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Human milk oligosaccharides and Hispanic infant weight gain in the first 6 months

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Abstract

Objective: The aim of this study was to determine whether human milk oligosaccharides (HMOs) at 1 month predicted infant weight gain at 6 months, and if associations varied by HMO secretor status.

Methods: Participants were 157 Hispanic mother-infant pairs. Human milk samples were collected at 1 month. Nineteen individual HMOs were analyzed using high-performance liquid chromatography, and secretor status was determined by the presence of 2'-fucosyllactose or lacto-N-fucopentaose (LNFP) I. Infant weight was measured at 1 and 6 months. Path analysis was used to test effects of HMO composition on infant weight gain, adjusting for maternal age, pre-pregnancy BMI, infant age, sex, and birthweight.

Results: In the total sample, higher LNFPII predicted lower infant weight gain ($g_f = -4.1$, $P = 0.004$); this was observed in both non-secretor ($g_f = -3.0$, $P = 0.006$) and secretor groups ($g_f = -4.7$, $P = 0.014$). In the non-secretor group, higher lacto-N-neotetraose (LNnT) ($g_f = 7.6$, $P = 0.011$) and disialyllacto-N-tetraose (DSLNT) ($g_f = 14.3$, $P = 0.002$) predicted higher infant weight gain. There were no other associations in the secretor group.

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PKB conceptualized the manuscript, analyzed and interpreted data, drafted the article, and revised the article critically for important intellectual content.

MIG and LB conceptualized and designed the study, coordinated and supervised acquisition of data, and revised the article critically for important intellectual content.

JHR analyzed and interpreted data and revised the article critically for important intellectual content.

JFP, RBJ, TLA, and CY coordinated acquisition of data, interpreted data, and revised the article critically for important intellectual content.

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Conclusions: Our data suggest that higher LNFPII in human milk may decrease obesity risk across all infants, whereas higher LNnT and DSLNT may increase obesity risk in infants of non-secretors only.

Keywords

Pediatrics; obesity; breastfeeding; oligosaccharides

Introduction

Breastfeeding, or human milk feeding, is the optimal source of infant nutrition, with a myriad of benefits to intestinal ^{1, 2}, immune ³, and cognitive development ⁴, as well as physical growth ^{5, 6}. Notably, human milk feeding promotion as a target for obesity prevention has received considerable attention and a great deal of study. Human milk feeding is known to temper weight gain compared to formula ^{2, 7}, and is most beneficial in the first 6 months. For example, higher infant weight gain within this window was associated with the highest prevalence of obesity in mid-childhood (i.e., up to 35%) ⁸. However, the extent to which human milk feeding may reduce infant obesity risk varies across studies. Differences may be attributed to a preponderance of data on the quantity (i.e., duration and frequency) rather than the quality (i.e., composition) of human milk in relation to infant weight gain. Because the composition of human milk is complex and highly variable ^{9, 10}, it is worthwhile to identify specific components that contribute to infant weight gain, which may improve our understanding of disparities in infant obesity risk and opportunities for intervention.

One component of human milk that warrants attention in relation to infant weight gain is a group of complex carbohydrates known as oligosaccharides. Animal studies provide evidence that exposure to oligosaccharides may diminish weight gain, adiposity, and caloric intake ¹¹. These studies posit that oligosaccharides are a source of prebiotics to flourish the gut microbiome, with several purported effects: in mice, bovine oligosaccharides diminished dysbiosis (imbalance) and inflammation in the immature intestine that perpetuates obesity development ¹¹. Oligosaccharides may also enhance fermentation of molecules that influence appetite regulation ^{12, 13}; in vitro, fucosylated oligosaccharides (e.g., 2'-fucosyllactose, 2'FL), lead to the production of lactate and short chain fatty acids that alter appetite signals. Though these mechanisms hold promise to link oligosaccharides with infant weight outcomes, this has not been examined in humans.

