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Mini Review

Effects of Human Milk Oligosaccharides on Microbiome

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Abstract

Human Milk Oligosaccharides (HMOs), the complex, unique glycans present in human milk, are essential for infants' early development and provide many health benefits to humans. Although the effects of all the individual HMOs are not fully understood, studies have revealed many health benefits of HMOs acting as prebiotics. They shape the gut microbiome, provide immune-modulatory and anti-adhesive effects to enhance innate and acquired immunity in the host and modulate the epithelial barrier. The effects of HMOs are not only limited to the infant gut microbiome but also affect the microbiome of breast milk and adults. This mini review highlights the recent findings on the effects of HMOs on the microbiome. It discusses the benefit of synthesizing and identifying the structures of individual glycans for further improvement in human health.

Keywords: Oligosaccharides; Glycans; Microbiome; Breast milk; Prebiotics

Introduction

Breast milk, the gold standard for infant nutrition, is rich in many bioactive components with complete nutrition that leads to the healthy development of the newborn. Human milk is rather a dynamic and complex nutrition source essential for the infant's growth and health that may change with some factors such as maternal diet (1), bacterial composition in milk (1), secretor and non-secretor status of the mother (2, 3, 4, 5) and environmental conditions (6). So, it is recommended by the World Health Organization (WHO) to feed the newborn exclusively with breast milk for at least six months.

Breast milk consists of lactose, fatty acids, and proteins, and among these, human milk oligosaccharides (HMOs) are considered the third most abundant macromolecular component after lactose and fat. HMOs are a complex group of glycans made out of glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc), and N-acetylneuraminic acid (Neu5Ac) or sialic acid. They are mainly indigestible sugars by the infant and unique only to humans. Emerging evidence shows that more than 200 different structures of HMOs are present in human milk. More frequent feedings can ingest a high amount of HMOs into the infant, causing significant positive associations with the gut microbiome (4).

The gut microbiome is the total of microorganisms and their collective genetic materials in the gastrointestinal system. A healthy gut microbiome causes the child to be physically and mentally healthy. Certain gut microorganisms utilize HMOs in specific ways, such as a carbon source for their growth (6, 7), and HMOs play an essential role in the development of gut microbiota, acting as decoys for pathogens,

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preventing disease and infections (6), enhancing gut-barrier function and developing innate (8) and acquired immunity in infants. Now the research field is advancing to identify more functions of HMOs for the better and long-term health of infants. In infants and adults, including the mother, HMOs play a major role in giving many health benefits (9, 10). HMOs such as 2'FL and LNnT restore homeostasis in dysbiotic adults by promoting the growth of beneficial Bifidobacteria and Actinobacteria and reducing the abundance of Firmicutes and Proteobacteria (9). These HMOs promote the gut barrier function in adults strengthening the gut barrier (10). Due to these benefits and future application in therapeutic purposes and as food additives, research is continuing to identify the structures and effects of HMOs, how to synthesize them, and the therapeutic benefits of individual HMOs. The recent findings on the effects of HMOs on the microbiome of infants, alteration of microbial composition in mother's milk (11), regulation of immune function in adults (10), fight against pathogens, and important recent findings to consider in further studies related to HMOs are discussed in this mini review.

Methods

Original, recent research articles were searched using relevant keywords in PubMed and Mendeley. Next, the articles were sorted out considering the most pertinent epidemiological studies, published year from 2014 onwards, and the article type as journal articles. Then, the selected articles were examined thoroughly to gain much insight into the effects of human milk oligosaccharides on the microbiome. The relevant findings were included in the mini review.

Results

Human Milk Oligosaccharides

Human milk oligosaccharides are complex, unconjugated glycans, highly concentrated in human milk and unique only to humans. Very high HMO concentrations can be observed in the colostrum (12). HMOs are incorporated of Glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc) and sialic acid (Sia), and there is evidence of more than 200 HMOs that have been identified with 3-22 units of monosaccharides (13). Since HMOs are not absorbed or metabolized in the gastrointestinal tract and enter the lower section of the gastrointestinal tract without any change (13). They act as human milk prebiotics, serve as antiadhesives, act as antimicrobials, and alter epithelial and immune cell responses (12).

Effects of HMOs on infant microbiome

HMOs are well known to support beneficial microorganisms such as *Bifidobacteriaceae,* and they are an abundant group in the gut microbiome of infants and toddlers (4, 6, 14). HMOs have strong bifidogenic effects (14), causing a long-term influence on infant and toddler health as prebiotics. They maintain the presence and activity of Bifidobacteria during the diversification of the gut microbiome following weaning. HMOs act as metabolic substrates for commensal bacteria (1). They digest complex carbohydrates and dietary fibers beneficial for human health. HMOs distinctly modulate intestinal microbiome composition and activity by promoting the growth of *Bacteroides* and Bifidobacteria (15). This group produces acetate, a major metabolite of fermentation of 2'-FL, one of the main oligosaccharides found in milk. 2'-FL and sialyl lactose increase the production of butyrate (13), propionate and lactate (10, 15), leading to many health benefits in the host.

