of SA in acidic oligosaccharides such as SL introduces a negative charge that is critical to some of the benefits of HMOs.[12,166] Food ingredients containing SA, for example as part of the milk-derived caseinoglycomacropeptide or

milk fat globule membrane, are on the market. However, in these products, SA is mainly protein bound, rather than being part of oligosaccharides. Because of the important biological functions of SA, and considering that most of the effects of HMOs are highly structure specific, routes are being explored to develop HMO structures, such as SL and other sialylated oligosaccharides, for application in infant formula.[1,167] A number of methods for the development of SL and sialylated oligosaccharides have been studied, among which are isolation and microbial and enzymatic methods.[167–169] As soon as larger amounts of sialylated oligosaccharides become available, human studies can be started to confirm whether SL and sialylated oligosaccharides indeed contribute to the various health aspects described in this review. With the availability of larger amounts of these structures, supplementation of infant formula can become feasible as well.

CONCLUSION

There is high interest in exploring the functionalities of HMOs, including sialylated oligosaccharides. Sialylated oligosaccharides, including SL, undoubtedly play an important role in a diverse range of health aspects. The most promising health targets for these structures are resistance to infectious disease (especially intestinal infectious disease), the immune function, and neonatal brain development. In addition, they may have effects on gut microbiota, gut maturation, and inflammation. Many initial studies that have addressed these health outcomes show positive effects and pave the way for follow-up research.

So far, evidence of beneficial effects of sialylated oligosaccharides is limited mainly to in vitro and animal studies. While there is a strong need for substantiation of health effects in humans, current research progression is hampered by overall limited availability of (purified) sialylated oligosaccharides, including SL. For similar reasons, these oligosaccharides are currently not used for application in infant formula. SA can be synthesized by most mammals, including humans, but external supplies (e.g., via consumption of sialylated oligosaccharides) are likely needed during periods of high demand, such as neonatal development. Therefore, if further evidence can be generated for the health effects in human infants, sialylated oligosaccharides may be an interesting component for fortification of infant formula in the near future.

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REFERENCES

1. Bode L. Human milk oligosaccharides: prebiotics and beyond. Nutr Rev. 2009;67(Suppl 2):S183–S191.
2. Ronnestad A, Abrahamsen TG, Medbo S, et al. Late-onset septicemia in a Norwegian national cohort of extremely premature infants receiving very early full human milk feeding. Pediatrics. 2005;115:e269–e276.
3. Leung AK, Sauve RS. Breast is best for babies. J Natl Med Assoc. 2005;97:1010–1019.
4. Hoddinott P, Tappin D, Wright C. Breast feeding. BMJ. 2008;336:881–887.
5. Adlerberth I. Factors influencing the establishment of the intestinal microbiota in infancy. Nestle Nutr Workshop Ser Pediatr Program. 2008;62:13–29; discussion 29–33.
6. Arslanoglu S, Moro GE, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. J Nutr. 2007;137:2420–2424.
7. Newburg DS, Ruiz-Palacios GM, Morrow AL. Human milk glycans protect infants against enteric pathogens. Annu Rev Nutr. 2005;25:37–58.
8. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125:1401–1412.
9. Ben XM, Li J, Feng ZT, et al. Low level of galacto-oligosaccharide in infant formula stimulates growth of intestinal *Bifidobacteria* and *Lactobacilli*. World J Gastroenterol. 2008;14:6564–6568.
10. Moro G, Arslanoglu S, Stahl B, et al. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Arch Dis Child. 2006;91:814–819.
11. Kunz C, Rudloff S, Baier W, et al. Oligosaccharides in human milk: structural, functional, and metabolic aspects. Annu Rev Nutr. 2000;20:699–722.
12. Boehm G, Stahl B. Oligosaccharides from milk. J Nutr. 2007;137(Suppl 2):847S–849S.
13. Kobata A. Structures and application of oligosaccharides in human milk. Proc Jpn Acad Ser B Phys Biol Sci. 2010;86:731–747.
14. McVeagh P, Miller JB. Human milk oligosaccharides: only the breast. J Paediatr Child Health. 1997;33:281–286.
15. Urashima T, Saito T, Nakamura T, et al. Oligosaccharides of milk and colostrum in non-human mammals. Glycoconj J. 2001;18:357–371.
