Associations between human milk oligosaccharides and infant body composition in the first 6 mo of life[1,2]

Tanya L Alderete,[3] Chloe Autran,[4] Benjamin E Brekke,[3] Rob Knight,[4,5] Lars Bode,[4] Michael I Goran,[3] and David A Fields[6]*

[3]Department of Preventive Medicine, University of Southern California, Los Angeles, CA; Departments of [4]Pediatrics and [5]Computer Science and Engineering, University of California, San Diego, CA; and [6]Department of Pediatrics, University of Oklahoma Health Sciences Center and Children's Medical Research Institute Metabolic Research Program, Oklahoma City, OK

ABSTRACT

Background: Evidence linking breastfeeding to reduced risk of developing childhood obesity is inconclusive, yet previous studies have not considered variation in specific components of breast milk that may affect early development.

Objective: We examined whether differences in the composition of human milk oligosaccharides (HMOs) correlate with infant growth and body composition at 1 and 6 mo of age.

Design: Twenty-five mother-infant dyads were recruited from the University Hospital at the University of Oklahoma Health Sciences Center. Infants were breastfed for 6 mo. Breast-milk and infant measures were obtained at 1 and 6 mo of infant age. HMO composition was analyzed by high-pressure liquid chromatography, and infant growth (length and weight) and body composition (percentage fat, total fat, lean mass) were measured by dual-energy X-ray absorptiometry. Relations between HMOs and infant growth and body composition were examined by using multiple linear regression. A priori covariates included maternal prepregnancy body mass index, pregnancy weight gain, and infant age and sex.

Results: Higher HMO diversity and evenness at 1 mo were associated with lower total and percentage fat mass at 1 mo. At 1 mo, each 1-μg/mL increase in lacto-N-fucopentaose (LNFP) I was associated with a 0.40-kg lower infant weight ($P = 0.03$). At 6 mo, each 1-μg/mL increase in LNFPI was associated with a 1.11-kg lower weight ($P = 0.03$) and a 0.85-g lower lean mass ($P = 0.01$). At 6 mo, each 1-μg/mL increase in LNFPI was associated with a 0.79-g lower fat mass ($P = 0.02$), whereas disialyl-lacto-N-tetraose and LNFPII were associated with a 1.92-g ($P = 0.02$) and 0.42-g ($P = 0.02$) greater fat mass, respectively. At 6 mo, each 1-μg/mL increase in fucosyl-disialyl-lacto-N-hexaose and lacto-N-neotetraose was associated with 0.04% higher ($P = 0.03$) and 0.03% lower ($P < 0.01$) body fat, respectively.

Conclusion: These findings support the hypothesis that differences in HMO composition in mother's milk are associated with infant growth and body composition. This trial was registered at clinicaltrials.gov as NCT02535637. *Am J Clin Nutr* 2015;102:1381–8.

Keywords: HMOs, LNFPI, human milk oligosaccharides, infant body composition, microbiome

INTRODUCTION

Childhood overweight and obesity rates have increased dramatically over the past 3 decades and remain high, with a prevalence of nearly 23% in children 2–5 y of age (1). Once established, obesity and its health consequences are difficult to treat and often persist into adulthood (2–4). Therefore, it is critical to identify factors in early childhood that increase or mitigate the risk of excessive weight gain and obesity to help develop early interventions.

Multiple studies have shown that breastfeeding decreases the risk of developing obesity in childhood and adolescence (5–10). However, not all studies support this view (11–14), and a comprehensive systematic review of 71 articles found only a modest protective effect of breastfeeding on obesity prevention in which breastfeeding reduced the prevalence of overweight and obesity by ~10% (15). These conflicting findings may be partially due to the diverse composition of human breast milk, which contains macronutrients, micronutrients, and a host of other bioactive compounds (cytokines, adipokines, chemokines, cofactors) that are only now being discovered and studied and that vary both among women and over time. Human milk contains high concentrations of unconjugated glycans, complex sugars called human milk oligosaccharides (HMOs),[7] that are currently not present in infant formula (16, 17). HMOs carry lactose at their reducing end that can be elongated by disaccharides of galactose and N-acetylglucosamine and modified by fucose and/or sialic acid (17). More than 150 structurally distinct HMOs have been identified so far, and many of their biological effects are highly structure specific (16).

Once ingested, HMOs resist degradation in the small intestine and persist in the colon, where different HMOs serve as specific metabolic substrates for certain bacteria and help shape the infant

[1] Mead Johnson Nutrition provided financial support for the milk collection.

