Part 3

HMOs – beyond a single function

Hayley Kuter, BHSc MRes RD, Medical Science Liaison, Medical & Scientific Affairs, Abbott's Nutrition Business

Human milk oligosaccharides (HMOs) are a diverse group of more than 200 very complex carbohydrates found in breastmilk.[1] Understanding the key roles that HMOs play in infant health and immunity has evolved over many decades of research.[2] First termed the 'bifidus factor', HMOs are the original prebiotics.[2] HMOs are now recognised for providing additional benefits for the developing infant.[3]

Part 1 and 2 of this series outlined the structure and function of HMOs (see **Figure 1** for summary of key known functions). This final article, part 3, focuses on the potential for HMOs to modulate the infant's immune system and highlights studies which demonstrate their effects.

Breastmilk protects

Breastmilk is the 'gold standard' for infant nutrition – ensuring optimal growth and a multitude of benefits.[5,6] Epidemiologically-demonstrated advantages for the breastfed infant include: decreased risk of gastroenteritis, respiratory tract infections, atopic dermatitis, asthma, obesity, type 1 and type 2 diabetes, necrotising enterocolitis (NEC), and sudden infant death syndrome (SIDS).[6]

In addition to complete nutrition, breastmilk contains non-nutritive, bioactive substances that promote the growth and development of the intestinal and immune systems.[7] The development of innate immunity and gastrointestinal microbiota at precisely the same time as lactation is unlikely to be a mere coincidence.[7]

HMOs – a significant component in breastmilk

HMOs are a type of glycan that have gained a great deal of attention due to both their quantity and functions in breastmilk. HMOs represent the third largest solid component of breastmilk, after lactose and lipids.[8] They constitute up to 2% w/v – often greater than protein.[7] They are energetically costly for the mammary gland to produce, and yet are indigestible in the infant.[7]

What then, is their purpose?

HMOs are unable to be broken down by digestive enzymes and therefore they reach the colon intact.[1] In the colon, they are metabolised by bacteria within the intestinal microbiome. In this sense, HMOs are prebiotics. They promote the growth of a favourable microbiota – specifically certain *Bifidobacteria* taxa and *Bacteroides* species.[5] In fact, HMOs are the sole carbon source of certain *Bifidobacterial* taxa.[5]

Figure 1: Key Functions of Human Milk Oligosaccharides[4]

1. Prebiotics: selectively enrich beneficial gut bacteria
2. Enhance gut barrier function
3. Prevent adhesion of certain pathogens
4. Directly support immune responses

Bifidobacteria and *lactobacilli* are responsible for the production of lactic acid and the short-chain fatty acids (SCFA) butyrate, acetate and propionate.[3] These metabolites provide energy for colonocytes and are involved in modulating inflammation.[1,5] Interestingly, a lack of *Bifidobacterium* has been shown to be associated with necrotising enterocolitis (NEC), and dysbiosis of the microbiota is associated with long-term health consequences, such as obesity, diabetes and inflammatory bowel disease.[5] The acquisition of appropriate intestinal microbiota is essential to intestinal health and prevention of inflammation and disease.[5]

More than just prebiotics

Although HMOs can indirectly affect the immune system by fuelling beneficial gut microbiota or affecting the intestinal epithelial cell response, HMOs may act directly to modulate immune responses.[1,9] This occurs either locally, on cells of the mucosa-associated lymphoid tissues or systemically; around 1% are absorbed and reach the systemic circulation.[1,9] HMOs have been detected in the plasma of breastfed infants at concentrations of 1-133 mg/L.[10]

Many immune receptors recognise HMOs.[10] HMOs have been shown to modulate immune signalling pathways and decrease acute phase inflammatory cytokine pathways.[5] Many cytokines, including anti-inflammatory cytokines (such as IL-10, TGF-β, IL1-RA and TNF-α) are already present in breastmilk.[5] However, animal models have shown that the addition of HMOs alone result in reduced intestinal inflammation.[5]

Emerging research on additional roles that HMOs play in infant development include that of the nervous system and gut-brain axis: HMOs are a source of sialic acid which is an essential nutrient for brain development and cognition.[11] 2'-fucosyllactose (2'-FL) affects cognitive measures and improves learning and memory in rodents.[12] Colon motor contractions have been found to be affected by fucosylated HMOs in *ex vivo* models; could HMOs be beneficial for conditions of disordered motility and gut pain, such as functional gut disorders and infantile colic?[13]