While oligosaccharides are the third most predominant component of human milk ⁹, not much is known about their possible associations with infant obesity risk. We conducted a small pilot study in non-Hispanic white mother-infant pairs (N=25), which revealed that individual fucosylated and sialylated oligosaccharides were related to infant adiposity at 6 months ¹⁴. This is in line with more recent findings in a separate but similar cohort and sample size (N=30) ¹⁵. To our knowledge, no prospective studies have been conducted to examine the extent to which oligosaccharides in early postpartum influence infant weight gain in later postpartum, nor have any assessed associations separately among secretors and non-secretors. For context, secretor status is genetically determined: mothers with an active

secretor locus that encodes for a functional fucosyltransferase 2 enzyme are secretors, and have milk with higher concentrations of fucosylated oligosaccharides than non-secretors^{16–18}. It is conceivable that this mother-to-mother variation could thereby influence infant outcomes.

Therefore, the primary aim of this study was to determine whether human milk oligosaccharides (HMOs) at 1 month predicted Hispanic infant weight gain at 6 months, and if associations varied by secretor status. Secretor status was confirmed through the presence or near absence of HMOs 2'FL or lacto-N-fucopentaose (LNFP) I^{19, 20}.

Methods

Subjects

Participants in this study were 157 mother-infant pairs recruited from maternity clinics in Los Angeles County. The study was limited to mothers who self-identified as Hispanic: these mother-infant pairs tend to be most susceptible to obesity, and so there was the potential to gain insight into this racial/ethnic disparity²¹. As previously described in Berger et al.²², additional inclusion criteria were as follows: 1) 18 years old at delivery; 2) gave birth to a healthy, full-term, singleton newborn; 3) enrolled within 1 month postpartum; 4) intended to breastfeed for 6 months postpartum; and 5) able to read English or Spanish at a 5th grade level to understand procedures²². Mother-infant pairs were excluded if they were taking medications or had a medical condition that could affect physical or mental health (i.e., diabetes mellitus, hypertension, thyroid disease, and eating disorders), nutritional status, or metabolism, used tobacco or recreational drugs, and had a clinical diagnosis of fetal abnormalities²². The Institutional Review Board of Children's Hospital Los Angeles at the University of Southern California approved all procedures. Participants provided written informed consent prior to data collection²².

Study design

As previously described in Berger et al.²², mother-infant dyads completed two visits at 1 and 6 months for the purposes of this study. At 1 month, historical health information was collected through self-reported questionnaires (i.e., family health history, maternal health history, maternal age, pre-pregnancy BMI, infant age, sex, birthweight, and human milk feedings per day). At 1 and 6 months, infant weight was measured following standard procedures²². Mothers were weighed with and without holding the infant on an electronic scale, and the difference in the mother's weight with and without holding the infant was calculated and recorded²². The average of two measurements was recorded and also used to determine weight-for-age z scores (WAZ) based on WHO standards^{22, 23}.

Human milk collection and HMO analysis

At 1 month, human milk was collected and analyzed following standard procedures^{20, 24, 25}. Mothers were instructed to refrain from eating for 1 hour and feeding and/or pumping human milk for 1.5 hours beforehand. Mothers were encouraged to pump the entire contents of a single breast expression using an electric breast pump to ensure the collection of fore, mid, and hind milk. Collection was standardized from the right breast, unless mothers could

only provide a sample from the left breast. Approximately 20 to 50 mL of human milk was collected during the visit. Aliquots were stored at -80°C until HMO analysis at the University of California San Diego.

Raffinose was added to each sample as an internal standard for absolute quantification. HMOs were isolated with high-throughput solid-phase extraction, fluorescently labeled, and measured using high-performance liquid chromatography²⁰. Nineteen HMOs were quantified based on standard retention times and mass spectrometric analysis. These individual HMOs account for >90% of total HMO composition, and include the following: 2'FL, 3-fucosyllactose (3FL), 3'-sialyllactose (3'SL), 6'-sialyllactose (6'SL), difucosyllactose (DFLac), lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), LNFPI, LNFPII, LNFPIII, sialyl-LNT (LST) b, LSTc, difucosyl-LNT (DFLNT), disialyllacto-N-tetraose (DSLNT), lacto-N-hexaose (LNH), fucosyl-LNH (FLNH), difucosyl-LNH (DFLNH), fucosyl-disialyl-LNH (FDSLNH), and disialyl-LNH (DSLNH).