[2] Preliminary data in 19 subjects irrespective of maternal BMI from this study were published previously [Fields DA, Demerath EW. Relationship of insulin, glucose, leptin, IL-6 and TNF-alpha in human breast milk with infant growth and body composition. Pediatr Obes 2012;7(4):304–12].

*To whom correspondence should be addressed. E-mail: goran@usc.edu.

[7] Abbreviations used: DSLNT, disialyl-lacto-N-tetraose; FDSLNH, fucosyl-disialyl-lacto-N-hexaose; HMO, human milk oligosaccharide; LNFP, lacto-N-fucopentaose; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; LST, sialyl-lacto-N-tetraose; 2′-FL, 2′-fucosyllactose; 3′-SL, 3′-sialyllactose.

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gut microbiome. For example, HMOs and their fucosylated components support increased bifidobacteria, which dominate the microbiota of breastfed infants (18–21). The gut microbiome has been associated with overweight and obesity (18–20, 22), leading us to speculate that specific HMOs might affect the development of overweight and obesity indirectly by altering the structure or function of the gut microbiome (23). HMOs might also have a more direct effect on infant growth and body composition, because they affect epithelial cell responses in the gut (16, 24–26) and are absorbed and reach the circulation where they might exert systemic effects (5, 27, 28).

Because HMO composition varies between women and over the course of lactation (29–32), we hypothesized that differences in HMO composition in mother's milk are associated with infant growth and body composition. To test this hypothesis, we examined a cohort of 25 mother-infant pairs at 1- and 6-mo postpartum and analyzed relations between HMO composition and infant growth and body composition.

METHODS

Study overview

Thirty-seven mother-infant dyads were initially enrolled at 1 mo (±5 d) of age with the intent of careful follow-up to 6 mo of age in a breast-milk growth study. The overall study design and preliminary results on infant growth and body composition in a subset of 19 subjects were described previously in which we examined relations between breast-milk hormones and inflammatory markers with infant growth and body composition (33). Participants were instructed to arrive at the University of Oklahoma Health Sciences in Oklahoma City between 0800–1000 h with the mother fasted. On arrival, a complete breast-milk expression from a single breast was obtained, which was followed by a whole-body dual-energy X-ray absorptiometry scan in the infant. These measures were repeated when the infant was 6 mo of age. All of the subjects signed both a consent and Health Insurance Portability and Accountability Act form before any data collection. The University of Oklahoma Institutional Review Board approved all testing procedures.

Study participants

The 37 mother-infant dyads enrolled into the study were breastfed (defined as no formula supplementation) and 31 mother-infant dyads returned for their 6-mo visit. The following inclusion and exclusion criteria were used to determine eligibility into the study. Inclusion criteria included the following: *1*) maternal age of 18–45 y at the time of delivery, *2*) gestation ≥37 wk, *3*) singleton birth, and *4*) a postpartum hospital stay for mother and infant of <3 d. Exclusion criteria were as follows: *1*) mother using any tobacco, *2*) alcohol consumption defined as >1 drink/wk, *3*) type 1 or 2 diabetes before or during pregnancy, and *4*) presumed or known congenital birth defects. All maternal demographic information (age, parity, prepregnancy weight, and gestational weight gain) was collected by medical chart abstraction when possible.

Anthropometric and body-composition variables

Crown-to-heel length was obtained by using a Seca 416 infantometer (Seca) in duplicate with both measures having to be within 0.1 cm; in the event they fell outside of these predefined parameters, a third measure was obtained with the 2 closest values averaged. Nude body weight was obtained by using a Seca 728 scale in duplicate with both measures having to be within 10 g; in the event they fell outside of these predefined parameters, a third measure was obtained with the 2 closest values averaged.

TABLE 1
Physical characteristics of mother-infant pairs[1]

	Infant age		
	1 mo	6 mo	Change
Mother			
Age, y	29.5 ± 4.80	—	—
BMI,[2] kg/m^2	25.5 (10.7)	—	—
Pregnancy weight gain, kg	10.8 ± 7.92	—	—
Gestational age, wk	39.6 ± 1.23	—	—
Secretor, *n* yes/no (%)	18/7 (72)	—	—
Infant			
Sex, *n* F/M (%)	8/17 (32)	—	—
Age,[2,3] d	39.0 (6.00)	167.0 (4.00)	128.0 (7.00)‡
Birth weight, kg	3.56 ± 0.48	—	—
Birth length, cm	52.0 ± 2.30	—	—
Weight, g	4720.84 ± 757.9	7193.6 ± 1191.3	2472.8 ± 729.9‡
Length,[2,3] cm	55.7 (2.60)	65.8 (2.60)	9.30 (2.50)‡
Fat mass, %	23.7 ± 2.97	32.6 ± 3.67	8.84 ± 3.33‡
Total fat mass, g	1190.5 ± 318.3	2457.8 ± 615.8	1267.0 ± 455.3‡
Lean mass, g	3693.2 ± 526.3	4865.4 ± 719.5	1172.2 ± 518.6‡
Total trunk fat mass,[2,3] g	371.0 (109.0)	723.0 (468.0)	360.0 (376.0)‡