16. Urashima T, Odaka G, Asakuma S, et al. Chemical characterization of oligosaccharides in chimpanzee, bonobo, gorilla, orangutan, and siamang milk or colostrum. Glycobiology. 2009;19:499–508.
17. Chaturvedi P, Warren CD, Altaye M, et al. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. Glycobiology. 2001;11:365–372.
18. Coppa GV, Pierani P, Zampini L, et al. Oligosaccharides in human milk during different phases of lactation. Acta Paediatr Suppl. 1999;88:89–94.
19. Ninonuevo MR, Park Y, Yin H, et al. A strategy for annotating the human milk glycome. J Agric Food Chem. 2006;54:7471–7480.
20. Thurl S, Munzert M, Henker J, et al. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. Br J Nutr. 2010;104:1261–1271.
21. Tao N, DePeters EJ, Freeman S, et al. Bovine milk glycome. J Dairy Sci. 2008;91:3768–3778.
22. Sundekilde UK, Barile D, Meyrand M, et al. Natural variability in bovine milk oligosaccharides from Danish Jersey and Holstein-Friesian breeds. J Agric Food Chem. 2012;60:6188–6196.
23. Aldredge DL, Geronimo MR, Hua S, et al. Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. Glycobiology. 2013;23:664–676.
24. Wang B. Sialic acid is an essential nutrient for brain development and cognition. Annu Rev Nutr. 2009;29:177–222.
25. Wang B, Brand-Miller J. The role and potential of sialic acid in human nutrition. Eur J Clin Nutr. 2003;57:1351–1369.
26. Rudloff S, Kunz C. Protein and nonprotein nitrogen components in human milk, bovine milk, and infant formula: quantitative and qualitative aspects in infant nutrition. J Pediatr Gastroenterol Nutr. 1997;24:328–344.
27. Coppa GV, Gabrielli O, Pierani P, et al. Changes in carbohydrate composition in human milk over 4 months of lactation. Pediatrics. 1993;91:637–641.
28. Kunz C, Rudloff S. Biological functions of oligosaccharides in human milk. Acta Paediatr. 1993;82:903–912.
29. Viverge D, Grimmonprez L, Cassanas G, et al. Variations of lactose and oligosaccharides in milk from women of blood types secretor A or H, secretor Lewis, and secretor H/nonsecretor Lewis during the course of lactation. Ann Nutr Metab. 1985;29:1–11.

30. Neeser JR, Golliard M, Del Vedovo S. Quantitative determination of complex carbohydrates in bovine milk and in milk-based infant formulas. J Dairy Sci. 1991;74:2860–2871.
31. Urashima T, Kitaoka M, Terabayashi T, et al. Milk oligosaccharides. In: Gordon NS, ed. *Oligosaccharides: Sources, Properties and Applictions*. Hauppauge, NY: Nova Science Publishers, Inc; 2011:1–58.
32. Martin-Sosa S, Martin MJ, Garcia-Pardo LA, et al. Sialyloligosaccharides in human and bovine milk and in infant formulas: variations with the progression of lactation. J Dairy Sci. 2003;86:52–59.
33. Wang B, Brand-Miller J, McVeagh P, et al. Concentration and distribution of sialic acid in human milk and infant formulas. Am J Clin Nutr. 2001;74:510–515.
34. Qiao Y, Feng J, Yang J, et al. The relationship between dietary vitamin A intake and the levels of sialic acid in the breast milk of lactating women. J Nutr Sci Vitaminol (Tokyo). 2013;59:347–351.
35. Carlson SE. N-acetylneuraminic acid concentrations in human milk oligosaccharides and glycoproteins during lactation. Am J Clin Nutr. 1985;41:720–726.
36. Spichtig V, Michaud J, Austin S. Determination of sialic acids in milks and milk-based products. Anal Biochem. 2010;405:28–40.
37. Martin MJ, Martin-Sosa S, Garcia-Pardo LA, et al. Distribution of bovine milk sialoglycoconjugates during lactation. J Dairy Sci. 2001;84:995–1000.
38. Sanchez-Diaz A, Ruano MJ, Lorente F, et al. A critical analysis of total sialic acid and sialoglycoconjugate contents of bovine milk-based infant formulas. J Pediatr Gastroenterol Nutr. 1997;24:405–410.