Individual concentrations of HMOs were summed to estimate total concentration. To assess overall diversity of HMO composition, Simpson's Diversity index was calculated as the reciprocal sum of the square of the relative abundance of each HMO. To determine evenness of HMO composition, Simpson's Equitability was calculated by dividing the actual diversity index for each sample by the maximum diversity index, defined as the theoretical case that all measured HMOs have the same relative abundance¹⁴. As described earlier, secretor status was defined by the presence or near absence of 2'FL or LNFPI. This was used as a covariate in the path analysis model, and as a grouping variable in the multiple-group path analysis model²⁰.

Statistical analysis

Descriptive statistics are presented as mean $\pm$ standard deviation (SD) for continuous variables and as frequency (percentage) for categorical variables. Normal distribution and homogeneity of variances were confirmed by Shapiro–Wilks W and Levene's tests, respectively. Differences between HMO secretor status groups at 1 month (HMO non-secretors vs. secretors) were tested by analysis of variance for continuous variables and by Mantel–Haenszel linear-by-linear association chi-square tests for categorical variables.

To address the primary aim, the following path analysis models were applied: 1) a path analysis, in which the model considered HMO secretor status group as a covariate (Figure 1A); and 2) a multiple-group path analysis, in which the model considered HMO non-secretor vs. secretor groups (Figure 1B). In both analyses, the mean difference in infant weight was modeled with infant weight at 6 months as the outcome and infant weight at 1 month as a covariate. This method is equivalent to using a change score²⁶, and for this reason the outcome is herein referred to as 'infant weight change' or 'infant weight gain.' In addition, the same path analysis models were conducted for the mean difference in infant WAZ, which was also modeled with infant WAZ at 6 months as the outcome and infant WAZ at 1 month as a covariate. Infant weight change was the primary outcome for ease of interpretation, whereas infant WAZ change was a secondary outcome to demonstrate consistent findings across two measures that are widely reported in studies of infant growth^{15, 22, 27–29}.

Assumptions of path analysis modeling (i.e., normality, outliers, and multicollinearity) were checked and showed no violations. To evaluate the path analysis models, root mean square error of approximation (RMSEA), comparative fit index (CFI), and standardized root mean squared residual (SRMR) were computed and compared to a ‘good’ (i.e., RMSEA <0.60, CFI >0.95, and SRMR <0.80) or ‘acceptable’ (i.e., RMSEA <0.80 and CFI >0.90) range. We also determined R-squared values for infant weight at 1 and 6 months and concentrations of HMOs at 1 month for significant path coefficients. In the path analysis models, concentrations of HMOs were multiplied by 0.01 to scale as appropriate. The same analyses were conducted replacing infant weight at 6 months with WAZ at 6 months (i.e., to determine WAZ change) as the outcome (secondary outcome). All models were adjusted for the following covariates: maternal pre-pregnancy BMI, age at delivery, infant sex, age, and birthweight. Analyses were conducted with SPSS software (version 24; IBM SPSS Statistics, Chicago, IL) and Mplus software (version 8; Muthén & Muthén, Los Angeles, CA).

Results

Mothers were 28.7 ± 6.1 years old at delivery and had a pre-pregnancy BMI of 28.2 ± 5.7 kg/m² (32.0% normal weight, 33.2% overweight, 34.8% obese). Maternal reports indicated that 76.4% of infants received 7 or more human milk feedings per day, which falls within the recommended range of exclusive human milk feeding^{29, 30}. HMO analysis revealed that 86.0% of mothers were classified as HMO secretors, which falls within the reported range of prior studies (i.e., 51.0% to 89.1%)^{15, 20, 3118}. Characteristics of the mother-infant pairs grouped by HMO secretor status at 1 month are described in Table 1. Distribution of infant age, sex, birthweight, weight at 1 month, weight at 6 months, and change in weight at 6 months did not differ between groups. Concentrations of HMOs grouped by secretor status at 1 month are presented in Table 2. Almost all concentrations of HMOs were significantly different between non-secretors and secretors, except for 3FL, 3'SL, LNnT, LSTc, DSLNT, LNH, and FLNH.

