Perspective: Human Milk Oligosaccharides: Fuel for Childhood Obesity Prevention?

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ABSTRACT

Obesity begins early but has lifelong consequences for health and well-being. Breastfeeding is thought to be preventive against obesity, but the extent and cause of this association are not well understood. Human milk oligosaccharides (HMOs) are abundant in human milk and not present in commercially available infant formula. These complex sugars are thought to contribute to the development of the infant gut microbiome and immune system. Recently, they have been investigated as a potential link between breastfeeding and lower obesity risk. So far, only a few human studies have examined HMO composition of human milk in association with the infant's concurrent anthropometry or subsequent growth in infancy, with conflicting results. However, HMOs have been shown to modulate the gut microbiome profile by selectively promoting the growth of specific bacteria, such as bifidobacteria. Moreover, there are differences in the gut microbiome of lean and obese humans, and there is some evidence that the early composition of the gut microbiome can predict later obesity. Although it seems that HMOs might have a role in infant growth and adiposity, there is not enough consistent evidence to understand their potential role in obesity prevention. More data, particularly from large or longitudinal studies, are needed to clarify the functions of HMOs and other breast-milk components in determining long-term health. *Adv Nutr* 2020;11:35–40.

Keywords: BMI, breastfeeding, human milk oligosaccharides, microbiome, obesity

Early Childhood Obesity: No Small Problem

Childhood obesity is a global issue with pervasive, long-lasting consequences for individual and societal health. Approximately 35% of children in a cross-sectional US study were overweight in 2015–2016, and 18.5% were obese, with older children more likely to be obese (1). In New Zealand, 1 in 3 preschool children are overweight and/or obese (2). Children with obesity experience a greater burden of health and psychological problems than their peers. These include cardiometabolic disorders (e.g. hypertension, impaired glucose tolerance, hyperinsulinism, and abnormal liver function) and a range of dysfunctions affecting mood, behavior, and sleep (3). Furthermore, being overweight as a child more than doubles the likelihood of being overweight or obese as an adult (4), and is associated with an increased risk of cardiometabolic morbidity and mortality later in life (5).

Adult obesity often has its origins in early life. Infants who experience rapid weight gain are approximately 4 times more likely to be overweight or obese in childhood, and twice as likely to be overweight or obese as adults, especially when the weight gain occurs in the first year of life (6). Obesity is notoriously difficult to treat once established; although estimates of magnitude vary considerably between studies, as many as 83% of overweight children become obese adults (7). As such, early prevention of obesity has become the focus of public health efforts (8).

Is Breastfeeding Protective against Childhood Obesity?

The short-term benefits of breastfeeding for infant mortality and morbidity are undeniable (9). Meta-analyses have also

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Abbreviations used: BMO, bovine milk oligosaccharide; HMO, human milk oligosaccharide; LNFP-I, lacto-*N*-fucopentaose 1; LNFP-II, lacto-*N*-fucopentaose 2; 2′FL, 2′-fucosyllactose.

demonstrated associations between breastfeeding and a wide range of beneficial longer-term outcomes. These include lower risk of type 2 diabetes (10), life-threatening or common childhood infections (11, 12), leukemia (13), and allergic disease (14), as well as better cognitive function (15), dental alignment and health (16, 17), and also a lower risk of ovarian or breast carcinoma and type 2 diabetes for breastfeeding mothers (18).

The role that dietary intake in the first months of life might have in the development of later obesity is more equivocal. A number of studies have suggested that breastfeeding is associated with a reduction in the risk of obesity later in life. A meta-analysis reported that children who were breastfed as infants were 22% less likely to be obese, with a longer duration of breastfeeding providing greater protection against childhood obesity (19). The reduced risk of later overweight and childhood obesity appears to be generally consistent yet modest among studies. Additionally, a range of factors contribute to the development of obesity in childhood, which are often intersecting, including complex associations between breastfeeding and later feeding preferences. Furthermore, the authors of a meta-analysis have suggested that there is some publication bias involving the reports of breastfeeding studies (20).

Limiting the inclusion in meta-analyses to studies that tightly controlled for confounding variables, estimates of the effect of breastfeeding on subsequent obesity risk are more conservative. Whereas a 2015 meta-analysis reported that children who were breastfed were 26% less likely to be overweight or obese than those who were formula fed, this effect halved to 13% when only high-quality studies were considered (10). Moreover, because of the ethical limitations of randomizing infant feeding practices, these conclusions rely on observational data. Nonetheless, a study that randomly allocated parents to usual care compared with promotion of breastfeeding did not find any effect of increased breastfeeding uptake on offspring adiposity at age 6.5 y (21). However, that study had several limitations, including a large overlap in feeding practices between the control and intervention group, so the results should not be considered conclusive.