Why HMOs are 'new'

New technologies for rapid and high-throughput HMO analysis, isolation and commercial production of HMOs have pushed the understanding and identification of over 200 HMOs.[1, 14] Industrial synthesis of isolated HMOs has taken over 20 years of research and several HMOs can now be produced synthetically to have molecular structures identical to those in breastmilk.[11] This has greatly changed what is known about these compounds, and also opens the possibility for them to be applied to target groups – such as those who are immune compromised or at high risk of infection.[10]

In striking contrast to breastmilk, cow's milk contains very few oligosaccharides and (as a result) infant formula contains none.[14] The European Food Safety Authority (EFSA) positively assessed 2'-FL as an addition to infant and follow-on formula in 2015, and since then, several more have been approved for use in formula.[15, 16] Synthetic HMOs were first added to infant formulas in 2016.[17] Unlike probiotics, biosynthesised HMOs are resistant to pasteurisation and freeze-drying.[11] There have been no adverse events reported to date.[11]

Safety and clinical outcomes of biosynthesised HMOs

The functional benefits of adding biosynthesised HMOs to infant formula have been reported in several intervention studies.[18] The most widely studied biosynthesised HMOs to date are 2'-FL and lacto-N-neotetraose (LnNT), and 2'-FL, in particular, is one of the most abundant HMOs in most mother's breastmilk.[11, 19]

Marriage *et al.* (2015) conducted a prospective, randomised, controlled, multi-centre study to examine growth and tolerance of an infant formula supplemented with 2'-FL.[20] Four hundred and twenty-four healthy full-term infants were enrolled by day 5 of life.[20] Three infant formulas were trialled (GOS only, or with 2'-FL at 0.2 g/L or 1.0 g/L) and a non-randomised breastfed group was also enrolled as a reference.[20] No significant differences were observed among any groups in growth parameters (weight, length, or head circumference) over the four-month study period.[20] 2'-FL was present in the plasma and urine of infants fed the two formulas containing 2'-FL.[20] All formulas were well tolerated and comparable in terms of average stool consistency, number of stools per day, and the percentage of feedings associated with spitting up or vomiting.[20] Interestingly, in a *post-hoc* analysis there were fewer parent-reported respiratory tract infections and eczema in the formula group supplemented with 2'-FL.[21]

Goehring *et al.* (2016) investigated biomarkers of immune function using a sub-group of the Marriage *et al.* (2015) study.[22] The infants fed the formula containing 2'-FL exhibited significantly different inflammatory cytokine profiles from those of the group fed the control formula (only GOS) ($p \leq 0.05$) but not different from those of breastfed infants.[22]

Puccio *et al.* (2017) randomised 175 healthy full-term infants to receive a formula supplemented with two biosynthesised HMOs: 2'-FL and LnNT for 6 months.[8] The supplemented formula was found to be safe and well tolerated, and supported age-appropriate growth.[8] The HMO-supplemented group had lower rates of parent-reported morbidity (particularly bronchitis) and medication use.[8] Berger *et al.* (2020) further reported on stool microbiota and antibiotic use (as a proxy measure of infection rates) in the same group until the age of 12 months.[23] At 3 months, those infants who received the HMO-supplemented formula had a microbiota profile more similar to breastfed infants than those fed the control formula with no HMOs.[23] Antibiotic use was less by the age of 12 months in the HMO-supplemented groups compared to the control.[23]

The future of HMO science

Breastmilk offers multiple layers of non-nutritive protection to the newborn by supporting healthy gut microbes, protecting the infant from infections, and facilitating intestinal and immune development.[10] Major advances in human milk research and analytical techniques, including high mass accuracy and identification and quantification of the diverse glycan structures, and novel methods including nano-chip technology, has greatly improved our understanding of HMOs.[7] Commercially produced, biosynthesised HMOs are becoming increasingly available and research has shown they are safe and tolerated.[10] Furthermore, research supports the role that biosynthesised HMOs may have in supporting the gut microbiota and in building immunity in infants who require a breastmilk alternative. Future research and clinical intervention studies may yet uncover additional benefits of HMOs and their metabolic products – not only in reducing the disease risk linked to gut ecology but in novel areas such as in brain development and neuronal transmission.[11, 23]

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