Path analysis (Figure 1A) coefficients for the total sample are presented in Supplementary Table 1 for all HMOs, and in Table 3 for LNFPII only. Higher LNFPII at 1 month predicted lower infant weight gain at 6 months ($g_f = -4.1$, $P = 0.004$). The model fit indexes were RMSEA= 0.020, CFI= 0.998, and SRMR= 0.033, and indicated that the model met the ‘good’ range criteria. R-squared values for infant weight at 1 and 6 months and LNFPII at 1 month were 0.410, 0.384, and 0.351, respectively, and indicated that the variability in outcome and exposure variables were well explained by the model (41.0%, 37.8%, and 35.1%, respectively). Similar results were observed after substituting infant WAZ at 6 months as the outcome (i.e., to determine WAZ change).

Multiple-group path analysis (Figure 1B) coefficients revealed that higher LNFPII at 1 month predicted lower infant weight gain in the non-secretor group ($g_f = -3.0$, $P = 0.006$) and secretor group ($g_f = -4.7$, $P = 0.014$) (Figure 2). The model fit indexes were RMSEA= 0.079, CFI= 0.968, and SRMR= 0.062, and indicated that the model met the ‘good’ range criteria. R-squared values in the non-secretor group for infant weight at 1 and 6 months and LNFPII at 1 month were 0.537, 0.889, and 0.085, respectively. R-squared values in the secretor

group for infant weight at 1 and 6 months and LNFPII at 1 month were 0.398, 0.353, and 0.011, respectively. Analyses also revealed that higher LNnT ($g_f = 7.6$, $P = 0.011$) and DSLNT ($g_f = 14.3$, $P = 0.002$) at 1 month predicted higher infant weight gain in the non-secretor group only. R-squared values in the non-secretor group for LNnT and DSLNT at 1 month were 0.149 and 0.342, respectively. Similar results were observed after substituting infant WAZ at 6 months (i.e., to determine WAZ change) as the outcome.

Discussion

In this study of Hispanic mother-infant pairs, we found that individual HMOs in early postpartum influenced infant weight change in later postpartum, a pattern that has the potential to impact obesity and related co-morbidities that extend from childhood to adulthood^{8, 32}. Specifically, higher LNFPII at 1 month predicted lower infant weight gain at 6 months, regardless of HMO secretor status. In contrast, higher LNnT and DSLNT at 1 month predicted higher infant weight gain in non-secretors, but not in secretors. These findings provide evidence that specific human milk components impact infant weight trajectory, and may offer insight into disparities in obesity and metabolic abnormalities that may be, in part, attributed to secretor status of Hispanic mothers.

Though HMOs comprise a considerable fraction of carbohydrates in human milk^{33, 34}, there is a lack of human data on HMOs in relation to infant obesity risk. As mentioned, this gap was first addressed through our proof-of-concept study in non-Hispanic white participants at 1 and 6 months¹⁴. In this small cohort ($N=25$), we found only one prospective association between HMOs and change in infant body composition: higher LNFPII at 1 month predicted lower infant fat mass at 6 months¹⁴. This initial finding lends support to our current finding in a separate and much larger cohort, as we also observed that higher LNFPII at 1 month predicted lower infant weight gain at 6 months in a sample of Hispanic participants. It is important to note that this was the only finding that was consistent across studies: because the present study found this association regardless of secretor status, it follows that the prior study also made this observation without having separated the results by secretor status.