[1]Values are means ± SDs unless otherwise indicated for the 25 mother-infant pairs included in this study. Paired *t* test on the change or log change (where noted) from ages 1 to 6 mo was used. ‡$P < 0.0001$.

[2]Values are medians with IQRs in parentheses for variables that were not normally distributed.

[3]Log changes are shown.

The relative percentage of fat and absolute total body composition (total fat and lean mass) and trunk fat mass were collected by using a Lunar iDXA scanner (GE Healthcare) described previously (33, 34) while the infant wore only a diaper and was swaddled with the use of a light receiving blanket provided by the study team to ensure minimal movement. The principal investigator (DAF) positioned all of the infants and performed subsequent scan analyses.

Breast-milk collection and HMO analysis

Mothers were encouraged to pump the entire contents of a single breast expression for the analyses of breast milk described by our group previously (33). HMO analysis was performed at the University of California, San Diego, as previously described, by using HPLC after fluorescent derivatization (35). Raffinose was added to each milk sample as an internal standard for absolute quantification. The total concentration of HMOs was calculated as the sum of the specific oligosaccharides detected. The following 16 HMOs were detected on the basis of retention time comparison with commercial standard oligosaccharides and mass spectrometry analysis: 2′-fucosyllactose (2′-FL), 3-fucosyllactose, 3′-sialyllactose, lacto-N-tetraose (LNT), lacto-N-neotetraose (LNnT), lacto-N-fucopentaose (LNFP) I, LNFPII, LNFPIII, sialyl-LNT (LST) b, LSTc, difucosyl-LNT, disialyl-LNT (DSLNT), fucosyl-lacto-N-hexaose, difucosyl-lacto-N-hexaose, fucosyl-disialyl-lacto-N-hexaose (FDSLNH), and disialyl-lacto-N-hexaose. Secretor status was defined by the presence of 2′-FL or LNFP. Simpson's Diversity index D was calculated as the reciprocal sum of the square of the relative abundance of each of the measured HMOs. Simpson's Equitability (evenness, E) was calculated by dividing the actual D index for each sample by Dmax (maximum D index in the theoretical case that all measured HMOs have the same relative abundance). E can be between 0 and 1; the closer E is to 1, the more even the HMO composition. E reflects the distribution of various HMOs based on concentrations in micrograms per milliliter. A paired *t* test on the change or log change from ages 1 to 6 mo was used.

Statistical analysis

Of the 37 mother-infant pairs enrolled in this study, 25 were included in the final analysis. One participant did not have enough breast milk for analysis, 4 were excluded due to missing data for weight gain during pregnancy, 6 due to missing measures at 6 mo, and 1 due to missing profiling of breast milk at 1 mo. Changes or log changes in infant variables and HMOs were examined by using paired *t* test on the means. A priori covariates included mother's prepregnancy BMI (kg/m^2), pregnancy weight gain, sex, and age (in days) of the infant at 1 mo.

Relations between HMOs and infant's weight and body composition were examined by using multiple linear regression. There were 16 distinct HMOs of interest as well as HMO diversity and evenness. Outcomes included infant weight, length, lean mass, fat mass, and percentage fat. Therefore, we examined 90 models to identify possible relations between HMOs and infant growth and body composition at 1 and 6 mo. At this stage of analysis, only single HMOs were included in each model. When examining 6-mo dependent variables, the baseline dependent variable was included in the model and 6-mo age was used in place of 1-mo age. The inclusion of breast-milk insulin concentrations in each model at 1 and 6 mo did not significantly change our results and was therefore not included in the final model (data not shown). For outcomes at 1 and 6 mo, we adjusted for all a priori covariates and conducted a univariate analysis of HMOs. Those with a $P \leq 0.20$ were further investigated within a compressive model containing all HMOs that were identified from the initial univariate analysis. Analyses were performed in SAS version 9.4 and an a priori significance level was set at $P < 0.05$. All assumptions of multiple linear regression were satisfied, and results are presented as means ± SDs unless noted otherwise. Given our small sample size, we report results unadjusted for multiple comparisons, because results were nonsignificant on the basis of the Holm procedure.