The current evidence derived from observational data makes it difficult to estimate the potential protective effect of breast milk against childhood obesity. Overall, available data still indicate that reduction in obesity risk can be considered among the long-term benefits of breastfeeding.

What Makes Human Milk Special?

Human milk has evolved as a very complex and highly variable fluid to supply infants with the nutrients necessary for their development, as well as containing bioactive molecules with some antimicrobial and immunomodulatory properties. Human milk composition can be affected by several factors, including the stage of lactation, maternal nutrition, and the health status of mother and infant (22–24). Thus, it is widely thought that human milk is tailored by the mother to fit the requirements of her infant (25). Human milk oligosaccharides (HMOs) are a group of complex sugars that are unique to human milk. HMOs are the third most abundant solid component of human milk, and are more abundant and more variable than oligosaccharides found in the milk of other mammals (26, 27). All HMOs comprise lactose attached to a simple or branching monosaccharide chain; they can be fucosylated or sialylated through the addition of a fucose or sialic acid molecule, respectively, and can be neutral or acidic (28, 29). Whereas >200 different HMOs have been identified, 20 of these account for >90% of HMOs in human milk (30). Notably, HMOs are not believed to have nutritional value and are poorly digested by infants. Instead, they pass unchanged through the infant's upper gastrointestinal tract and begin to be metabolized by the developing infant microbiome in the lower gastrointestinal tract (28, 31).

Some of the benefits of human milk (compared with formula) are thought to be produced by HMOs. Whereas oligosaccharides appear to be present in the milk of all placental mammals, the milk from other animals commonly used in infant formula contains negligible amounts of oligosaccharides, which also lack the complex structures of HMOs (32). Soy formula accounts for ∼10% of the infant formula market in New Zealand (33), and contains only very small quantities of oligosaccharides. Importantly, oligosaccharides are sometimes removed from the product altogether due to their potential to cause flatulence and diarrhea in susceptible infants (33). HMOs are not the only constituents of human milk that differentiate it from bovine- or soy-based formula alternatives: leptin, insulin-like growth factor I, fatty acids, protein, and various micronutrients are among many substances that have been studied for their potential role in infant growth, but data are scarce at present (34).

The quantity and composition of HMOs in human milk are highly variable among lactating mothers and over time. In general, colostrum contains 20–25 g/L of HMOs, whereas breast milk produced during days 5 to 90 of lactation contains 5–20 g/L (35). There is geographical variation in which HMOs are most abundant in breast milk, but the mean total concentration of HMOs in breast milk is similar between many countries (36). However, the total concentration of HMOs can vary almost 4-fold between individual mothers at the same stage of lactation (30).

Individual variations in structure and abundance of some HMOs are thought to be largely genetic, but the magnitude of environmental effects has not been fully explored. In particular, enzymes that catalyze HMO fucosylation are differentially expressed on the basis of secretor and Lewis blood group genes (28). Around 80% of Caucasians express fucosyltransferase 2 and are described as "secretors," whereas nonsecretor status is more common among African or African-American populations (37, 38). The HMO profile of secretor milk is dominated by α1–2 fucosylated HMOs, primarily 2′-fucosyllactose (2′FL) with smaller amounts of lacto-*N*-fucopentaose 1 (LNFP-I) and difucosyllactose (39). Nonsecretors tend to have a lower proportion of fucosylated

HMOs and lower total HMO concentration (37). However, the reported magnitude of this difference ranges from almost negligible to as much as 30% (40). It should also be noted that both the range of HMOs measured and the analytical methods used varied between studies, which could have led to the contrasting observations. Moreover, 2 studies have reported variations in HMO composition in association with a range of factors, such as parity, geographical location, season, and introduction of formula or solid foods to the infant's diet (30), as well as maternal BMI (41). A recent international study of HMO profiles found differences in HMO composition based on geographic location but only modest contributions of BMI, parity, and maternal age to HMO profiles (36).

How Could HMOs Be Related to Obesity?

The role that HMOs might play in infant growth is a relatively new field with a paucity of published data. A few animal models have provided some insight into possible effects of HMOs in humans. Dietary supplementation with bovine milk oligosaccharides (BMOs) reduced weight gain and adiposity in mice fed a high-fat diet (42). In contrast, sialylated BMOs increased body weight and lean body mass gain in mice and piglets, but these animals were reared on a Malawian diet that was linked to stunting in human infants (43). However, there is some evidence that HMOs are digested in the small intestine in rats, whereas this is not thought to take place in humans (44). Thus, HMOs are likely to be digested and metabolized differently in animals than in humans, so it is unclear whether any observed effects of HMOs in animal models can be extrapolated to humans (44).

