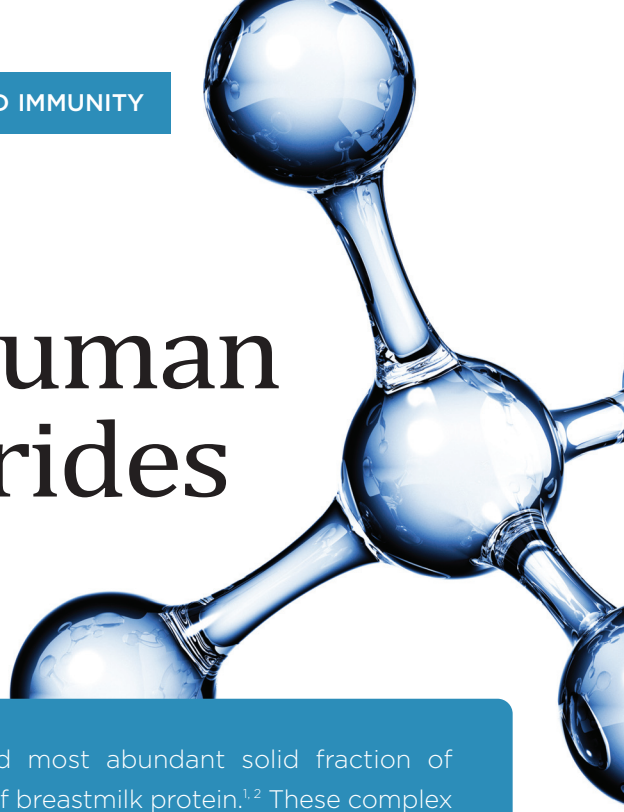

Part 1

Understanding Human Milk Oligosaccharides

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Human milk oligosaccharides (HMOs) account for the third most abundant solid fraction of breastmilk; at 5-20 g/litre, they often exceed the total amount of breastmilk protein.[1,2] These complex sugars, however, are virtually absent from cow's milk and other mammal milks.[3]

What is the role of HMOs in breastmilk and how do they affect infant health and the immune system? This three-part series will begin with a background and understanding of HMO structure, metabolism and function. Part 2 will focus on the immune-modulating effects of HMOs. Part 3 will explore clinical studies focusing on specific HMOs.

Introduction

Breastmilk unequivocally provides the best nourishment for a baby.[4] This message is by no means new, but the science that underpins it has come a long way in recent decades. Observational studies consistently show better health outcomes of breastfed infants, including reduced neonatal infection and non-communicable diseases involving the immune system and immune tolerance, such as asthma, eczema, allergic rhinitis, and other immune disorders.[5] The immune benefits of breastfeeding can be attributed to the dynamic and multi-dimensional components of breastmilk (see **Figure 1**) – now with a strong case for the key role of human milk oligosaccharides in neonatal immune defence and maturation.[6]

In the 1950s, the nutritional scientist Paul Gyorgy described a "bifidus factor" unique to breastmilk.[7] His research identified a substance in breastmilk that specifically supported the growth of *Lactobacillus bifidus* species and furthermore inhibited pathogen binding.[7] Since then, years of research into the complex structures and functions of HMOs have shown they exert specific prebiotic effects and play an essential role in the development of a healthy microbiome, but also have wider and targeted effects on the immune system.[8]

Figure 1: Selected Bioactive Compounds in Breastmilk[5]

- Oligosaccharides
- Microorganisms
- Bioactive proteins: cytokines, growth factors, immunoglobulins, caseins, whey proteins
- Polyunsaturated fatty acids (Ω-3 and Ω-6)
- Nucleotides, hormones, vitamins, minerals, immune cells

What are human milk oligosaccharides?

Human milk oligosaccharides (HMOs) are the third most abundant class of biomolecules in breastmilk, after lactose and lipids.[1] They are complex soluble carbohydrate (glycan) structures of which around 200 have been characterised to date – although thousands are likely to exist.[1]

HMOs consist of 5 basic monosaccharide building blocks: galactose (Gal), glucose (Glc), N-acetylglucosamine (GlcNAc), fucose (Fuc) and the sialic acid (Sia) derivative N-acetylneuraminic acid (Neu5Ac).[3] The core monosaccharide units carry lactose (Gal β1-4 Glc) at the reducing end and fucose at the non-reducing end.[9] HMOs are elongated linearly or branched in a myriad of different ways using glycosidic linkages to produce either neutral or acidic HMOs.[1] The diversity of the structures of HMOs results in various roles in infant health and immune development, as will be explained below.

Not all breastmilk is the same

This may seem an obvious fact, but every mother carries her own breastmilk 'fingerprint'. HMO concentrations and profiles vary significantly between women and over the course of lactation.[10]

Genetic factors have largely been attributed to HMO differences between women; the Lewis (Le) blood group and secretor (Se) status can partly explain this variance.[11] The presence or absence of the Se gene - FUT2 (galactoside 2-α-L-fucosyltransferase 2) and the Le gene - FUT3 (galactoside 3/4-L-fucosyltransferase) results in 4 milk types: Se-Le+, Se+Le+, Se-Le-, Se+Le-.[8] So-called 'secretors' make up ~70-80% of the European and American population and these mothers express genes capable of producing all the known HMOs and in the greatest concentrations.[12]

A recent international study observed that HMOs from ethnically similar mothers varied geographically – for example, mothers living rurally or in a city displayed different HMO profiles.[11] This suggests an additional environmental and/or dietary influence on HMO biosynthesis; obesity, hyperglycaemia and malnutrition changed HMO profiles.[2] This suggests that individual manipulation of HMOs may be also be possible.