RESULTS

Table 1 displays the mean physical characteristics of the 25 mother-infant pairs. Overall, mothers were ~29.5 y of age, overweight (BMI: 27.9 ± 7.5) before conception, and gained an average of 10.8 kg during pregnancy. As expected, infants significantly increased their weight, length, and measures of fat and lean mass over the 6-mo study period ($P < 0.001$). As shown in **Table 2**, almost all of the measured HMOs significantly changed from 1 to 6 mo. Only 2′-FL, LSTb, fucosyl-lacto-N-hexaose, and Simpson's Diversity and Simpson's Equitability (evenness) were unchanged over the course of the study.

TABLE 2
HMOs[1]

	Infant age		
HMO	1 mo, μg/mL	6 mo, μg/mL	Change, μg/mL
2′-FL	2750.0 (3738.7)	2430.9 (2798.8)	−13.5 (932.5)
3-FL[2]	162.7 (130.2)	515.7 (439.1)	291.0 (229.9)[‡]
3′-SL[2]	756.3 (432.1)	1125.4 (284.3)	408.4 (400.5)[‡]
DFLNH[2]	235.4 (145.7)	41.0 (49.7)	−160.7 (183.6)[‡]
DFLNT[2]	1219.8 (1157.9)	901.8 (688.6)	−260.2 (354.5)[†]
DSLNH[2]	191.9 (160.0)	43.6 (54.8)	−127.5 (137.8)[‡]
DSLNT[2]	247.0 (244.7)	183.7 (133.7)	−51.5 (125.3)[§]
FDSLNH[2]	84.8 (61.4)	45.3 (39.4)	−40.3 (53.2)[†]
FLNH	66.9 (77.0)	68.0 (35.8)	8.3 (88.8)
LNFPI	971.4 (1,537.2)	437.9 (181.5)	−436.0 (708.8)[†]
LNFPII[2]	1340.1 (1111.9)	1616.1 (1459.0)	342.8 (586.2)[†]
LNFPIII[2]	71.1 (46.6)	161.4 (99.0)	93.4 (120.2)[‡]
LNnT	39.2 (32.1)	105.3 (65.9)	55.6 (78.2)[†]
LNT[2]	1422.6 (1250.7)	1226.5 (1043.1)	−436.2 (522.2)[‡]
LSTb[2]	96.2 (97.6)	148.9 (165.7)	35.5 (74.6)
LSTc	206.0 (136.3)	76.8 (57.6)	−131.7 (123.0)[‡]
Sum	11,285.8 (2555.1)	10,408.1 (1645.6)	−877.6 (1403.3)[†]
Diversity	5.14 (1.18)	5.20 (1.29)	0.06 (0.80)
Evenness	0.32 (0.07)	0.33 (0.08)	0.004 (0.05)

[1]Values are medians (IQRs) when variables were not normally distributed for the 25 mothers included in this study. Paired *t* test on the change or log change (where noted) from ages 1 to 6 mo. [‡]$P < 0.0001$, [§]$P \leq 0.002$, [†]$P \leq 0.005$. DFLNH, difucosyl-lacto-N-hexaose; DFLNT, difucosyl-lacto-N-tetraose; DSLNH, disialyl-lacto-N-hexaose; DSLNT, disialyl-lacto-N-tetraose; FDSLNH, fucosyl-disialyl-lacto-N-hexaose; FLNH, fucosyl-lacto-N-hexaose; LNFP, lacto-N-fucopentaose; LNnT, lacto-N-neotetraose; LNT, lacto-N-tetraose; LST, sialyl-lacto-N-tetraose; 2′-FL, 2′-fucosyllactose; 3-FL, 3-fucosyllactose; 3′-SL, 3′-sialyllactose.

[2]Log changes are shown.