39. McJarrow P, van Amelsfort-Schoonbeek J. Bovine sialyl oligosaccharides: seasonal variations in their concentrations in milk, and a comparison of the colostrums of Jersey and Friesian cows. Int Dairy J. 2004;14:571–579.
40. Veh RW, Michalski JC, Corfield AP, et al. New chromatographic system for the rapid analysis and preparation of colostrum sialyloligosaccharides. J Chromatogr. 1981;212:313–322.
41. Nakamura T, Kawase H, Kimura K, et al. Concentrations of sialyloligosaccharides in bovine colostrum and milk during the prepartum and early lactation. J Dairy Sci. 2003;86:1315–1320.
42. Fong B, Ma K, McJarrow P. Quantification of bovine milk oligosaccharides using liquid chromatography-selected reaction monitoring-mass spectrometry. J Agric Food Chem. 2011;59:9788–9795.
43. Gopal PK, Gill HS. Oligosaccharides and glycoconjugates in bovine milk and colostrum. Br J Nutr. 2000;84(Suppl 1):S69–S74.
44. Asakuma S, Akahori M, Kimura K, et al. Sialyl oligosaccharides of human colostrum: changes in concentration during the first three days of lactation. Biosci Biotechnol Biochem. 2007;71:1447–1451.
45. Bao Y, Zhu L, Newburg DS. Simultaneous quantification of sialyloligosaccharides from human milk by capillary electrophoresis. Anal Biochem. 2007;370:206–214.
46. Wang B, McVeagh P, Petocz P, et al. Brain ganglioside and glycoprotein sialic acid in breastfed compared with formula-fed infants. Am J Clin Nutr. 2003;78:1024–1029.
47. Wang B, Miller JB, Sun Y, et al. A longitudinal study of salivary sialic acid in preterm infants: comparison of human milk-fed versus formula-fed infants. J Pediatr. 2001;138:914–916.
48. Duncan PI, Raymond F, Fuerholz A, et al. Sialic acid utilisation and synthesis in the neonatal rat revisited. PLoS ONE. 2009;4:e8241. doi: 10.1371/journal.pone.0008241.
49. Viverge D, Grimmonprez L, Cassanas G, et al. Discriminant carbohydrate components of human milk according to donor secretor types. J Pediatr Gastroenterol Nutr. 1990;11:365–370.
50. Kunz C, Rudloff S. Potential anti-inflammatory and anti-infectious effects of human milk oligosaccharides. Adv Exp Med Biol. 2008;606:455–465.
51. Nakano T, Sugawara M, Kawakami H. Sialic acid in human milk: composition and functions. Acta Paediatr Taiwan. 2001;42:11–17.
52. Brand-Miller JC, McVeagh P, McNeil Y, et al. Digestion of human milk oligosaccharides by healthy infants evaluated by the lactulose hydrogen breath test. J Pediatr. 1998;133:95–98.
53. Newburg DS. Oligosaccharides in human milk and bacterial colonization. J Pediatr Gastroenterol Nutr. 2000;30(Suppl 2):S8–S17.
54. Gnoth MJ, Kunz C, Kinne-Saffran E, et al. Human milk oligosaccharides are minimally digested in vitro. J Nutr. 2000;130:3014–3020.
55. Nohle U, Schauer R. Metabolism of sialic acids from exogenously administered sialyllactose and mucin in mouse and rat. Hoppe Seylers Z Physiol Chem. 1984;365:1457–1467.
56. Engfer MB, Stahl B, Finke B, et al. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. Am J Clin Nutr. 2000;71:1589–1596.
57. Dickson JJ, Messer M. Intestinal neuraminidase activity of suckling rats and other mammals. Relationship to the sialic acid content of milk. Biochem J. 1978;170:407–413.
58. Coppa GV, Pierani P, Zampini L, et al. Characterization of oligosaccharides in milk and feces of breast-fed infants by high-performance anion-exchange chromatography. Adv Exp Med Biol. 2001;501:307–314.
59. Sabharwal H, Sjoblad S, Lundblad A. Sialylated oligosaccharides in human milk and feces of preterm, full-term, and weaning infants. J Pediatr Gastroenterol Nutr. 1991;12:480–484.
60. Albrecht S, Schols HA, van den Heuvel EG, et al. CE-LIF-MS n profiling of oligosaccharides in human milk and feces of breast-fed babies. Electrophoresis. 2010;31:1264–1273.