Charbonneau et al. (43) reported that human milk from Malawian mothers with stunted infants had lower concentrations of total, sialylated, and fucosylated HMOs compared with the milk of Malawian mothers of infants with healthy growth at 6 mo of age. Interestingly, comparing the HMO abundance only among secretor mothers revealed no differences between the 2 groups; in contrast, there were more pronounced differences in nonsecretor mothers, with 2′FL and sialyllacto-*N*-tetraose b being the most discriminatory HMOs (43). These results support the authors' suggestion that a certain amount of both fucosylated and sialylated HMOs could be beneficial for infant growth when adequate nutrition is not readily available, and that composition of breast milk could be particularly important for nonsecretors who produce lower amounts of fucosylated HMOs. However, because milk samples were only collected at 6 mo postpartum, it is not known whether the differences in HMO content of milk preceded differences in growth. In addition, maternal nutrition was not assessed in the study as a possible confounder in these associations.

To our knowledge, only 3 other human studies have investigated the associations between HMOs and infant growth, weight, or fat mass. In Singapore, Sprenger et al. (39) found no differences in weight and height velocities across the first 120 d of life between 34 infants consuming milk with high concentrations of 2′FL and 16 with low concentrations. In contrast, Alderete et al. (45) and Davis et al. (46) described complex associations between the composition of HMOs in milk and the infants' weight and fat mass. Alderete et al. reported that a balanced and diverse composition of HMOs at 1 mo predicted lower total body fat percentage more strongly than maternal prepregnancy BMI in 25 infants. A higher milk LNFP-I content at 1 mo predicted lower infant weight and fat mass, whereas higher dia-lacto-*N*-tetraose content at 6 mo was associated with greater fat mass. However, there appeared to be paradoxical effects of lacto-*N*-fucopentaose 2 (LNFP-II) content on infant adiposity. Whereas greater LNFP-II concentrations at 1 mo were associated with lower infant weight and fat mass at 6 mo, higher LNFP-II concentrations at 6 mo were conversely associated with greater adiposity at that age. Davis et al. (46) examined 33 mother-infant dyads in The Gambia, but in contrast to Alderete et al., fucosylated HMOs did not predict weight-for-age *z*-score at 20 wk. Instead, the sialylated HMO 3′-sialyllactose was a positive predictor and sialyllacto-*N*-neotetraose a negative predictor of weight-for-age *z*-score (46).

These studies all reported data for a relatively small number of infants, but overall provide compelling evidence that HMOs play some role in growth that could differ between populations or even individuals. The participants in the studies of both Alderete et al. (45) and Davis et al. (46) had different overall HMO profiles, likely due to population differences in secretor and Lewis blood group prevalences between West Africa and the United States (37). This could explain the lack of consensus between the 2 studies as to which HMOs might modulate growth. Sprenger et al. (39) analyzed only 5 HMOs and assessed the effect of secretor status (rather than individual HMOs) on infant growth status by using 2′FL content of milk as a proxy. It is possible that in nonsecretor women, different HMOs fulfill the same role that fucosylated HMOs otherwise would in a secretor woman. Taking into account the findings of Charbonneau et al. (43), it seems that HMO composition within these groups could have more influence on infant growth than secretor status alone. Considering the prominent differences observed in gut microbiome composition across geographical areas even during infancy (47), the results of previous studies could be better interpreted if the infants′ gut microbiome had also been analyzed.

It should be noted that none of the studies discussed above accounted for the fat, protein, and micronutrient content of the breast milk that infants consumed, nor their total caloric intake, which are important factors for infant growth. To our knowledge, no published data have directly compared the fat and protein composition of breast milk with its HMO profile or the mother′s secretor status. Obtaining this information alongside infant growth data could help clarify the potential contribution of HMOs to infant growth. However, there are many challenges in measuring total milk consumption of breastfed infants, or accounting for the changes in breast-milk composition that occur from day to day and even across 1 feed (48).

Because of the complex and dynamic nature of human milk, infant formula supplementation with an individual or small number of HMOs has the potential to clarify the role of HMOs in isolation from other human milk components. As formula supplementation with HMOs is a relatively recent innovation, few studies have adopted this approach. So far, comparisons between nonsupplemented infant formula and formula supplemented with 2′FL and lacto-*N*-neotetraose have not revealed any differences in infant growth (49, 50). However, both studies had a relatively short follow-up period (4 mo), they used supplemented formulas that still contained a lower content of 2′FL (1 g/L) and markedly lower content of total HMOs than would be expected in typical mature human milk, and also did not assess the infants′ body composition (35, 49, 50). In addition, galacto-oligosaccharides, which have been associated with changes to the gut microbiome when added to infant formula (51), were included in both control and experimental formulas in much higher amounts than 2′FL in 1 study (50). The potential for randomized studies of HMOs using supplemented formula has yet to be realized, but as the safety of supplementation is established this will be a useful tool for testing hypotheses based on data obtained from studies comparing HMO content of breast milk with infant growth.