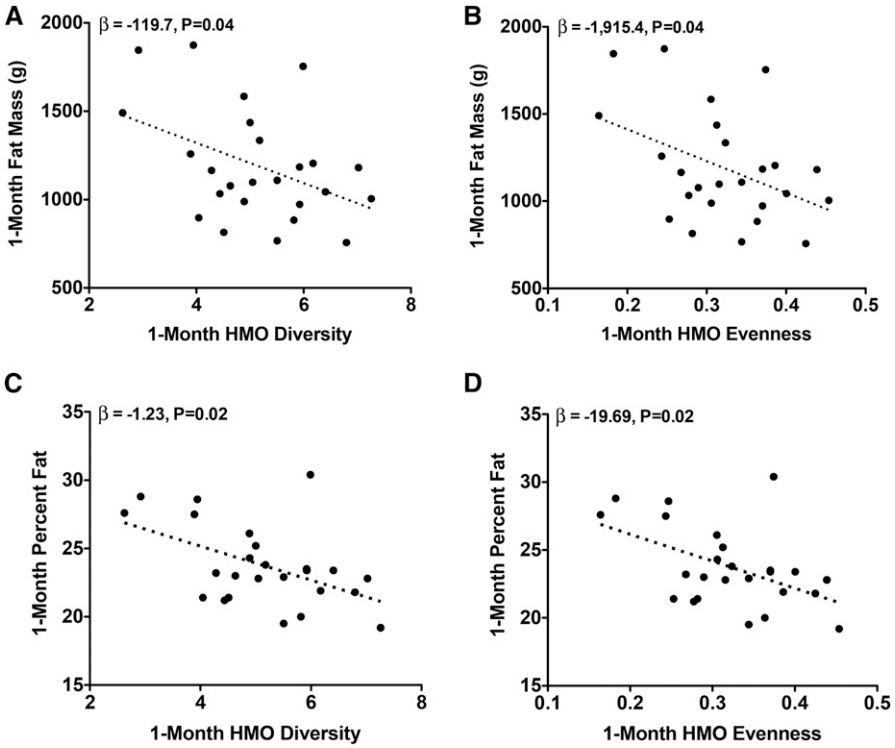

FIGURE 1 One-month adiposity is inversely related to 1-mo HMO diversity and evenness. Unadjusted values for infant fat mass (A and B) and percentage fat (C and D) compared with breast-milk evenness and diversity are shown. Multiple linear regression was performed to obtain the parameter estimates (β) after adjustment for mother's prepregnancy BMI, pregnancy weight gain, sex, and 1-mo infant age (n = 25). HMO, human milk oligosaccharide.

HMOs, infant growth, and adiposity at age 1 mo

At 1 mo, each 1-μg/mL increase in LNFPI was associated with a 0.40-g lower infant weight ($\beta = -0.40$, $P = 0.03$). As shown in **Figure 1** and **Table 3**, HMO diversity and evenness at 1 mo were inversely related to 1-mo fat mass ($\beta = -119.7$, $P = 0.04$, and $\beta = -1915.4$, $P = 0.04$, respectively) and 1-mo percentage fat ($\beta = -1.23$, $P = 0.02$, and $\beta = -19.69$, $P = 0.02$, respectively). No other HMOs at 1 mo were related to 1-mo growth or body composition.

HMOs, infant growth, and adiposity at age 6 mo

At 6 mo, each 1-μg/mL increase in DSLNT was associated with a 0.01-cm increase in 6-mo length ($\beta = 0.01$, $P = 0.04$). As shown in **Figure 2,** each 1-μg/mL increase in 6-mo LNFPI was associated with a 1.11-g lower weight ($\beta = -1.11$, $P = 0.03$), a 0.85-g lower lean mass ($\beta = -0.85$, $P = 0.01$), and a 0.79-g lower fat mass ($\beta = -0.79$, $P = 0.02$). Conversely, as shown in **Figure 3**, each 1-μg/mL increase in 6-mo DSLNT and LNFPII was associated with a 1.92-g ($\beta = 1.92$, $P = 0.02$) and 0.42-g ($\beta = 0.42$, $P = 0.02$) greater fat mass, respectively. Interestingly, a 1-μg/mL increase in 1-mo LNFPII predicted a 0.33-g lower fat mass ($\beta = -0.33$, $P = 0.03$). As shown in **Figure 4**, each 1-μg/mL increase in 6-mo LNnT was associated with a 0.03% lower body fat ($\beta = -0.03$, $P < 0.01$) and a 1-μg/mL increase in 6-mo FDSLNH was associated with a 0.04% higher body fat ($\beta = 0.04$, $P = 0.03$). No other HMOs at 6 mo were related to 6-mo growth or body composition.

HMOs and variance in body composition at ages 1 and 6 mo

As shown in Table 3 and **Table 4**, even after adjustment for maternal BMI and gestational weight gain, specific HMOs accounted for a significant increase in the percentage of the observed variance in infant body composition in our sample. At 1 mo, including LNFPI in the model explained 18% more of the variance in 1-mo infant weight ($R^2 = 0.20$ compared with 0.38;

TABLE 3
Cumulative R^2 for infant body composition at 1 mo[1]

Model	β	Cumulative R^2	R^2 P value
Weight			
Base model		0.20	
+1-mo LNFPI[2]	−0.40*	0.38	0.03
Fat mass			
Base model		0.17	
+ Diversity	−119.7*	0.34	0.04
Base model		0.17	
+ Evenness	−1915.4*	0.34	0.04
Percentage fat			
Base model		0.25	
+ Diversity	−1.23*	0.45	0.02
Base model		0.25	
+ Evenness	−19.69*	0.45	0.02

[1]Multiple linear regression was performed to examine the change in the R^2 with the addition of each human milk oligosaccharide. The base model included sex, prepregnancy BMI, weight gain during pregnancy, and 1-mo infant age. Parameter estimates (β) are from the full model (n = 25). *$P < 0.05$.