61. Gnoth MJ, Rudloff S, Kunz C, et al. Investigations of the in vitro transport of human milk oligosaccharides by a Caco-2 monolayer using a novel high performance liquid chromatography-mass spectrometry technique. J Biol Chem. 2001;276:34363–34370.
62. Jantscher-Krenn E, Marx C, Bode L. Human milk oligosaccharides are differentially metabolised in neonatal rats. Br J Nutr. 2013;110:640–650.
63. Rudloff S, Pohlentz G, Borsch C, et al. Urinary excretion of in vivo (13)C-labelled milk oligosaccharides in breastfed infants. Br J Nutr. 2012;107:957–963.
64. Coppa GV, Gabrielli O, Giorgi P, et al. Preliminary study of breastfeeding and bacterial adhesion to uroepithelial cells. Lancet. 1990;335:569–571.
65. Rudloff S, Pohlentz G, Diekmann L, et al. Urinary excretion of lactose and oligosaccharides in preterm infants fed human milk or infant formula. Acta Paediatr. 1996;85:598–603.
66. Obermeier S, Rudloff S, Pohlentz G, et al. Secretion of 13C-labelled oligosaccharides into human milk and infant's urine after an oral [13C]galactose load. Isotopes Environ Health Stud. 1999;35:119–125.
67. Tram TH, Brand Miller JC, McNeil Y, et al. Sialic acid content of infant saliva: comparison of breast fed with formula fed infants. Arch Dis Child. 1997;77:315–318.
68. Kolling G, Wu M, Guerrant RL. Enteric pathogens through life stages. Front Cell Infect Microbiol. 2012;2:114. doi: 10.3389/fcimb.2012.00114.
69. Vester Boler BM, Rossoni Serao MC, Faber TA, et al. In vitro fermentation characteristics of select nondigestible oligosaccharides by infant fecal inocula. J Agric Food Chem. 2013;61:2109–2119.
70. Wolin MJ, Miller TL, Yerry S, et al. Changes of fermentation pathways of fecal microbial communities associated with a drug treatment that increases dietary starch in the human colon. Appl Environ Microbiol. 1999;65:2807–2812.
71. Backhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial mutualism in the human intestine. Science. 2005;307:1915–1920.
72. Stoddart LA, Smith NJ, Milligan G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: pharmacology and pathophysiological functions. Pharmacol Rev. 2008;60:405–417.
73. Wong JM, de Souza R, Kendall CW, et al. Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol. 2006;40:235–243.
74. Ward RE, Ninonuevo M, Mills DA, et al. In vitro fermentability of human milk oligosaccharides by several strains of bifidobacteria. Mol Nutr Food Res. 2007;51:1398–1405.
75. Sela DA, Chapman J, Adeuya A, et al. The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. Proc Natl Acad Sci U S A. 2008;105:18964–18969.
76. Yu ZT, Chen C, Newburg DS. Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. Glycobiology. 2013;23:1281–1292.
77. Ruiz-Moyano S, Totten SM, Garrido DA, et al. Variation in consumption of human milk oligosaccharides by infant gut-associated strains of *Bifidobacterium breve*. Appl Environ Microbiol. 2013;79:6040–6049.
78. Servin AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol Rev. 2004;28:405–440.
79. Lievin V, Peiffer I, Hudault S, et al. Bifidobacterium strains from resident infant human gastrointestinal microflora exert antimicrobial activity. Gut. 2000;47:646–652.
80. Hart AL, Lammers K, Brigidi P, et al. Modulation of human dendritic cell phenotype and function by probiotic bacteria. Gut. 2004;53:1602–1609.
81. Favier CF, de Vos WM, Akkermans AD. Development of bacterial and bifidobacterial communities in feces of newborn babies. Anaerobe. 2003;9:219–229.
82. LoCascio RG, Ninonuevo MR, Freeman SL, et al. Glycoprofiling of bifidobacterial consumption of human milk oligosaccharides demonstrates strain specific, preferential consumption of small chain glycans secreted in early human lactation. J Agric Food Chem. 2007;55:8914–8919.
83. Sela DA, Li Y, Lerno L, et al. An infant-associated bacterial commensal utilizes breast milk sialyloligosaccharides. J Biol Chem. 2011;286:11909–11918.