Indeed, the link between higher LNFPII and lower infant weight gain has potential clinical significance for several reasons. First, there are certain HMOs that have received a modicum of study in infants (e.g., DSLNT, LNnT)^{15, 35, 36}: LNFPII, however, has not been one of them. Second, there are several HMOs that differ by maternal factors: while studies have shown that modifiable factors were largely unassociated with individual HMOs (e.g., maternal pre-pregnancy BMI), non-modifiable factors tended to be associated with individual concentrations (e.g., HMO secretor status, maternal age), which included LNFPII²⁰. However, we found that the influence of higher LNFPII was evident across all infants in our cohort, which would indicate that the concentration of LNFPII was adequate regardless of HMO secretor status. It is unclear, however, how this could change over the course of lactation to influence infant weight trajectory^{14, 20}. What is clear from our findings is that greater exposure to LNFPII at 1 month conferred benefits to our cohort of infants at 6 months, a critical period of postnatal growth and development⁸.

While higher LNFPII was protective in all infants, we also found that higher DSLNT and LNnT at 1 month were predictive of higher infant weight gain in non-secretors only. This was not expected because DSLNT and LNnT did not differ by secretor status, and smaller studies have reported discrepant findings from ours in relation to infant weight gain offer some insight into our observations. First, low birthweight infants fed DSLNT had a reduced risk of necrotizing enterocolitis^{1, 18}, a condition in the gut that decreases tolerance to feedings³⁷. It stands to reason that higher DSLNT may increase tolerance to feedings and, in turn, infant weight gain. Second, normal birthweight infants fed LNnT (with 2'FL, produced in secretors only) had similar weight gain compared to controls³⁵. This may indicate that other variations in HMOs offset the influence of DSLNT and LNnT on infant weight gain in secretors compared to non-secretors²⁰. Though there were no differences in infant weight status at 6 months in our cohort, this may emerge over time and should be monitored. It is also worth noting that we had a very small sample of non-secretors (n= 22), which could have contributed to our outcomes.

HMOs may influence infant weight change through multiple means³⁸. HMOs have garnered the most attention, however, as a source of prebiotics to flourish the gut microbiome. This may influence infant weight gain through a number of mechanisms. First, HMOs can decrease (or increase) gut dysbiosis and inflammation³⁹; this could influence fat tissue formation. Second, HMOs can maximize (or minimize) production of metabolites that function in energy balance to influence infant weight change: this includes lactate and short chain fatty acids that alter appetite signals^{13, 40}. Moreover, HMOs can decrease (or increase) proliferation of the gut epithelium to function in digestion, absorption, and metabolism. In vitro, LNFPII (i.e., fucosylated HMO) increased apoptosis, whereas DSLNT (i.e., sialylated HMO) increased differentiation of gut epithelial cells that mimicked the infant intestine⁴¹. This aligns with our understanding that HMOs may be separated into fractions (e.g., fucosylated vs. sialylated) defined by the presence of residues that dictate distinct functions⁴². Future reports from this cohort will assess the influence of LNFPII and individual HMOs on infant weight gain in combination with influences of the gut microbiome.

This study is not without limitations. The prospective observational design cannot be used to establish causality. Historical health information (e.g., maternal pre-pregnancy BMI, infant birthweight, human milk feedings per day) was collected through self-report, which is subject to bias. Analyses were done with each HMO, which may not capture shared information or synergy among HMOs. Infant body composition was not assessed in all infants in our cohort, which would have bolstered our findings with infant anthropometry. In addition, findings are limited to a relatively homogenous sample of Hispanic mothers and infants in the Southwestern United States: therefore, differences in socioeconomic status, built environment, and food choice of our study population may limit generalizability of our findings, as well as HMO profile, which has been shown to vary by geographic location⁴³.

Conclusion

In conclusion, findings from this study revealed that higher LNFPII in human milk may decrease weight gain across all infants in the first 6 months, whereas higher DSLNT and

LNnT may increase weight gain in infants of non-secretors only. These findings provide evidence that specific HMOs contribute to infant weight trajectory, and may help explain disparities in the influence of human milk feeding on infant weight gain as a function of HMO composition and secretor status. This information may guide interventions to optimize infant exposure to individual HMOs for healthy growth and development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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What is known about this subject?

