

Human Milk Interactions With the Developing Infant Microbiome

David A. Sela, PhD

Organisms have evolved to cooperate in nutritional feeding strategies. We see evidence of this in the animal kingdom, such as marine birds that loiter around whales while they acquire food from the ocean. The same principle has evolved in microbial communities that colonize humans, often referred to collectively as the gut microbiome. Nutrition interventions seek to extend this ecological concept toward enhancing human health through optimizing joint feeding strategies within the gut microbiome. Ultimately nutrition provides a set of solutions to achieve one's full health potential, including optimal growth and development during childhood and maintenance of quality of life with advancing age. Optimal nutrition precisely tailored to the individual's genome, lifestyle, and even microbiome all have the potential to mitigate pathological states and bolster prophylactic strategies. The interconnectedness of the gut microbiome with our physiology provides a tractable target to improve human health.

Our group specifically researches the interactions between molecules dissolved in human milk and the early establishment and function of the infant gut microbiome. At one point, human milk was solely characterized as providing a benefit through direct nutrition and modulation of the immune system, in addition to panoply of benefits to the infant. However, we now know that the infant microbiota is influenced by, and interacts with, human milk molecules that are not fully hydrolyzed in the upper gut and arrive at the lower gastrointestinal tract.¹ Human milk oligosaccharides (HMOs) are produced in the mammary gland at a significant energetic cost to the mother and remain intact once ingested. Upon transit to the colon, prominent bifidobacterial populations within the infant gut consume HMOs. Bifidobacteria are often enriched within the infant gut, and formula-fed infants exhibit greater diversity within the entire gut bacterial microbiome.² The differences between breastfed and formula-fed infants are also evident in the differential metabolites produced by the microbiota depending on feeding mode.³ Interestingly, more amino acids and fewer amino acid degradation products were found in circulation in formula-fed infants, compared to breastfed controls, although impact to host physiology remains speculative.³ Benefits that bifidobacteria provide to the developing infant include pathogen inhibition, production of molecules that modulate host processes, and metabolic benefits.⁴

Approximately 200 distinct HMO structures have been identified across human milk samples, and each mother is capable of producing around 60 different HMOs.⁵ HMOs comprise up to five monosaccharide residues, with a variety of combinations, glycosidic linkages, and degrees of polymerization. An example of a simple yet abundant HMO

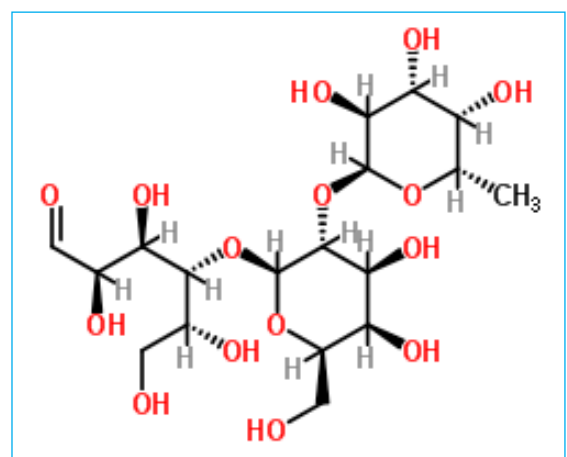


Fig 1. 2'-fucosyllactose (2'-FL).

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is 2'-fucosyllactose (2'-FL), a trisaccharide comprised of glucose, galactose, and fucose (Fig 1). HMOs are generally planar in molecular orientation and may have linear or branched structures. That there are only ~200 different HMO species identified suggest a functional constraint that has coevolved between the mother and infant mediated by the microbiome. In general, infant colonizing bifidobacteria utilize HMOs to a greater extent than adult-associated taxa such as *Bifidobacterium adolescentis*. This is evident in the *in vitro* growth phenotype and the identification of the specific HMOs consumed by *Bifidobacterium longum* subsp. *infantis* (*B. infantis*).^{1,6} These growth assays are conducted with HMO as a sole carbohydrate source to determine if the bacterium is capable of utilizing it as energy. *B. infantis* is the archetypical HMO consumer that serves as the experimental model for this physiological phenomenon. In general, *B. infantis* consumes the most abundant HMO species that are also the lowest in molecular weight and degree of polymerization. This provides several implications for the judicious modulation of the developing neonate microbiome.