[2]LNFPI, lacto-N-fucopentaose I.

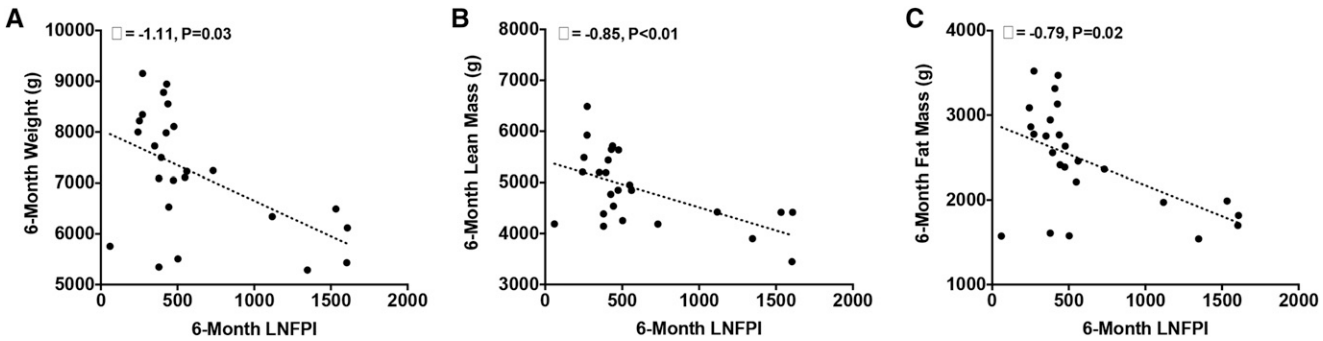

FIGURE 2 Six-month infant body composition is inversely related to 6-mo LNFPI. Unadjusted values for weight (A), lean mass (B), and fat mass (C) compared with breast milk LNFPI at 6 mo are shown. Multiple linear regression was performed to obtain the parameter estimates (β) after adjustment for mother's prepregnancy BMI, pregnancy weight gain, and infant sex, 6-mo infant age, and the dependent variable at 1 mo (n = 25). LNFPI, lacto-N-fucopentaose I.

P = 0.03). In addition, including both 1-mo HMO diversity and evenness in the model explained 20% more of the variance in 1-mo percentage fat (R^2 = 0.25 compared with 0.45; P = 0.02). At 6 mo, the inclusion of DSLNT in the model explained 7% more of the variance in 6-mo length (R^2 = 0.68 compared with 0.75; P = 0.04). The inclusion of LNFPI at 6 mo explained 6% more of the variance in 6-mo weight (R^2 = 0.76 compared with 0.82; P = 0.03) and 13% more of the variance in lean mass (R^2 = 0.66 compared with 0.79; P = 0.01). When examined in the same model, LNFPI, DSLNT, and FDSLNH explained 33% (R^2 = 0.54 compared with 0.87) more of the variance in infant fat mass. Last, the inclusion of LNnT in the model at 6 mo explained 23% of the variance in 6-mo percentage fat (R^2 = 0.34 compared with 0.57; $P < 0.01$).

DISCUSSION

To our knowledge, this is the first study to consider variation in HMOs and how they relate to infant growth and obesity during early development. Increasing evidence suggests that nonnutritive substances in human breast milk affect growth and body composition early in life (33, 36, 37). In a group of 25 breastfeeding mother-infant pairs, our primary objective was to test whether HMOs were related to infant growth and body composition at 1 and 6 mo of infant age. Although far from conclusive, emerging data also suggest that increased leptin (38–42) and insulin in breast milk may influence infant body weight (43). Building on this work, our study suggests that HMOs at both 1 and 6 mo are associated with infant body weight, fat mass, and lean mass. With regard to body composition, results from this study indicate that 6-mo LNFPII and 6-mo DSLNT were associated with 6-mo fat mass. Specifically, a 1-SD increase in LNFPII and DSLNT was associated with a 398- and 856-g greater fat mass, which was ~16% and 35% of the average fat mass observed at 6 mo. When examined collectively, LNFPI, DSLNT, and FDSLNH explained 33% more of the variance in infant fat mass than did sex, prepregnancy BMI, weight gain during pregnancy, and 6-mo infant age alone.

