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The functional biology of human milk oligosaccharides

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A B S T R A C T

Human milk oligosaccharides (HMOs) are a group of complex sugars that are highly abundant in human milk, but currently not present in infant formula. More than a hundred different HMOs have been identified so far. The amount and composition of HMOs are highly variable between women, and each structurally defined HMO might have a distinct functionality. HMOs are not digested by the infant and serve as metabolic substrates for select microbes, contributing to shape the infant gut microbiome. HMOs act as soluble decoy receptors that block the attachment of viral, bacterial or protozoan parasite pathogens to epithelial cell surface sugars, which may help prevent infectious diseases in the gut and also the respiratory and urinary tracts. HMOs are also antimicrobials that act as bacteriostatic or bacteriocidal agents. In addition, HMOs alter host epithelial and immune cell responses with potential benefits for the neonate. The article reviews current knowledge as well as future challenges and opportunities related to the functional biology of HMOs.

Contents

1. What are human milk oligosaccharides?

Human milk oligosaccharides (HMOs) are unconjugated complex glycans (sugars and carbohydrates) that are highly abundant in human milk, but not in infant formula [1]. One liter of mature human milk contains 10–15 g HMO, which often exceeds the total amount of protein and is 100- to 1000-fold higher than the concentration of oligosaccharides in bovine milk, which is the basis of most infant formula. Concentrations are even higher in human colostrum.

HMOs consist of five monosaccharide building blocks: galactose (Gal), glucose (Glc), N-acetylglucosamine (GlcNAc), fucose (Fuc) and the sialic acid (Sia) derivative N-acetyl-neuraminic acid. All HMOs carry lactose (Galβ1-4Glc) at the reducing end, which can be elongated in β1-3- or β1-6-linkage by two different disaccharides, either Galβ1-3GlcNAc (type 1 chain) or Galβ1-4GlcNAc (type 2 chain). HMOs with more than 15 disaccharide units have been described, forming complex structural backbones that can be further modified by the addition of Fuc or/and Sia.

1.1. HMO fucosylation is related to Lewis blood group antigens

Fuc can be added to the HMO backbone in α1-2-, α1-3- or α1-4-linkage. HMO fucosylation is highly dependent on the mother's Lewis

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blood group status [2–5]. An enzyme called fucosyltransferase 2 (FUT2) catalyzes the addition of Fuc in α1-2-linkage on Lewis blood group epitopes as well as on HMOs [2]. FUT2 is actively expressed in over 70% of Caucasian women (Secretors). The milk of Secretor women contains high concentrations of α1-2-fucosylated HMOs, *e.g.* 2′-fucosyllactose (2′FL) and lacto-N-fucopentaose 1 (LNFP1). Nonsecretors, however, do not express an active FUT2 and the milk of Nonsecretor women lacks α1-2-fucosylated HMOs like 2′FL or LNFP1.

A separate enzyme called fucosyltransferase 3 (FUT3) catalyzes the addition of Fuc in α1-3/4-linkage (depending on the type of the underlying HMO backbone), and FUT3 can also be inactive in parts of the population (Lewis negative) [3]. The milk of Lewis negative women has markedly reduced concentrations of α1-3/4-fucosylated HMOs.

Depending on the expression of active FUT2 and FUT3 enzymes, women can be separated into four groups: 1. Lewis positive Secretors (FUT2 active, FUT3 active), 2. Lewis negative Secretors (FUT2 active, FUT3 inactive), 3. Lewis positive Nonsecretors (FUT2 inactive, FUT3 active), and 4. Lewis negative Nonsecretors (FUT2 inactive, FUT3 inactive). Accordingly, the oligosaccharide composition in the milk of women from these four groups varies significantly [4,5].

1.2. Variation in HMO sialylation is more subtle than the variation in HMO fucosylation

Sia can be added to the HMO backbone in α2-3- or α2-6-linkage, either to the terminal Gal or to internal GlcNAc. Sia contains a carboxyl-group, which introduces a negative charge to HMOs. Therefore, sialylated (or acidic) HMOs carry one or more negative charges depending on the number of Sia linked to the HMO backbone. Several sialyltransferases are involved in catalyzing the addition of Sia to the HMO backbone. As described above, the fucosyltransferases FUT2 and FUT3 can be inactive and certain fucosylated HMOs can be entirely absent. A complete loss of sialyltransferases and corresponding sialylated HMO has not yet been described. Instead, more variations in HMO sialylation are likely due to subtle interindividual expression variations in sialyltransferases or other enzymes and transporters important in sialylation pathways.