- The benefits of human milk feeding on infant obesity risk are well-known, yet the extent to which human milk is protective varies across studies.
- Recently, human milk oligosaccharides (HMOs) have received attention as a mechanism to influence infant outcomes, including weight gain.

What are the new findings in this manuscript?

- This is the first study to report that HMO lacto-N-fucopentaose (LNFP) II at 1 month may be protective against infant weight gain over 6 months, regardless of HMO secretor status.
- This suggests that early exposure to LNFP II could be a critical temporal window to offset early obesity risk.

How might the results change the direction of research or focus of clinical practice?

- Findings may lend support for programs on the initiation, duration, and exclusivity of human milk feeding, and inform the design and formulation of supplements.
- This has the potential to optimize early infant growth and minimize later obesity risk.

Figure 1. Path analysis models to examine effects of HMOs at 1 month on infant weight change. Path analysis model in total sample, with secretor status as a covariate (A). Multiple-group path analysis model, with secretor status as a grouping variable for non-secretors vs. secretors (B).

Figure 2. HMO LNFPII at 1 month related to infant weight change in non-secretors vs. secretors. Associations between LNFPII at 1 month and infant weight change were examined using path analysis models, with infant weight at 6 months as the outcome and infant weight at 1 month as a covariate. Models were also adjusted for maternal age, pre-pregnancy BMI, infant age, sex, and birthweight.

Table 1.Characteristics of Hispanic mother-infant pairs.^{ab}

	Total	Non-secretor	Secretor	<i>p</i> ^c
<i>n</i>	157	22	135	
Mothers				
Age at delivery (years)	28.7 ± 6.1	28.8 ± 5.5	28.7 ± 6.2	0.94
BMI, pre-pregnancy (kg/m ²)	28.2 ± 5.7	28.0 ± 4.9	28.2 ± 5.8	0.89
Caesarean delivery (%) ^d	25.6	27.3	25.4	0.85
Infants				
Female (%) ^d	53.2	59.1	52.2	0.55
Age (days)	32.5 ± 4.5	32.5 ± 4.1	32.6 ± 4.6	0.93
Birthweight (kg)	3.39 ± 0.4	3.33 ± 0.5	3.40 ± 0.4	0.48
Human milk feedings per day, 1 month (%) ^d				0.77
6 per day	23.6	18.2	24.4	
7 per day	76.4	81.8	75.6	
Weight, 1 month (kg)	4.59 ± 0.5	4.58 ± 0.6	4.59 ± 0.5	0.89
Weight, 6 months (kg)	8.02 ± 0.8	8.11 ± 0.8	8.02 ± 0.8	0.68
Change in weight, 1–6 months (kg)	3.44 ± 0.7	3.62 ± 0.5	3.41 ± 0.7	0.26
WAZ, 1 month	0.42 ± 0.8	0.41 ± 0.9	0.42 ± 0.8	0.98
WAZ, 6 months	0.44 ± 0.8	0.56 ± 0.8	0.42 ± 0.8	0.51
Change in WAZ, 1–6 months	0.02 ± 0.8	0.30 ± 0.7	−0.02 ± 0.8	0.15

Abbreviations: BMI, body mass index; WAZ, weight-for-age z score.

^aValues are mean ± SD or %.^bSecretor status defined by presence or absence of 2'-fucosyllactose or lacto-N-fucopentaose.^cTests of significance between groups were calculated with ANOVA.^dTests of significance between groups were based on chi-square test.

