Human Milk Oligosaccharides: The Bridge Between Human Milk and Infant Formula

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Breastfeeding is commonly regarded as the gold standard for supplying nutrition to infants and has been a natural method of feeding infants since ancient times. The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months after birth. On one hand, breastfeeding supplies infants with essential nutrients for their healthy growth. On the other hand, it offers protection against infections, particularly gastrointestinal and respiratory ones, and lowers the risk of various conditions like obesity, diabetes, atopy, and asthma.The structure of breast milk is complex and dynamic, comprising macronutrients (carbohydrates, proteins, and fats), micronutrients (such as calcium, thiamine, and zinc), hormones, insulin-like growth factors, and cytokines.Human milk oligosaccharides (HMOs), the third most abundant solid component in human milk, are resistant to digestion in upper small intestine and the majority of HMOs reach the large intestine (Zhang *et al.*, 2021).

HMOs, a class of non-digestible carbohydrates, constitute a significant component of human breast milk. The concentration of HMOs varies, with levels ranging from 20 to 25 g/L in colostrum and 5 to 15 g/L in mature human milk. One of the defining features of HMOs is their resilience against processes such as pasteurization and freeze-drying. Structurally, HMOs are built upon a lactose base that elongates through the addition of N-acetyllactosamine units, accompanied by fucoylation and/or sialylation. This intricate process results in three major categories: fucosylated neutral HMOs(35-50%), non-fucosylated neutral HMOs(42-55%), and sialylated acidic HMOs (12-14%).Neutral HMOs make up over 75% of total HMOs, with 2'-fucosyllactose (2'-FL), a trisaccharide consisting of glucose, galactose, and fucose, being the most prevalent (accounting for almost 30% of all HMOs).HMOs have a fundamental structural design, despite the fact that they come in several forms and perform a range of tasks (Figure 1). The basic building components of HMOs are five monosaccharides: glucose, galactose, N-acetylglucosamine, fucose, and N-acetylneuraminic acid(Ray *et al.*, 2019).

Functions and health benefits of HMOs

Numerous research endeavors have supported the positive impacts of HMOs. HMOs have been recognized as active prebiotics that withstand digestion by human enzymes and foster the proliferation of advantageous bacteria. For instance, *Bifidobacterium longum subsp. infantis* (B. infantis) thrives on HMOs as its exclusive carbohydrate source.Furthermore, various strains of bifidobacteria have demonstrated the capability to utilize HMOs for their growth stimulation. The proliferation of bifidobacteria results in the

production of short-chain fatty acids that create an environment conducive to the expansion of beneficial bacteria, thus discouraging the growth of potential pathogens.

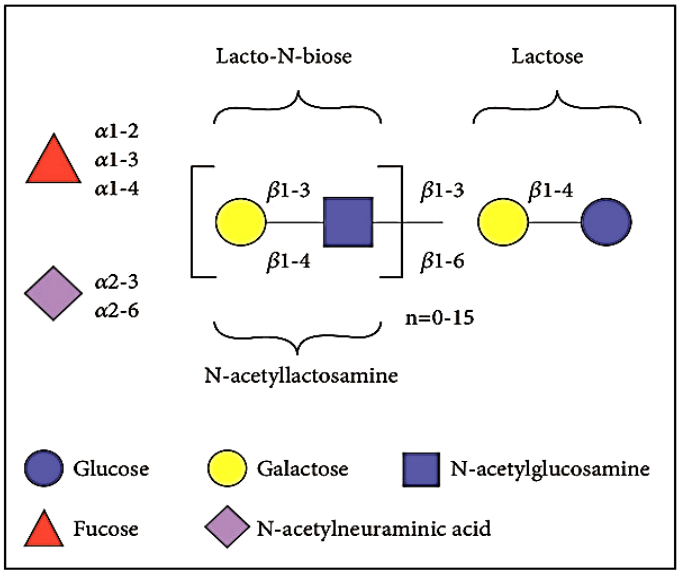

Fig 1: Structural blueprint of HMOs (adapted from Ray *et al.*, 2019)

HMOs possess a significance beyond merely being "nutrition for microorganisms". Evolutionarily, it is speculated that oligosaccharides in milk might have played a role in the antimicrobial defence system. Research indicates that certain specific HMOs can directly impede the growth of group B Streptococcus (GBS), a predominant cause of invasive bacterial infections in newborns typically acquired during childbirth due to maternal vaginal colonization. Moreover, HMOs exhibit the ability to modulate GBS growth and the formation of biofilms. Detailed microscopic analysis revealed disruptions in the arrangement of biofilm cells in bacterial cultures treated with particular HMOs, indicating the bacteriostatic impact of HMOs by hampering bacterial growth kinetics (Asakuma*et al.*, 2011).

HMOs also exhibit an antiadhesive effect. Pathogens like viruses, bacteria, and parasites often require adherence to mucosal surfaces to colonize and cause diseases. HMOs function as decoy receptors, thwarting microbe attachment to epithelial cells. Notable studies in animals demonstrated HMO-mediated inhibition of Campylobacter colonization and protection against infectious diarrhoea. Another HMO, lacto-N-neotetraose (LNnT), administered intratracheally alongside pneumococcus, exhibited the potential to mitigate the progress of pneumococcal pneumonia and prevent nasopharyngeal colonization in animal models.