What Do We Know about HMOs?

HMOs have been described as the first prebiotics (31). They provide a substrate for bacterial growth in the neonate′s gastrointestinal tract that is thought to favor beneficial bacterial taxa (e.g., *Bacteroidaceae* and *Bifidobacteriaceae*) (28, 52). Accordingly, the fecal microbiota of breastfed infants differs from that of infants who are formula fed, with a higher relative abundance of *Bifidobacteriaceae* and fewer *Firmicutes* and *Bacteroidaceae*, as well as an apparent resistance to the loss of microbial diversity associated with diarrheal episodes (53). HMO metabolism by bacteria is structure specific (28, 52), so the HMO composition of breast milk is thought to correspondingly influence the bacterial composition of the infant′s microbiome. An in vitro study demonstrated that many, but not all, *Bifidobacterium* spp. and *Bacteroides* spp. from infant stool cultures grew well in the presence of fucosylated HMOs including 2′FL, but fewer species grew in the presence of sialylated HMOs (54). Research of this theory in vivo is limited but there is some evidence that the infant fecal microbiome is influenced by the HMOs in the infant's diet. For example, in 1 study LNFP-I in milk consumed by infants—previously described to be associated with lower infant weight and fat mass in a recent study (45)—was associated with increased *Bifidobacterium* spp. in the fecal microbiota, whereas 2′FL was negatively associated with abundance of this bacterial genus (26).

A comprehensive review of the role of HMOs in immune development is beyond the scope of this article, but in short, HMOs are thought to promote immune function through interactions with epithelial and immune cells, alteration of nasopharyngeal microbiota, and direct interactions with pathogens (55–57). 2′FL in particular has been associated with both lower numbers of inflammatory markers and fewer episodes of diarrheal illness in infants (57, 58). As indirect evidence, antibiotic drugs are used less frequently in children who have been breastfed (9), and the antibiotic properties of HMOs might partially explain this association (28). Antibiotics appear to disrupt the microbiome, and have been demonstrated to contribute to obesity in animal models (59). In humans, antibiotic use (particularly in the first 6 mo of life) is associated with a modest increase in the risk of childhood obesity (60). However, there are still limited data to ascertain whether this association is mediated by effects on the microbiome, or confounded by differences between infants who require antibiotics in early life and those who do not (60).

It is widely theorized that associations between HMOs and future obesity risk are mediated by the effect of the former on the infant microbiome. Differences in microbiome have been described between obese and lean subjects (59, 61). Such changes to the bacterial population of the lower gastrointestinal tract can impact on the amount of energy harvested from food (59, 61). As previously mentioned, infants who are not exclusively breastfed have a microbiome populated with a relatively higher proportion of *Firmicutes* than exclusively breastfed infants (26, 53). A higher ratio of *Firmicutes* to *Bacteroidetes* has been associated with obesity in several animal studies (62), and observed in obese adults and children, though not universally (61, 63). The microbiome changes with BMI, and there is evidence that a change in microbiome can precede weight gain in mice, where animals gained weight more rapidly after receiving a fecal transfer from an overweight human donor (64). Stanislawski et al. (65) provided further evidence that the microbiome could influence weight gain in a Norwegian birth cohort. Adjusting for potential confounding variables, the gut microbiome of 165 infants at ages as young as 10 d predicted their BMI at age 12 y (65). The association with future BMI was stronger for the microbiome at age 2 y, whereas BMI *z*-scores at the same age were not significantly higher in children who later became overweight or obese (65). To our knowledge, no studies in humans have simultaneously examined data on HMO intake, microbiome, and growth and/or weight gain. However, Charbonneau et al. (43) reported that the increase in weight and lean body mass, as well as the alterations in metabolic parameters, in mice and piglets fed sialylated BMOs were associated with changes in their gut microbiome. The body mass differences between BMO-treated mice and control mice were not attributable to differences in food consumption, so might have been mediated by a more efficient utilization of nutrients for anabolic functions (43).

Conclusions

The potential effects of HMOs on growth and body composition are still being explored. Animal models of the potential biological effects of HMOs might be limited in their translation to humans due to differences in sugar metabolism between species (44), but can provide some insights into the

mechanisms underlying the potential associations between HMOs and growth. Nonetheless, from the limited number of small studies conducted to date, there is currently no consistent strong evidence for the effects of HMOs on growth and adiposity. In particular, data from large studies or long-term follow-up are not yet available, nor is information about how HMO content of milk might affect its overall composition and nutritional value. As we better understand the biological actions of specific nutrients in breast milk, it might be possible to identify the long-term health benefits of its individual components such as HMOs.

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