Table 2.Concentrations of HMOs (ug/mL) at 1 month.^a

	Total (n=157)	Non-Secretors (n=22)	Secretors (n=135)	<i>P</i> ^b
2'FL	2716 ± 1579	46.6 ± 69.9	3155 ± 1238	<0.01
3FL	201.7 ± 172	244.5 ± 386	194.6 ± 103	0.21
3'SL	355.6 ± 357	295.1 ± 246	365.5 ± 372	0.39
6'SL	625.1 ± 209	729.4 ± 249	607.9 ± 198	0.01
DFLac	279.7 ± 217	28.2 ± 33.8	321.0 ± 206	<0.01
LNT	806.4 ± 516	1068 ± 808	763.5 ± 440	0.01
LNnT	440.8 ± 253	388.5 ± 378	449.4 ± 227	0.30
LNFP I	1498 ± 957	392.8 ± 636	1680 ± 876	<0.01
LNFP II	873.0 ± 565	1669 ± 874	742.2 ± 359	<0.01
LNFP III	60.1 ± 36.1	86.2 ± 38.9	55.8 ± 33.9	<0.01
LSTb	89.1 ± 57.0	126.9 ± 58.7	82.8 ± 54.5	<0.01
LSTc	318.2 ± 140	263.6 ± 107	327.2 ± 144	0.05
DFLNT	1481 ± 754	1072 ± 704	1548 ± 743	0.01
DSLNT	477.1 ± 228	454.9 ± 240	480.7 ± 226	0.62
LNH	90.1 ± 57.1	97.3 ± 69.4	89.0 ± 55.1	0.53
FLNH	147.8 ± 92.8	162.4 ± 115	145.5 ± 89.0	0.43
DFLNH	229.2 ± 196	100.9 ± 129	250.3 ± 197	<0.01
FDSLNH	315.1 ± 387	915.7 ± 671	216.4 ± 185	<0.01
DSLNH	436.5 ± 185	566.6 ± 213	415.2 ± 172	<0.01
Sum	11441 ± 1356	8709 ± 1158	11889 ± 702	<0.01
Diversity	5.50 ± 1.9	6.88 ± 1.7	5.23 ± 1.8	<0.01
Evenness	0.29 ± 0.1	0.36 ± 0.1	0.28 ± 0.1	<0.01

Abbreviations: 2'-fucosyllactose (2'FL), 3-fucosyllactose (3FL), 3'-sialyllactose (3'SL), 6'-sialyllactose (6'SL), difucosyllactose (DFLac), human milk oligosaccharide (HMO), lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), lacto-N-fucopentaose (LNFP) I, LNFP II, LNFP III, sialyl-LNT (LST) b, LSTc, difucosyl-LNT (DFLNT), disialyl-LNT (DSLNT), lacto-N-hexaose (LNH), fucosyl-LNH (FLNH), difucosyl-LNH (DFLNH), fucosyl-disialyl-LNH (FDSLNH), disialyl-LNH (DSLNH).

^aValues are mean ± SD.

^bTests of significance between groups were calculated with ANOVA (P<0.05).

Table 3.

Path analysis model of HMO LNFPII at 1 month on infant weight change in total sample of participants.

	Estimate	SE	P	R-squared
HMO LNFPII at 1 month on				0.351
Maternal age at delivery	0.068	0.060	0.252	
Maternal pre-pregnancy BMI	−3.574	2.075	0.085	
Maternal HMO secretor status	−16.513	3.922	0.000	
Infant weight at 1 month on				0.410
Maternal HMO secretor status	−0.040	0.088	0.647	
Infant birthweight	0.695	0.074	0.000	
Infant sex	0.194	0.063	0.002	
Infant age	0.019	0.007	0.007	
Infant weight at 6 months on				0.384
HMO LNFPII at 1 month *	−0.041	0.015	0.004	
Maternal HMO secretor status	−0.499	0.217	0.021	
Maternal age at delivery	−0.008	0.010	0.419	
Maternal pre-pregnancy BMI	0.152	0.130	0.243	
Infant weight at 1 month	0.830	0.162	0.000	
Infant birthweight	0.095	0.183	0.605	
Infant sex	0.215	0.129	0.097	
Infant age	−0.009	0.014	0.550	

Abbreviations: human milk oligosaccharide (HMO), lacto-N-fucopentaose (LNFPII) II

Note:

* indicates the rescale of HMO LNFPII by multiplying 0.01. Thus, it should be interpreted with the original scale (i.e., −4.1).