The *B. infantis* genome sequence encodes a 40 kbp gene cluster that enables the bacterium to capture small mass oligosaccharides from the extracellular environment and metabolize it within the cytoplasm.⁷ This gene cluster contains all the glycosyl hydrolases and transporters that are predicted to interact with HMOs. Subsequent research has supported this model by characterizing several molecular mechanisms involved in HMO utilization.^{8,9} Moreover, *B. infantis* exhibits a global gene expression profile that varies when consuming specific HMO structures and across time.¹⁰ In addition, early HMO consumption *in vitro* induces a gene expression profile distinct from later timepoints. Moreover, gene expression while *B. infantis* consumes lactose is more similar to HMO utilization than 2'-FL. This is surprising as lactose is structurally similar to 2'-FL as the latter is identical with the exception of an additional fucosyl group. This may have implications for prebiotic/synbiotic approaches as pre-culturing carbohydrate choice (e.g., lactose or 2'-FL) may be used to optimize probiotics prior to delivery to the consumer. Moreover, there are distinct differences between the utilization of lacto-*N*-

tetraose (LNT) and lacto-*N*-neotetraose (LNnT). This is significant as these two tetrasaccharide HMO species differ by only one beta linkage between the terminal galactose and *N*-acetylglucosamine (Fig 2). This is consistent with observed strain-dependent utilization

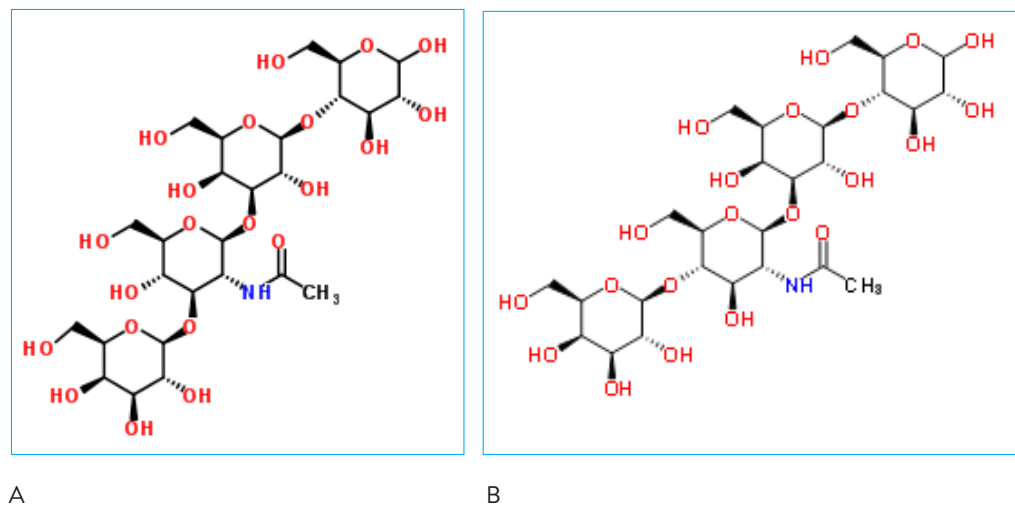


Fig 2. (A) Lacto-*N*-tetraose (LNT); (B) lacto-*N*-neotetraose (LNnT).

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of LNT versus LNnT. Bifidobacteria isolated from non-human primates are capable of utilizing both LNT and LNnT in contrast to bacteria isolated from the pig gut (unpublished data). This suggests that the bifidobacteria that colonize phylogenetically similar hosts to humans (i.e., primate) exhibit a similar HMO utilization pattern than more divergent hosts. The ability to utilize both HMOs is consistent with the repertoire of glycosyl hydrolase genes present in the primate microbiome. Furthermore, strains exhibit growth responses that are distinct depending on the HMOs that are utilized (unpublished data). This potentially has implications for prebiotic strategies to specifically enrich targeted bifidobacterial populations within the infant gut.

The goal of this research is to understand the fundamental physiology of bifidobacterial utilization of HMOs. This will extend not only to bifidobacterial populations as well as interactions with other members of the developing infant microbiome. For example, other non-bifidobacteria secrete butyrate that bifidobacteria do not synthesize. This short-chain fatty acid provides energy to colonocytes and possesses anti-inflammatory properties. Ultimately, translation of these findings requires testing hypotheses in infants to conclude there is indeed a benefit to infant health.

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