HMOs in human colostrum also influence the human milk microbiota, mainly bifidobacteria, subsequently altering the infant gut microbiota after breastfeeding (11). It has been discovered that various microbial groups such as streptococci, staphylococci, enterococci and bifidobacteria have strong positive correlations with different HMOs (11). In infant cultures, where the effects of Sialyllactose (SL) were investigated by impaling SL to the fecal cultures, specific growth of *B.prevotella* group and *B.thetaiotaomicron* have been induced by SL and in adults, SL is reported to induce the growth of *F.prausnitizii* (15). These Bacterioides and Prevotella genera belong to the Bacteroidetes phylum, which mainly inhabits the human gut and have proven to be influenced by several HMOs.

There are significant intrinsic differences between the luminal and mucosal compartments in the microbiome compositions in the various gut regions, such as the proximal and distal colon. So, the effects of HMOs, such as 2'-FL (2'-O-fucosyllactose), show minor differences due to the variation of microbial community composition in these regions (14), and the effects are HMO specific (16). The SHIME model, a multi-compartment dynamic simulator of the human gut, 2'-FL increases *Bifidobacterium* population and increases *B.coccoides/E.rectale* in the proximal colon in adults and the distal vessel, only the *Bifidobacterium* population increases with regards to *B.coccoides/E.rectale* (10). The effects of HMOs are structure and bacterial strain-dependent as different HMOs such as 2'-FL, 3'-FL(3-fucosyllactose), 6'-SL (6-sialyllactose) and LNT2 (lacto-N-triose) affect differently in the growth patterns of various bacterial strains (16). From the mentioned HMOs, 6'-SL modulates the growth of *B.longum infantis* subspecies, and further growth is seen in co-cultures. So, HMOs selectively stimulate beneficial microbial communities' growth and metabolic activity, which means the type of HMO predicts the growth of specific types of bacteria (5). Though many studies reveal the growth improvement of microbiota, another study has discovered that pooled HMOs have the ability to inhibit bacterial growth. HMOs act as antibiofilm agents against Group B *Streptococcus* (GBS), a bacterial pathogen (17).

Fucose, one of the building blocks of HMO can be added to the HMO backbone in α 1-2- linkage, which is catalyzed by an enzyme fucosyltransferase 2 (FUT2). FUT2 active expression with high concentrations of α 1-2- fucosylated HMOs in milk can be seen in secretor mothers. Non-secretor mothers can be identified with a lack of α 1-2-fucosylated HMOs, such as 2'FL or lacto-N-fucopentaose 1 (LNFP1), having no active expression of FUT2 (12). A study has shown that the mother's secretor status does not significantly impact the infant's gut microbiota (2) and causes fewer effects on the milk microbiome (3). However, another study has discovered that the abundance of *Bifidobacterium* spp. is significantly higher in the secretor mothers' infant gut (3). Also, it has been revealed, that non-secretor mothers who are low in detectable concentrations of α-1-2-linked fucosylated HMOs in milk can have infants with increased abundance of enteropathogens and decreased abundance of *Bifidobacterium* due to the influence of gut microbial factors such as fortificant iron (2).

Fucosylated milk glycans shape the gut microbiome of infants (3). The microbiome composition of infants is lower than adults but shows a relatively high amount of Bifidobacteria (15). HMOs, like prebiotics, exert a strong bifidogenic effect in infants with the proliferation of *Bifidobacterium infantis, B.breve* and *B.bifidum* strains (6). These strains metabolize HMOs into short-chain fatty acids, giving the child many health benefits such as providing energy for the colon epithelial cells, maintaining the gut barrier, and showing immunomodulatory properties (13). Microbiota of older infants degrade HMOs efficiently, and they are more adapted for using indigestible HMOs for energy metabolism (4).

It has been proved that low Bifidobacteria levels cause gastrointestinal disorders in infants. But, some studies have shown that a high amount of *Prevotella* causes low morbidity in infants and there are no correlations between bifidobacteria and morbidity (6). In the studies that analyzed morbidity of infants, α (1-2) fucosylation of LNT (Lacto-N-tetraose) to LNFP I (lacto-N-fucopentaose I) in mother's milk has been reported to have an association with lower morbidity of a child by protecting from infections directly or indirectly (6). The *in-vitro* studies have discovered that breast-fed infants have fewer respiratory infections due to the anti-adhesive properties of HMOs (8).