84. Kavanaugh DW, O'Callaghan J, Butto LF, et al. Exposure of *Bifidobacterium longum* subsp. *infantis* to milk oligosaccharides increases adhesion to epithelial cells and induces a substantial transcriptional response. PLoS ONE. 2013;8:e67224. doi: 10.1371/journal.pone.0067224.
85. Kiyohara M, Tanigawa K, Chaiwangsri T, et al. An *exo*-α-sialidase from bifidobacteria involved in the degradation of sialyloligosaccharides in human milk and intestinal glycoconjugates. Glycobiology. 2011;21:437–447.
86. Brown WR. Relationships between immunoglobulins and the intestinal epithelium. Gastroenterology. 1978;75:129–138.

87. Gill RK, Mahmood S, Nagpaul JP, et al. Functional role of sialic acid in IgG binding to microvillus membranes in neonatal rat intestine. Biol Neonate. 1999;76:55–64.
88. Chu SH, Walker WA. Developmental changes in the activities of sialyl- and fucosyltransferases in rat small intestine. Biochim Biophys Acta. 1986;883:496–500.
89. Dall'Olio F, Malagolini N, Di Stefano G, et al. Postnatal development of rat colon epithelial cells is associated with changes in the expression of the β1,4-*N*-acetylgalactosaminyltransferase involved in the synthesis of Sd^a antigen of α2,6-sialyltransferase activity towards *N*-acetyl-lactosamine. Biochem J. 1990;270:519–524.
90. Donovan SM. Human milk oligosaccharides – the plot thickens. Br J Nutr. 2009;101:1267–1269.
91. Kawashima N, Yoon SJ, Itoh K, et al. Tyrosine kinase activity of epidermal growth factor receptor is regulated by GM3 binding through carbohydrate to carbohydrate interactions. J Biol Chem. 2009;284:6147–6155.
92. Dvorak B. Milk epidermal growth factor and gut protection. J Pediatr. 2010;156(Suppl):S31–S35.
93. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2095–2128.
94. Lane JA, Marino K, Naughton J, et al. Anti-infective bovine colostrum oligosaccharides: *Campylobacter jejuni* as a case study. Int J Food Microbiol. 2012;157:182–188.
95. Sakarya S, Gokturk C, Ozturk T, et al. Sialic acid is required for nonspecific adherence of *Salmonella enterica* ssp. *enterica* serovar Typhi on Caco-2 cells. FEMS Immunol Med Microbiol. 2010;58:330–335.
96. Coppa GV, Zampini L, Galeazzi T, et al. Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli*, *Vibrio cholerae*, and *Salmonella fyris*. Pediatr Res. 2006;59:377–382.
97. Finlay BB, Fry J, Rock EP, et al. Passage of *Salmonella* through polarized epithelial cells: role of the host and bacterium. J Cell Sci Suppl. 1989;11:99–107.
98. Salcedo J, Barbera R, Matencio E, et al. Gangliosides and sialic acid effects upon newborn pathogenic bacteria adhesion: an in vitro study. Food Chem. 2013;136:726–734.
99. Martin-Sosa S, Martin MJ, Hueso P. The sialylated fraction of milk oligosaccharides is partially responsible for binding to enterotoxigenic and uropathogenic *Escherichia coli* human strains. J Nutr. 2002;132:3067–3072.
100. Schwertmann A, Schroten H, Hacker J, et al. S-fimbriae from *Escherichia coli* bind to soluble glycoproteins from human milk. J Pediatr Gastroenterol Nutr. 1999;28:257–263.
101. Angeloni S, Ridet JL, Kusy N, et al. Glycoprofiling with micro-arrays of glycoconjugates and lectins. Glycobiology. 2005;15:31–41.
102. Burger O, Weiss E, Sharon N, et al. Inhibition of *Helicobacter pylori* adhesion to human gastric mucus by a high-molecular-weight constituent of cranberry juice. Crit Rev Food Sci Nutr. 2002;42:279–284.
103. Evans DG, Evans DJ, Jr, Moulds JJ, et al. N-acetylneuraminyllactose-binding fibrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor antigen. Infect Immun. 1988;56:2896–2906.
104. Simon PM, Goode PL, Mobasseri A, et al. Inhibition of *Helicobacter pylori* binding to gastrointestinal epithelial cells by sialic acid-containing oligosaccharides. Infect Immun. 1997;65:750–757.
