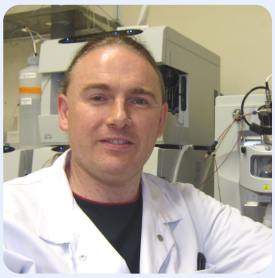
Expert Interview with Dr. Sean Austin and Dr. Norbert Sprenger

Human milk oligosaccharides (HMOs) has been considered the most abundant bioactive component which brings a myriad of health benefits to the newborn infants including the regulation of gut microbiome homeostasis, the stimulation of gut barrier functions as well as immunomodulating effects[1,2].

The dynamic and diverse profile of HMOs



Q&A session with Dr Sean Austin

Expert, Carbohydrate Analysis
Nestlé Institute of Food Safety and Analytical Sciences
Nestlé Research
Switzerland

Q: Question from WNSC HK **SA: Answer by Dr Austin**

Q: How many types of HMOs have been identified to date? Are they all quantifiable and at similar concentrations throughout lactation?

SA: To date, more than 160 unique HMO structures have been characterized. They are based on various core structures decorated by series of monosaccharide components. Evidence suggests more unknown HMOs are yet to be characterized[2]. Among all, quantitative data are only available for around 20-30 of the most abundant HMOs, quantitative work is hindered by a lack of analytical standards for HMO peak annotation and quantification. From existing studies, it has been observed that HMO concentrations vary during lactation. Majority of HMOs follow a descending trend throughout lactation while some remain at a relatively stable concentration plus a few exceptions, such as 3-Fucosyllactose (3-FL), which increases overtime[3-6].

Q: How are the diverse HMO structures formed?

SA: All oligosaccharides in human milk contain lactose as the basic unit. N-acetylglucosamine (GlcNAc) and galactose (Gal) residues are then attached to the starting lactose to create a range of "core structures". While the HMOs identified so far are derived from 13 core structures, it has been postulated that as many as 19 core structures may exist[2].

The simplest core structures include lactose itself and the tetrasaccharides Lacto-N-tetraose (LNT) and Lacto-N-neotetraose (LNnT). Core structures can be further decorated by fucose (Fuc) via α-1,2, α-1,3, or α-1,4 glycosidic linkages and/or N-acetylneuraminic acid (Neu5Ac, a type of sialic acids[7]) via α-2,3 or α-2,6 linkages. Examples of these HMOs include 2'-Fucosyllactose (2'-FL) and 3'-Sialyllactose (3'-SL).

Q: What are some tools available for assessing HMO diversity in human milk samples?

SA: The Shannon-Wiener diversity index has been used in recent HMO research[8,9]. This tool was originally established for assessing the diversity of populations in a given ecosystem. The Index serves as a unified measurement of both richness (i.e. how many different species are present) and evenness (their relative proportion in the ecosystem), with a larger value indicating a higher number of individual species present as well as a more even distribution of all species[10].

The underlying question is how the index can be best adapted to the context of HMO diversity. One approach is to simply count each individual HMO as a different "species". However, we know we are not measuring all the different species, so perhaps the results would not be representative of the whole. An alternative approach would be to focus on key structural features which ultimately defines the distinct HMO classes. Based on the corresponding molar concentration of each HMO measured, the "number" of structural features in a human milk sample can be estimated and hence be translated into the "richness" of structure species of its HMO profile (note that one HMO may contain several key structural features). Assuming that the HMOs measured are representative of all the HMOs in the milk, then using the structural feature approach may provide a more meaningful estimate of diversity.

The significance of HMO diversity

Q&A session with Dr Norbert Sprenger

Senior Expert, Glycobiology
Nestlé Institute of Health Sciences
Nestlé Research
Switzerland

Q: Question from WNSC HK **NS: Answer by Dr Sprenger**

Q: How do *FUT-2* and *FUT-3* gene polymorphisms influence the HMO profile of lactating mothers?

NS: Lactose is produced in the mammary glands where it is also elongated with various monosaccharide units at different positions by glycosyltransferases, resulting in a diversity of HMO chain lengths, compositions and structures. Fucose is added primarily by the fucosyltransferase enzymes FUT-2 and FUT-3 encoded by the secretor and Lewis gene, respectively. Both are involved in the formation of the Lewis histo-blood group phenotypes. The distinct HMO profiles stem first from the presence or absence of FUT-2 or FUT-3, which determines whether certain HMOs are formed or not formed. This subsequently leads to altered monosaccharide donor substrate and oligosaccharide acceptor substrate availability. Hence, additional glycosyltransferase enzymes can use more of those substrates. Together, this explains part of the HMO profile differences observed in milk of different mothers[11].

Q: Is there any evidence suggesting the role of a diverse HMO profile on health outcomes of infants? In other words, how are HMO functions structure-specific?

NS: In breastfed infants, HMO functions are primarily investigated through the associations with health and disease. This allows us to gain important insights on possible physiological roles of different HMOs at different scales:

I. The expression of a functional FUT-2 gene determines the secretor status of lactating mothers and the presence of 2'-Fucosyl-HMOs in their milk. The maternal secretor status has been linked to their breastfed infants' gut microbiota establishment[12], promotion of Bifidobacterium dominance[13] as well as the reduction of respiratory infection risk[14]. Of note, FUT2-dependent HMOs were also shown to help delay IgE-associated eczema onset at 2 years of age in C-section born infants with a hereditary risk[12].

II. Individual HMOs have been associated with a spectrum of health outcomes. Apart from 2'-FL, a well-recognized key contributor to neonatal gastrointestinal health, recent observational data also reported relations of 3'-Sialyllactose (3'-SL) to cognitive development at 24 months of age . These reports were further supported by findings from basic research models as well as mechanistic studies with clinical samples which often indicate a very specific HMO structure-function relation.

III. That said, the importance of structural features should also be considered when investigating the biological role of HMOs. The reported predominant HMOs can be distinguished for example by 8 key differentiating structural features. Of those, 3 are key to define the shape of the HMO core structures and 5 additional ones define endpoint decorations. These terminal decorations together with the shape of the structures are generally the key determinants for biological functions. One example indicating that HMO diversity is important stems from a preterm infant trial, which found that HMO diversity was lower (as measured by Shannon-Wiener diversity index) in breastmilk fed to preterm subjects with necrotizing enterocolitis (NEC) as compared to those who did not experience NEC[15].

References:

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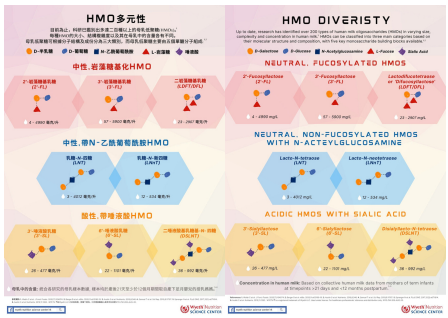

HMO Diversity Infographic

- Examples of top abundant types
- Three structural categories
- Monosaccharide blocks and overall structures
- Concentration ranges throughout lactation

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