Effects of HMOs on mother's milk microbiome

Besides the beneficial effects on the infant microbiome, HMOs impact the milk microbiome, and bifidobacteria utilize HMOs as their energy source (1, 11). The community and functional profile of the milk microbiome can be changed by the composition of specific HMOs by altering the maternal diet (1, 5). Research has proved that *B.longum* and some strains of *B.breve utilize* LNT and sialylated HMOs such as LST, respectively. So, individual HMOs influence the human milk microbiota in specific ways. The hypothesis has been made that HMO influence on milk microbiota causes the selective transfer of milk microbiome through breastfeeding (1, 11). So, pathogens in the mother's milk will minimally

transfer to the infant *via* breastfeeding. This happens because HMOs allow the growth of multiple *Streptococcus spp.,* which displaces *Staphylococcus aureus,* a common pathogen that causes mastitis. So, the bacterial diversity in mother's milk will be increased. Pushing away harmful pathogens and eventually reducing the pathogen population in the milk microbiome (1).

Genera such as *Lactobacillus* and *Bifidobacterium* are in lower abundance in breast milk, and *Streptococcus* spp., the dominant genus in the milk microbiome, alter the carrying capacity and grant a growth advantage in media with HMO (1). Due to the proliferation driven by HMOs in *Streptococcus aureus*, the mother can be prevented from having mastitis, which is associated with a reduction in milk microbiome diversity. The proliferation also establishes the complex commensal communities in the oral microbiome of the infant. The net effect of both these has been speculated as minimizing pathogens causing mastitis in maternal milk and the infant's mouth (1).

Effects of HMOs on the adult microbiome

HMOs provide many health benefits in adults by modulating the immune function, providing bifidogenic activity (9, 10), and modulating the gut barrier function (10). To restore and strengthen the gut homeostasis in adults, HMOs such as 2'-FL and LNnT (lacto-N-neotetraose) are proved to be safer and well-tolerated (9,10). The increase in the relative abundance of actinobacteria and bifidobacteria and the reduction in the relative abundance of Firmicutes and Proteobacteria in these adults highlight the specific modification of adult gut microbiota. The effect of giving powders of HMOs to be consumed with water as oral supplements to adults is dose-dependent (9). Adults subjected to Irritable Bowel Syndrome (IBS) or aging may develop a leaky gut or have an imbalanced microbiota. So, bacterial metabolites produced by fermenting HMOs are helpful in restoring and beneficially modulate the gut barrier function, restoring homeostasis in adults (9, 10). Supplementation of several HMOs than individual HMOs is considered more tolerated (9).

It has been reported in a study that the secretor status of a mother does not significantly influence the abundance of the sum of all pathogens in the maternal gut. But a higher abundance of *C.perfringens* can be observed in non-secretor mothers (2). It has been discovered that the mother can prevent pathogen colonization due to the HMO-driven proliferation of specific bacteria such as *Streptococcus* spp. in the human milk (1).

Effects of HMOs in enhancing immunity

Other than the effects on beneficial microorganisms, HMOs provide protection against pathogens by enhancing innate immunity. This is done by enhancing the growth of beneficial bacteria, epithelial cell maturation, and promoting epithelial barrier functioning (8, 10, 15). 2'-FL promotes the maturation of the immune system and exerts anti-inflammatory action (13). Similar to 2'-FL, each HMO shows unique effects on the pathogens such as viruses. Individual HMOs can only produce these effects. So, it has been discovered that specific HMOs reduce respiratory viral infections in human airway epithelial and peripheral blood mononuclear cells enhancing innate immunity (8). They interact directly with epithelial and immune cells to inhibit respiratory viral infections by inducing cytokines. HMOs, block pathogen binding to host cell surface glycans or receptors (8) as pathogen colonization starts by adhesion to the host cells. HMOs act as antimicrobial and antibiofilm agents against pathogens like Group B *Streptococcus* (17).

The type of HMO in mother's milk influences the overall immune status of the baby (5). It has been discovered that helper T cell (Th1) immunity can be stimulated by sulfonated and non-sulfonated HMOs (5,13). Specific HMOs or glycosyltransferase pathways change the milk composition allowing the infant to invest energy for growth by maintaining lower rates of infection and inflammation (6).