105. Unemo M, Aspholm-Hurtig M, Ilver D, et al. The sialic acid binding SabA adhesin of *Helicobacter pylori* is essential for nonopsonic activation of human neutrophils. J Biol Chem. 2005;280:15390–15397.
106. Mahajan S, Rodgers FG. Isolation, characterization, and host-cell-binding properties of a cytotoxin from *Campylobacter jejuni*. J Clin Microbiol. 1990;28:1314–1320.
107. Koketsu M, Nitoda T, Sugino H, et al. Synthesis of a novel sialic acid derivative (sialylphospholipid) as an antirotaviral agent. J Med Chem. 1997;40:3332–3335.
108. Takahashi K, Ohashi K, Abe Y, et al. Protective efficacy of a sulfated sialyl lipid (NMSO3) against human rotavirus-induced diarrhea in a mouse model. Antimicrob Agents Chemother. 2002;46:420–424.
109. Yang JC, Shun CT, Chien CT, et al. Effective prevention and treatment of *Helicobacter pylori* infection using a combination of catechins and sialic acid in AGS cells and BALB/c mice. J Nutr. 2008;138:2084–2090.
110. Newburg DS, Peterson JA, Ruiz-Palacios GM, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. Lancet. 1998;351:1160–1164.
111. Yolken RH, Peterson JA, Vonderfecht SL, et al. Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. J Clin Invest. 1992;90:1984–1991.
112. Idota T, Kawakami H, Murakami Y, et al. Inhibition of cholera toxin by human milk fractions and sialyllactose. Biosci Biotechnol Biochem. 1995;59:417–419.
113. Noguchi S, Malicdan MC, Nishino I. Animal model of distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy and preclinical trial with sugar compounds. Brain Nerve. 2010;62:601–607.
114. Mysore JV, Wigginton T, Simon PM, et al. Treatment of *Helicobacter pylori* infection in rhesus monkeys using a novel antiadhesion compound. Gastroenterology. 1999;117:1316–1325.
115. Hester SN, Chen X, Li M, et al. Human milk oligosaccharides inhibit rotavirus infectivity in vitro and in acutely infected piglets. Br J Nutr. 2013;110:1233–1242.
116. Opekun AR, El-Zaimaity HM, Osato MS, et al. Novel therapies for *Helicobacter pylori* infection. Aliment Pharmacol Ther. 1999;13:35–42.
117. Parente F, Cucino C, Anderloni A, et al. Treatment of *Helicobacter pylori* infection using a novel antiadhesion compound (3′sialyllactose sodium salt). A double blind, placebo-controlled clinical study. Helicobacter. 2003;8:252–256.
118. Rasko DA, Wilson TJ, Zopf D, et al. Lewis antigen expression and stability in *Helicobacter pylori* isolated from serial gastric biopsies. J Infect Dis. 2000;181:1089–1095.
119. Bryce J, Boschi-Pinto C, Shibuya K, et al. WHO estimates of the causes of death in children. Lancet. 2005;365:1147–1152.
120. O'Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet. 2009;374:893–902.
121. Van Woensel JB, van Aalderen WM, Kimpen JL. Viral lower respiratory tract infection in infants and young children. BMJ. 2003;327:36–40.
122. Tarrant M, Kwok MK, Lam TH, et al. Breast-feeding and childhood hospitalizations for infections. Epidemiology. 2010;21:847–854.
123. Gambaryan AS, Tuzikov AB, Chinarev AA, et al. Polymeric inhibitor of influenza virus attachment protects mice from experimental influenza infection. Antiviral Res. 2002;55:201–205.
124. Watanabe Y, Hashimoto Y, Shiratsuchi A, et al. Augmentation of fatality of influenza in mice by inhibition of phagocytosis. Biochem Biophys Res Commun. 2005;337:881–886.
125. Watanabe Y, Shiratsuchi A, Shimizu K, et al. Stimulation of phagocytosis of influenza virus-infected cells through surface desialylation of macrophages by viral neuraminidase. Microbiol Immunol. 2004;48:875–881.
126. Ukkonen P, Varis K, Jernfors M, et al. Treatment of acute otitis media with an antiadhesive oligosaccharide: a randomised, double-blind, placebo-controlled trial. Lancet. 2000;356:1398–1402.