One of the most prominent relations between HMOs and infant body composition was LNFPI, in which 1-mo LNFPI was inversely associated with 1-mo infant weight and 6-mo LNFPI was inversely associated with infant weight, lean mass, and fat mass. Results from this study show that at 6 mo, a 1-SD increase in LNFPI was associated with a 677-g lower weight, a 519-g lower lean mass, and a 482-g lower fat mass. Importantly, 1- and 6-mo LNFPI explained 18% and 6% more of the variance in infant weight at each time point than did other important covariates such as maternal prepregnancy BMI and pregnancy weight gain. A recent study in 10 mother-infant pairs found that certain HMOs, such as LNFPI, have better correlations between mother's milk and infant plasma than urine. As suggested by the authors, these findings may indicate selective intestinal or systemic utilization of LNFPI (5), which could contribute to some of our findings. The opposing associations between LNFPI and LNFPII with infant body composition were intriguing given that a recent

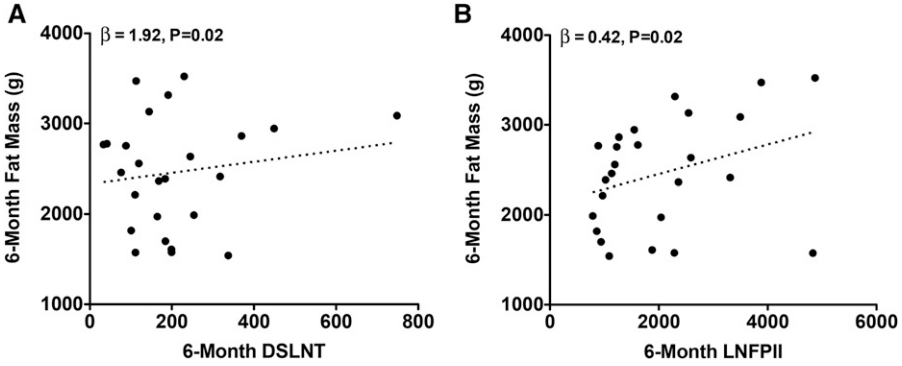

FIGURE 3 Six-month fat mass is positively related to DSLNT and LNFPII. Unadjusted values for body fat mass compared with breast-milk DSLNT and LNFPII at 6 mo are shown. Multiple linear regression was performed to obtain the parameter estimates (β) after adjustment for mother's prepregnancy BMI, pregnancy weight gain, and infant sex, 6-mo infant age, and the dependent variable at 1 mo (n = 25). DSLNT, disialyl-lacto-N-tetraose; LNFPII, lacto-N-fucopentaose II.

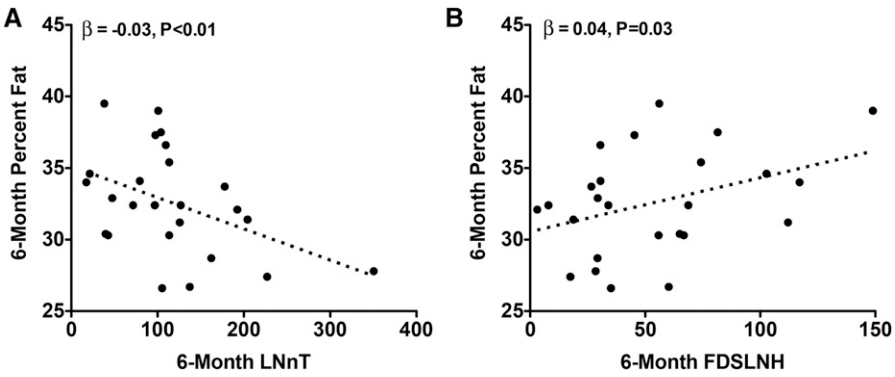

FIGURE 4 Six-month body fat percentage is inversely related to 6-mo LNnT and positively related to 6-mo FDSLNH. Unadjusted values for infant percentage fat compared with breast-milk LNnT (A) and FDSLNH (B) at 6 mo are shown. Multiple linear regression was performed to obtain the parameter estimates (β) after adjustment for mother's prepregnancy BMI, pregnancy weight gain, and infant sex, 6-mo infant age, and the dependent variable at 1 mo (n = 25). FDSLNH, fucosyl-disialyl-lacto-N-hexaose; LNnT, lacto-N-neotetraose.

proof-of-concept study found that the presence of these HMOs in infant stool dramatically changed during early life in which LNFPII increased whereas LNFPI decreased over 13 wk (44).