1.3. Genetic and environmental factors that contribute to HMO biosynthesis remain mostly unknown

As outlined for HMO fucosylation, HMO biosynthesis in the human mammary gland is in part genetically determined. Genes other than FUT2 and FUT3 that contribute to chain elongation, branching or sialylation might be differentially expressed in different women and lead to distinct HMO composition profiles. Whether or not other maternal factors like age, diet, general health status, use of medication and drugs, etc. affect HMO biosynthesis remains mostly unknown. Environmental factors that impact HMO composition are currently in the focus of HMO research.

2. HMO metabolism

Once ingested by the breast-fed infant, HMOs resist the low stomach pH as well as degradation by the infant's pancreatic and brush border enzymes [6,7]. Approximately, 1% of the ingested HMOs are absorbed, reach the infant's systemic circulation, and are excreted intact in the infant's urine [8–10]. The majority of HMOs are either metabolized by the infant's gut microbes or excreted intact with the infant's feces [11,12].

Since HMOs are absorbed and appear in the systemic circulation, they likely reach many organs other than the gut, including the liver and the brain, as well as the respiratory and the urinary tract. Thus, the biological functions of HMOs may not be localized to the gut; HMOs might impact the infant on multiple different levels throughout the neonate.

3. HMOs are human milk prebiotics

According to a definition by Roberfroid et al., "a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota, that confers benefits upon host well-being and health" [13]. HMOs serve as metabolic substrates for specific bacteria like *Bifidobacterium longum* subsp. *infantis*. As a consequence, these bacteria have a growth advantage and thrive. Other bacteria that cannot utilize HMOs have a disadvantage and do not grow as well or not at all. Thus, HMOs are the first prebiotics that humans encounter with their diet, usually from day one of life. A bacteria's ability to utilize HMOs requires an entire set of enzymes, transporters and other molecules. For example, certain bacteria have evolved together with HMOs and express sialidases that cleave Sia and fucosidases that cleave Fuc. Only very few bacteria are capable of degrading the entire set of HMOs [14–16]. Other bacteria may only be able to utilize a limited set of HMOs or specific epitopes on more complex HMOs. For example, bacteria with a certain fucosidase may be able to utilize Fuc, but not Sia. Some bacteria may be able to utilize HMOs only after other bacteria have removed the Fuc or Sia from the backbone, creating a "community feast" where multiple different bacteria may be able to degrade the entire set of HMOs, but only when they act together as a community.

While the sequential degradation of HMOs by different microbes needs to be further elucidated, it is evident that, based on their structural diversity, different HMOs can be metabolized by different bacteria. In other words, not all HMOs lead to the same changes in composition and/or activity in the gastrointestinal microbiota and have the same benefits upon host well-being and health. Prebiotic effects are likely structure-specific, and HMOs are a group of structurally diverse glycans. Since HMO composition varies between women, one can hypothesize that the milk of different women affects the infant gut mircobiome differently, which may relate to short-term infant health outcomes, but also have long-term consequences for health status and disease risk later on in life.

4. HMOs serve as antiadhesives

While the primary focus of HMO research has traditionally been on their prebiotic effects, HMOs are more than just "food for bugs". Many viral, bacterial or protozoan parasite pathogens need to attach to epithelial cell surfaces to proliferate and in some cases invade and cause disease. Often, the initial attachment is to epithelial cell surface sugars (glycans) also known as the glycocalyx. While these glycans are conjugated to proteins or lipids, HMOs resemble some of the glycan structures and serve as soluble decoy receptors that block pathogen binding to epithelial cells. Unbound pathogens can no longer attach to the cell surface and are washed out without causing disease. Norovirus and Rotavirus are examples of viral pathogens that bind to the epithelial glycocalyx; HMOs resemble the glycan binding partners and block viral attachment, providing one explanation for the reduced incidence of these viral infections in breast-fed infants compared to formula-fed infants. *Campylobacter jejuni* [17] and enteropathogenic *E. coli* [18] are examples of bacterial pathogens that follow the same principle and have significant impact on infant health as they are responsible for a majority of bacterial diarrheal episodes.

Our lab has recently shown that HMOs also prevent the attachment of the protozoan parasite *Entamoeba histolytica* [19]. Although uncommon in the US and Europe, *E. histolytica* infects more than 50 million people worldwide and causes the disease amebiasis, leading to more than 100,000 deaths annually [20]. *E. histolytica* expresses a lectin, a glycan-binding protein, which is a major virulence factor involved in *E. histolytica* attachment to intestinal epithelial cells [21,22]. The lectin is also involved in the subsequent killing and phagocytosis of these cells. HMOs block the lectin and prevent attachment, killing and phagocytosis. The effects are structure-specific and require a terminal Gal on