127. Johansson SM, Arnberg N, Elofsson M, et al. Multivalent HSA conjugates of 3′-sialyllactose are potent inhibitors of adenoviral cell attachment and infection. Chembiochem. 2005;6:358–364.
128. Hendricks GL, Weirich KL, Viswanathan K, et al. Sialylneolacto-N-tetraose c (LSTc)-bearing liposomal decoys capture influenza A virus. J Biol Chem. 2013;288:8061–8073.
129. Fievre S, Wieruszeski JM, Michalski JC, et al. Primary structure of a trisialylated oligosaccharide from human milk. Biochem Biophys Res Commun. 1991;177:720–725.
130. Yabe U, Sato C, Matsuda T, et al. Polysialic acid in human milk. CD36 is a new member of mammalian polysialic acid-containing glycoprotein. J Biol Chem. 2003;278:13875–13880.
131. Gamblin SJ, Skehel JJ. Influenza hemagglutinin and neuraminidase membrane glycoproteins. J Biol Chem. 2010;285:28403–28409.
132. Matsubara T, Onishi A, Saito T, et al. Sialic acid-mimic peptides as hemagglutinin inhibitors for anti-influenza therapy. J Med Chem. 2010;53:4441–4449.
133. Ogata M, Murata T, Murakami K, et al. Chemoenzymatic synthesis of artificial glycopolypeptides containing multivalent sialyloligosaccharides with a gamma-polyglutamic acid backbone and their effect on inhibition of infection by influenza viruses. Bioorg Med Chem. 2007;15:1383–1393.
134. Zenhom M, Hyder A, de Vrese M, et al. Prebiotic oligosaccharides reduce proinflammatory cytokines in intestinal Caco-2 cells via activation of PPARγ and peptidoglycan recognition protein 3. J Nutr. 2011;141:971–977.
135. Zenhom M, Hyder A, Kraus-Stojanowic I, et al. PPARγ-dependent peptidoglycan recognition protein 3 (PGlyRP3) expression regulates proinflammatory cytokines by microbial and dietary fatty acids. Immunobiology. 2011;216:715–724.
136. Eiwegger T, Stahl B, Haidl P, et al. Prebiotic oligosaccharides: in vitro evidence for gastrointestinal epithelial transfer and immunomodulatory properties. Pediatr Allergy Immunol. 2010;21:1179–1188.
137. Comstock SS, Wang M, Hester SN, et al. Select human milk oligosaccharides directly modulate peripheral blood mononuclear cells isolated from 10-d-old pigs. Br J Nutr. 2014;111:819–828.
138. Bohm S, Schwab I, Lux A, et al. The role of sialic acid as a modulator of the anti-inflammatory activity of IgG. Semin Immunopathol. 2012;34:443–453.
139. Videira PA, Amado IF, Crespo HJ, et al. Surface α2-3- and α2-6-sialylation of human monocytes and derived dendritic cells and its influence on endocytosis. Glycoconj J. 2008;25:259–268.
140. Cabral MG, Silva Z, Ligeiro D, et al. The phagocytic capacity and immunological potency of human dendritic cells is improved by α2,6-sialic acid deficiency. Immunology. 2013;138:235–245.

141. Redelinghuys P, Antonopoulos A, Liu Y, et al. Early murine T-lymphocyte activation is accompanied by a switch from *N*-glycolyl- to *N*-acetyl-neuraminic acid and generation of ligands for siglec-E. J Biol Chem. 2011;286:34522–34532.
142. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. Nat Rev Immunol. 2007;7:255–266.
143. Pillai S, Netravali IA, Cariappa A, et al. Siglecs and immune regulation. Annu Rev Immunol. 2012;30:357–392.
144. Benchimol EI, Fortinsky KJ, Gozdyra P, et al. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. Inflamm Bowel Dis. 2011;17:423–439.
145. Fuhrer A, Sprenger N, Kurakevich E, et al. Milk sialyllactose influences colitis in mice through selective intestinal bacterial colonization. J Exp Med. 2010;207:2843–2854.
146. Weiss GA, Hennet T. The role of milk sialyllactose in intestinal bacterial colonization. Adv Nutr. 2012;3:483S–488S.
147. Kurakevich E, Hennet T, Hausmann M, et al. Milk oligosaccharide sialyl(α2,3)lactose activates intestinal CD11c^{+} cells through TLR4. Proc Natl Acad Sci U S A. 2013;110:17444–17449.