Table 1: Effects of Human Milk Oligosaccharides on Microbiome

Overall effects of HMOs on the microbiome	• Selectively support beneficial microorganisms e.g. bifidobacteria • Act as prebiotics • Act as metabolic substrates for commensal bacteria • Modulate intestinal microbiome composition and activity • Inhibit some bacterial growth • Fucosylated HMOs reduce the abundance of enteropathogens in infants
Effects of HMOs on infant microbiome	• Fucosylated glycans shape the gut microbiome • Exert strong bifidogenic effects • Provide energy for colon epithelial cells • Support gut barrier maintenance • Show immune-modulatory properties • Utilize for energy metabolism • Protection from infections directly or indirectly
Effects of HMOs on mother's milk microbiome	• Utilize as sources of energy • Alter the community and functional profile of the microbiome • Selective transfer of milk microbiome through breastfeeding • Prevention of mother from having mastitis • Provide a media to grow
Effects of HMOs on the adult microbiome	• Modulate immune function • Modulate the gut barrier function • Restore and strengthen the gut homeostasis
Effects of HMOs in enhancing immunity	• Enhance the growth of beneficial bacteria • Epithelial cell maturation • Promote epithelial barrier functioning • Promote maturation of immune system • Exert anti-inflammatory action • Individually show unique effects on pathogens • Block pathogen binding to host cell surface glycans or receptors • Act as antimicrobial and antibiofilm agents against pathogens • Stimulate helper T cell immunity in mother's milk • Maintain lower rates of infection and inflammation in infants

Discussion

HMOs play a vital role in the early development and shaping of the microbiome since it is the only nutrition supplied to the infant during the first few months of life. The highest amount of HMO is present in the colostrum, and the content gradually decreases with time in human milk (2, 3). But with the increase in daily milk intake, the HMO concentration ingested becomes relatively stable in the infant (4). The gut

microbiome dramatically influences the health of the human and maintains health throughout life by developing the immune system, host metabolism, and fermentation of the dietary indigestible glycans to short-chain fatty acids, which exert many health benefits (14). Gut microbiome composition varies largely among individuals, mainly among children (14), and one of the major factors that cause this variation is HMOs.

In this review, the effects of HMOs have been discussed briefly, considering the effects of the total HMO composition and the individual HMOs. Most studies have been conducted by analyzing maternal milk samples, feces samples, the synthesized, high purity chemicals, and using *in-vitro* models such as the Human Intestinal Microbial Ecosystem (SHIME) simulator used to study the function of HMOs on the adult microbiome (10). Fecal samples of the donors have been used to analyze the utilization of HMOs by different bacterial strains (4). In clinical studies, the convenience of the study subjects has been considered, and the samples have been collected within allocated time periods.

Most studies of individual HMOs have been done *in-vitro* (8, 15) or in animal models (13). So, with the positive results obtained, we can consider conducting more *in-vivo* studies. The effects of HMOs give hope to using different HMOs for therapeutic purposes. Some studies have revealed that supplying HMOs in adults is also an excellent strategy to promote the growth of beneficial Bifidobacteria (9). Even the maternal diet plays a vital role in changing the HMO composition in the milk (1). These will provide means to give solutions to enhance the health of the mother and the infant, preventing many infectious diseases. Some studies, which were done among a small group of subjects, provide the need for a large cohort study (11). Some studies were specific to a particular community (3). Those results can be compared with other communities in different geographical areas to gain much insight into the particular effects of HMOs and the factors that contribute to those effects.

In this review, the mother's secretor status is also considered, as this is related to the amount and type of oligosaccharides in her milk (3, 4). 2'-FL is reported to be the most abundant HMO in a secretor mother, and it is absent or minimally available in a non-secretor mother. Some studies have discovered the beneficial effects of 2'-FL supplementation (7, 14). Since some infants are not supplied with the particular HMO, and the results of the study done by Paganini D. *et al.* (2019) (2) and the study by Bai Y. *et al.* (2018) (3) were not the same for the comparison of secretor status and infant microbiome composition, the need for more studies on the HMO composition considering the secretor status of the mother arises.

Since every child cannot be breastfed for various unavoidable reasons, more attention has been given to comparing the effects of formula feeding against HMOs due to the vast amount of beneficial effects these sugars exert. HMOs are unique carbohydrates only found in human milk, and a slight change in the structure causes a significant impact on the activity of the particular HMO (16). All the structures of HMOs in milk are not identified yet. So, more studies can be conducted to find the structures of HMOs and include them in formulas to enhance the health benefits in non-breast-fed infants.

Conclusion

HMOs play a major role in developing and maintaining humans' short-term and long-term health and exert HMO structure and microbial strain-specific effects on the infant and adult microbiome. They act as prebiotics supporting beneficial bacterial growth, providing anti-pathogenic, immune-modulatory effects, and strengthening the host's gut barrier; due to the beneficial effects of HMOs, more attention has been given to using them in infants formulas and as therapeutic agents.

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