Varying profiles of HMOs are thought to be associated with differential risk of infectious diseases – for example gastrointestinal diseases or urinary tract infections.[9] Perhaps an important survival strategy for human populations periodically confronted with newly emergent, virulent and/or deadly pathogens may be that some proportion of the population is innately resistant by virtue of their mother's HMO profiles.[9]

HMO metabolism

In contrast to lactose, HMOs are indigestible; they are resistant to the low gastric pH and enzyme digestion in the upper gastrointestinal tract and reach the lower gastrointestinal tract intact. Around 1% of HMOs will be directly absorbed where they will reach the systemic circulation.[3] HMOs can be detected in the urine and plasma of breastfed infants.[12] It is likely that HMOs reach many organs beyond the gut including the liver and brain and this provides some clues as to the extent of impact on the infant. The majority of HMOs though, are metabolised by the infant's gut microbes or are excreted intact in the infant's stools.[3]

Functions of HMOs: the gut and beyond

1. HMOs are 'bespoke' prebiotics

HMOs exert their main effects in the large intestine and colon where they act as specific substrates to those bacteria that express the essential glycosidic enzymes.[2] Breastfed infants display marked differences in their microbiota profiles in comparison to mixed or formula-fed infants.[13] The abundance of Bifidobacterium spp. in breastfed infants can be explained by the growth advantage that HMOs provide; certain bacteria have co-evolved with HMOs to express enzymes that cleave only Sia or Fuc for example.[3] In other words, different HMOs can be metabolised by different bacteria and not all HMOs lead to the same changes in the microbiome. Bacteria that cannot utilise HMOs will have a disadvantage and do not grow as well, if at all.[3] Part 2 of this series will explore the prebiotic effects of HMOs in more detail.

2. HMOs support the developing architecture of the gut barrier

As indicated above, HMOs appear to have co-evolved with beneficial strains of bacteria to allow a favourable microbiome to establish. This is important for the general health of the infant, and also for the development of a healthy gut barrier and immune system. The immune system of the gut – the gut-associated lymphoid tissue (GALT) - is responsible for the mucosal layer and immune responses.[14] Directly, isolated HMOs have been shown to promote differentiation and reduce proliferation of various intestinal epithelial cell cultures (HT-29 and Caco-2).[1] Some interesting research on animal models indicate that necrotising enterocolitis (NEC) may be reduced or inhibited by specific HMOs (such as 2'-fucosyllactose and 6'-sialyllactose).[15]

3. HMOs potentially inhibit adhesion of pathogens

Due to methodological and ethical issues, clinical studies on the anti-pathogenic function of HMOs are limited and often use animal or *in vitro* studies. It is known that many pathogenic viruses, bacteria or protozoan parasites need to be able to attach to the epithelial cell walls in order to proliferate to invade or cause disease.[3]

Most enteric bacteria and protozoan-parasitic pathogens use cell surface glycans (sugars) to identify and bind to their target cells, which is the critical first step in pathogenesis.[14] HMOs resemble some of the surface glycans – meaning they can act as floating 'decoys' that can block pathogen binding to receptor cells.[3] Group B Streptococcus – a leading cause of invasive bacterial infection in newborns, has been shown to be inhibited by non-sialylated neutral HMOs, with lacto-N-tetraose (LNT) causing the highest inhibition in a study by Lin *et al.* (2017).[16]

HMOs can also help prevent infection by viruses by competing for binding with receptors.[8] Rotavirus and norovirus are examples of viral pathogens that that bind to the epithelial glycans – this may provide one explanation for the reduced incidence of these viruses in breastfed compared to formula-fed infants.[3] A case-cohort analysis of HIV-uninfected infants from HIV-positive Zambian mothers found that higher levels of the breastmilk containing fucosyllated HMOs - 2'-fucosyllactose (2'-FL) and lacto-N-fucopentose 1 (LNFP1) resulted in better outcomes in terms of mortality.[17]

4. HMOs can directly alter immune responses

A small portion of HMOs are absorbed intact into the circulation where they remain in concentrations high enough to modulate the immunological system.[18] HMOs modulate lymphocyte cytokine production and enable a more balanced TH1/TH2 response.[19] In a randomised-controlled trial, the plasma concentration of 5 cytokines (IL-1ra, TNF-α, IL-1α, IL-1β and IL-6) were significantly lower in the breastfed infants and infants fed with experimental formula supplemented with the HMO 2'-FL than that in infants fed with a control formula.[20]

Summary

Breastmilk – in addition to its nutrient balance – is known for its anti-pathogenic, anti-infective, anti-inflammatory properties.[18] Human milk oligosaccharides make up a significant fraction of breastmilk and recent studies indicate that they may explain some of the beneficial effects of breastmilk. HMOs can act locally in the intestine to shape the developing intestinal microbiome and research shows they play a role in protecting infants from infections. Their absorption into the blood and ability to modulate cytokines offers a promising insight into immunological outcomes that human milk oligosaccharides offer.

This article has been commissioned and placed by Abbott. Part 2 of the 'Human Milk Oligosaccharides – Shaping infant health and immunity' series from Abbott will follow in Jul/Aug issue of CN Magazines.

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