Apart from shaping the gut microbiota composition, HMOs directly influence the responses of host intestinal epithelial cells. They can curtail cell growth while inducing differentiation and apoptosis in human intestinal cells. Some HMOs are also implicated in promoting the maturation of intestinal cells and enhancing barrier function. Another significant role of HMOs is their capacity for immunomodulation.HMOs have a dual impact on the immune system: they exert direct effects on immune cells systemically upon absorption into the bloodstream, while also indirectly influencing immune responses by altering the gut microbiota. Following ingestion, most HMOs resist intestinal digestion and reach the distal small intestine and colon, with a small fraction, approximately 1%, being absorbed and detected in the systemic circulation and urine. The exact mechanism of HMO absorption into the bloodstream remains partially understood; however, studies indicate that HMOs bind to endothelial cells, leading to interactions with

monocytes, lymphocytes, and neutrophils, thereby exerting systemic effects (Bode *et al.*, 2004).

HMOs possess a direct impact on intestinal epithelial cells, governing gene expression and prompting changes in cell surface glycans and other cellular responses. Lymphocyte cytokine production can also be influenced by HMOs, possibly leading to a more balanced Th1/Th2 response. Notably, one of the primary HMO components, 2'-fucosyllactose (2'-FL), plays a role in restraining inflammation during the invasion of enterotoxigenic *Escherichia coli* into intestinal epithelial cells. This is achieved through the attenuation of CD14 induction, which mediates the lipopolysaccharide-Toll-like receptor 4 stimulation of an inflammatory pathway.

Furthermore, the influence of HMOs extends to neurodevelopment and cognitive function. Animal studies have revealed that oral supplementation of 2'-FL enhances memory and learning. Human cohort studies have also indicated that increased early exposure to 2'-FL might contribute to infant cognitive development. Moreover, metabolic byproducts of HMOs, such as sialic acid, play a role in promoting brain development, neuronal transmission, and synaptogenesis(Cheng and Yeung, 2021).

Synthetic methods for HMO production

Research efforts have been concentrated on acquiring access to thesemacromolecules via synthetic pathways since scientific evidence strongly supports the role of HMO in a number of beneficial physiological effects in newborns.Indeed, by attracting new customers or by incorporating HMOs into other products, the need for HMOs may increase over the next years. As of now, four possible synthetic pathways—chemical synthesis, whole-cell biotransformation, enzymatic, and chemo-enzymatic pathways—have been suggested for the generation of HMOs(Pérez-Escalante*et al.*, 2022).

Incorporation of HMO to infant formula

Only 38% ofthe worldwide infant population has been exclusivelybreastfed. Presently, the composition of milk formulas for newborns has evolved, moving away from the conventional fortification with oligosaccharides like FOS and GOS, which were more prevalent in previous years. As a result, a novel approach to infant formula has arisen, with the industry striving to create formulations that closely resemble human milk. This is achieved by incorporating fats, proteins, and HMOs.

When maternal production of certain HMOs is insufficient or to provide additional disease protection in early childhood, integrating HMOs into milk formulas is recommended. Research supports the idea that well-formulated HMO compositions can yield similar microbiome profiles as those found in breastfed infants. For instance, a cow protein-based infant formula containing 2'-fucosyllactose (2'FL) and lacto-N-neotetraose (LNnT) at specific concentrations showed promising microbiome similarity to breastfed infants.Furthermore, studies have highlighted the good assimilation and tolerance of infants fed with formulas containing 2'FL and LNnT.

Studies investigating the safety and tolerance of HMOs in infant milk formulations have yielded positive results. Formulas enriched with 2'FL and LNnT were well-tolerated, with favorable absorption rates and no significant differences in anthropometric measurements when compared to formulas fortified with other oligosaccharides. Moreover, infants fed with 2'FL and LNnT-enriched formulas exhibited a

lower incidence of respiratory infections and reduced use of antipyretics and antibiotics.

Currently, HMOs are present in infant formulas developed by Nestlé and Abbott, mainly 2'-fucosyllactose (2'FL) and lacto-N-neotetraose (LNnT) incorporation (Bych *et al.*,2019). Eventhough many foreign countries have legally permitted HMO addition to infant formulas; Indian standards does not permit the same. Hence, formulas with HMOs are not available in Indian market (Pérez-Escalante*et al.*, 2022).

Conclusion

In conclusion, breast milk stands as the gold standard for infant feeding and nutrition, offering a range of benefits essential for optimal growth and development. Human Milk Oligosaccharides (HMOs), a distinctive component of breast milk, constitute a critical factor in differentiating between human milk and formula. While breastfeeding remains the optimal choice for infant nutrition and well-being, the incorporation of HMOs into infant formulas emerges as a viable alternative when breast milk is inadequate or unavailable. Although challenges in synthesizing certain HMOs persist, the continuous research and progress in this area hold the promise of further refining infant formulas and improving the overall well-being of infants in need. In coming years, HMOs will be considered as gold standard for premium infant formulas.

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