Part 2

HMOs – unique and dedicated prebiotics

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Breastmilk contains a diverse number of bioactive compounds that nature has designed to specifically support the developing infant. Human milk oligosaccharides (HMOs) account for the third largest fraction of breastmilk.[1] They are a complex mixture of indigestible carbohydrates with multiple functions (see **Figure 1**).[2] Increasing evidence shows the exceptional role HMOs play in supporting immunity and in defence against harmful pathogens.[3] Here, in the second of a three-part series, the beneficial effects of HMOs on the microbiome are explored in detail.

Starting with the microbiome

The gut microbiome is comprised of trillions of bacteria, their genomes, archaea, protozoa, fungi and viruses.[5, 6] The microbiome and the surrounding environment in the gastrointestinal tract has been the subject of intense research over the past few decades.[7] The microbiome's crucial role in digestion, synthesis of vitamins and minerals, colonisation resistance and immune modulation has even led it to be considered as a 'new organ' of the human body.[7]

Many questions remain unanswered about the exact workings of the microbiome.[8] It was only recently that the concept of a 'sterile womb' and the accepted belief that the neonatal microbiome is acquired after birth has been challenged: microbes have been detected in the placenta, amniotic fluid, foetal membrane, umbilical cord blood, and meconium – showing that the foetus is exposed to bacteria and metabolites *prior* to birth.[9, 10] HMOs have now been detected in the circulation of pregnant mothers *prior* to birth.[11]

Regardless, the neonate is born with a relatively limited set of microorganisms, and the development of the infant gut microbiota is a process that continues for at least the first 2-3 years of life.[3] The microbiome development during infancy is a critical period: it shapes the lifelong signature of the adult intestinal microbiome and future health.[12]

The assembly of the microbiota may be affected by factors such as prenatal exposure to antibiotics or toxins, the mode of delivery, antibiotic exposure after birth, diet (breastfeeding *versus* formula feeding), the introduction of solid food, and environmental factors, such as geography or climate.[6]

Following birth, the first major shift in intestinal microbiota composition was initially thought to be associated with the introduction of solid food during weaning.[5] Recent evidence has shown that it is the reduction or cessation of breastfeeding that causes a major change in the infant's gastrointestinal ecology.[5] This provides evidence of the significant role that breastmilk plays in the microbiome development.

See **Figure 2** for key differences between the microbiota and microbiome.

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

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

Figure 2: Microbiota or Microbiome – what's the difference?[8]

Often used interchangeably, although there is a difference between the microbiome and the microbiota.

- **Microbiota**
  The population of microorganisms colonised in a particular location, e.g. the gastrointestinal tract, skin, lungs.
- **Microbiome**
  The entire collection of genes, proteins, microproteins and metabolites of the microbiota.

Role of the microbiota in health and disease

The intestinal microbiota is a key driver of the gut and immune system development. An increasing body of evidence has shown that the early microbiota colonisation has a strong influence on later health outcomes. The mutually beneficial relationship between the host and the microorganisms cannot be undermined: disruptions (dysbiosis) to the microbiome in the neonate can lead to diseases, such as necrotising enterocolitis (NEC) or bacterial sepsis.[13] Disruption to the microbiome in animal models has been associated with higher incidence of chronic disease, such as obesity and metabolic syndrome, inflammatory conditions (e.g. inflammatory bowel disease) and allergy/atopy.[6]

What does a 'healthy' microbiome do?

A strict definition of a 'healthy' microbiome does not yet exist.[10] In adults, a highly diverse number of microbiota (i.e. more than 600,000 bacterial genes) is thought to be ideal.[10] We do know that the microbiome has several essential functions. These include the fermentation bacteria to produce into absorbable metabolites (i.e. short-chain fatty acids), the synthesis of essential vitamins, the removal of toxic compounds, the out-competition of pathogens, the strengthening of the intestinal barrier, and the stimulation and regulation of the immune system.[14]

HMOs: key drivers of early microbiome development

Next-generation sequencing of the human genome has greatly improved the understanding of the microbiome. Limitations of cultured experiments have largely been overcome with new sequencing techniques. For example, gut bacteria exist in a competitive mixed ecosystem; data from pure culture experiments cannot easily translate into 'real' outcomes.[15] The ecological differences between breastfed and formula-fed infants has therefore never been so obvious.

Studies have consistently shown that HMOs stimulate the growth of specific *Bifidobacterium* and *Bacteroides* species. These two groups are both involved in the production of short-chain fatty acids (SCFAs) and lactate, which lower the colonic pH, modulate the microbiota, and have other systemic properties.[16] *Bifidobacterium* is abundant in breastfed infants; a study by Fan *et al.* (2014) showed there was almost 15% difference in *bifidobacteria* species in 1-6 month old breastfed *versus* formula-fed infants.[12] *Bifidobacterium infantis* for example holds a matched set of genes that encode specific enzymes for HMO metabolism.[3]

In Part 1 of this HMO series, it was explained that the mother's genetic makeup partly influences the HMO composition of her breastmilk. Infants fed by mothers who secrete a complete set of HMOs ('secretors'), which include all the fucosylated HMOs, demonstrate establishment of bifidobacterial-predominant microbiota earlier than infants fed by non-secretor mothers.[16] Gastrointestinal infections, such as *Campylobacter* gastroenteritis, and allergic outcomes, for example eczema, have found to be lower in secretor mothers than non-secretors.[17] There is some suggestion though that non-secretor status may also confer some benefits, such as reduced *Helicobacter pylori* infections – showing that HMO secretor status may be under balancing selection.[16]

Lactose, GOS and FOS as drivers of microbiota?

Lactose is the main source of energy and carbohydrate in breastmilk and can also be used for energy by some bacteria, particularly the *Bifidobacteria* species and *Lactobacillus* species.[18] However, in infants (whose intestinal lactase is typically high) lactose does not often reach the terminal ileum and colon where most intestinal bacteria live.[18] Lactose is therefore unlikely to be a significant driver of microbiota diversity.[6]

The absence of HMOs in infant formulas have led to the addition of galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) (both short and long-chain) to infant formula. GOS and FOS are not found in breastmilk.[19] GOS is derived from enzymatic synthesis based on lactose, and FOS is derived from vegetable plants.[4, 20] They are relatively inexpensive to produce and have been found to mimic some of the prebiotic effects of breastmilk.[20]

In a simulated model comparing the effects on microbiota of GOS and lactose and one of the most abundant HMOs – 2'-fucosyllactose (2'-FL) – it was found that 2'-FL generated a more diverse microbiota.[21] The study showed how 2'-FL was capable of specificity as an energy source for microbes. Also, compared to GOS and lactose, 2'-FL resulted in production of intermediate levels of SCFA metabolites – acetate and lactate. High levels of acetate and lactate have been implicated in colic and osmotic diarrhoea, respectively.[21] GOS and FOS are structurally simpler than HMOs and are neither fucosylated nor sialylated, which means that they lack a negative charge crucial to HMO effects.[19]

Summary

Breastmilk provides the perfect nutrition for infants – its formulation perfected throughout millions of years of evolution. The rich mix of non-nutritive, bioactive substances is different in every mother but acts to provide infants with the best possible defence against immediate and future threats. HMOs are especially interesting for their ability to specifically enhance beneficial gut bacteria and play a key role in establishing a lifelong microbiome signature.[12] Whilst the clinical consequences of each of several hundred HMOs is still relatively unknown, accumulating evidence shows their significance in breastmilk.[4] Although great shifts have been made in its understanding, the importance of breastfeeding is once again highlighted.

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