148. Barclay AR, Russell RK, Wilson ML, et al. Systematic review: the role of breastfeeding in the development of pediatric inflammatory bowel disease. J Pediatr. 2009;155:421–426.
149. De Leoz ML, Gaerlan SC, Strum JS, et al. Lacto-*N*-tetraose, fucosylation, and secretor status are highly variable in human milk oligosaccharides from women delivering preterm. J Proteome Res. 2012;11:4662–4672.
150. Jantscher-Krenn E, Zherebtsov M, Nissan C, et al. The human milk oligosaccharide disialyllacto-N-tetraose prevents necrotising enterocolitis in neonatal rats. Gut. 2012;61:1417–1425.
151. Miller JB, McVeagh P. Human milk oligosaccharides: 130 reasons to breast-feed. Br J Nutr. 1999;82:333–335.
152. Nohle U, Schauer R. Uptake, metabolism and excretion of orally and intravenously administered, 14C- and 3H-labeled N-acetylneuraminic acid mixture in the mouse and rat. Hoppe Seylers Z Physiol Chem. 1981;362:1495–1506.
153. Carlson SE, House SG. Oral and intraperitoneal administration of *N*-acetylneuraminic acid: effect on rat cerebral and cerebellar *N*-acetylneuraminic acid. J Nutr. 1986;116:881–886.
154. Morgan BL, Oppenheimer J, Winick M. Effects of essential fatty acid deficiency during late gestation on brain N-acetylneuraminic acid metabolism and behaviour in the progeny. Br J Nutr. 1981;46:223–230.
155. Morgan BL, Winick M. Effects of environmental stimulation on brain N-acetylneuraminic acid content and behavior. J Nutr. 1980;110:425–432.
156. Morgan BL, Winick M. Effects of administration of N-acetylneuraminic acid (NANA) on brain NANA content and behavior. J Nutr. 1980;110:416–424.
157. Morgan BL, Winick M. The subcellular localization of administered N-acetylneuraminic acid in the brains of well-fed and protein restricted rats. Br J Nutr. 1981;46:231–238.
158. Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. Am J Clin Nutr. 1999;70:525–535.
159. Hiratsuka S, Honma H, Saitoh Y, et al. Effects of dietary sialic acid in *n*-3 fatty acid-deficient dams during pregnancy and lactation on the learning abilities of their pups after weaning. J Nutr Sci Vitaminol (Tokyo). 2013;59:136–143.
160. Scholtz SA, Gottipati BS, Gajewski BJ, et al. Dietary sialic acid and cholesterol influence cortical composition in developing rats. J Nutr. 2013;143:132–135.
161. Sprenger N, Julita M, Donnicola D, et al. Sialic acid feeding aged rats rejuvenates stimulated salivation and colon enteric neuron chemotypes. Glycobiology. 2009;19:1492–1502.
162. Sakai F, Ikeuchi F, Urashima T, et al. Effects of feeding sialyllactose and galactosylated N-acetylneuraminic acid on swimming learning ability and brain lipid composition in adult rats. J Appl Glycosci. 2006;53:249–254.
163. Wang B, Downing JA, Petocz P, et al. Metabolic fate of intravenously administered *N*-acetylneuraminic acid-6-14-C in newborn piglets. Asia Pac J Clin Nutr. 2007;16:110–115.
164. Colombo JP, Garcia-Rodenas C, Guesry PR, et al. Potential effects of supplementation with amino acids, choline or sialic acid on cognitive development in young infants. Acta Paediatr Suppl. 2003;92:42–46.
165. Marques TM, Wall R, Ross RP, et al. Programming infant gut microbiota: influence of dietary and environmental factors. Curr Opin Biotechnol. 2010;21:149–156.
166. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012;22:1147–1162.
167. Han NS, Kim TJ, Park YC, et al. Biotechnological production of human milk oligosaccharides. Biotechnol Adv. 2012;30:1268–1278.
168. Strum JS, Aldredge D, Barile D, et al. Coupling flash liquid chromatography with mass spectrometry for enrichment and isolation of milk oligosaccharides for functional studies. Anal Biochem. 2012;424:87–96.
169. Choi YH, Kim JH, Park JH, et al. Protein engineering of α2,3/2,6-sialyltransferase to improve the yield and productivity of in vitro sialyllactose synthesis. Glycobiology. 2014;24:159–169.