HMOs are thought to aid in the growth and metabolic efficacy of the developing infant microbiome (21). Traditionally, measures of diversity and evenness have been used to characterize the microbiome in which diversity is a measure of the variety of bacterial species and evenness is a measure of the relative proportion of each respective species. To our knowledge, this is the first study to explore HMO diversity and evenness and how it relates to infant growth and body composition. We showed robust inverse associations between 1-mo HMO diversity and evenness with measures of adiposity at 1 mo in which a 1-SD increase in either diversity or evenness was associated with an ~6.3% lower fat mass. At the same time, we found that HMO diversity and evenness explained between 17% and 20% more of the variance in adiposity than did other important factors such as maternal prepregnancy BMI and pregnancy weight gain.

Although we did not collect stool samples, preliminary findings observed in the current study may be partially attributed to the prebiotic effects of HMOs. In support of this, a recent study found a distinct fecal microbiota composition in breastfed compared with formula-fed infants, in which the fecal microbiota in breastfed infants correlated with the HMOs consumed (particularly LNFPI and DSLNT) (23). Another study in adults found that gut microbial richness correlated with obesity and metabolic markers (45), lending support to the theory that variations in breast-milk composition (46) may modulate the gut flora, alter gastrointestinal activities, and influence inflammatory processes, thereby affecting infant growth and body composition. Future studies will need to show whether HMO diversity and evenness are associated with microbiome diversity and evenness and whether those links contribute to early infant body composition.

Given the small sample size included in this study, our results do not provide definitive evidence of independent effects of HMOs on infant growth and body composition. Because the current findings did not withstand multiple correction testing, we conclude that the overall composition of HMOs in breast milk appears to be related to infant growth and body composition. Future work should be performed in larger samples with longer follow-up to identify the exact contribution of specific HMOs to infant development. Because data were only collected at ages 1 and 6 mo, we are unable to draw definitive conclusions with regard to the relations between HMOs and infant outcomes. HMO composition may have direct interactions with infant growth and body composition due to effects on epithelial cell responses in the gut (16, 24–26) or through systemic effects because they are absorbed and reach the circulation (5, 27, 28). For example, HMOs may also affect neural development, which

TABLE 4
Cumulative R^2 for infant body composition at 6 mo[1]

Model	β	Cumulative R^2	R^2 P value
Length			
Base model		0.28	
+1-mo length	0.94‡	0.68	<0.0001
+1-mo DSLNT	−0.0001	0.68	0.87
+6-mo DSLNT	0.01*	0.75	0.04
Weight			
Base model		0.11	
+1-mo weight	1.33‡	0.76	<0.0001
+1-mo LNFPI	0.44	0.76	0.73
+6-mo LNFPI	−1.11*	0.82	0.03
Lean mass			
Base model		0.17	
+1-mo lean mass	0.94†	0.66	<0.0001
+1-mo LNFPI	0.17	0.68	0.23
+6-mo LNFPI	−0.85†	0.79	0.01
Fat mass			
Base model		0.07	
+1-mo fat mass	1.39†	0.54	<0.001
+1-mo LNFPI	0.18	0.55	0.52
+6-mo LNFPI	−0.84†	0.67	0.02
+1-mo DSLNT	0.31	0.67	0.62
+6-mo DSLNT	1.66*	0.82	<0.01
+1-mo FDSLNH	−2.63*	0.87	0.04
+6-mo FDSLNH	1.84	0.87	0.43
Percentage fat			
Base model		0.18	
+1-mo percentage fat	0.35	0.33	0.06
+1-mo LNnT	0.001	0.34	0.65
+6-mo LNnT	−0.03†	0.57	<0.01

[1]Multiple linear regression was performed to examine the change in the R^2 with the addition of each human milk oligosaccharide. The base model included sex, prepregnancy BMI, weight gain during pregnancy, and 6-mo infant age. Parameter estimates (β) are from the full model (n = 25). *$P < 0.05$, ‡$P < 0.0001$, †$P \leq 0.01$. DSLNT, disialyl-lacto-N-tetraose; FDSLNH, fucosyl-disialyl-lacto-N-hexaose; LNFPI, lacto-N-fucopentaose I; LNnT, lacto-N-neotetraose.

is important for the control of infant feeding behavior. It is also possible that HMOs appear to be associated with infant growth and body composition due to natural variations over time. The generalizability of our results is limited because we were unable to explore whether these relations differed by obesity status or race-ethnicity. Furthermore, additional work is needed to fully understand the mechanistic links between HMOs and infant development as well as the involvement of the infant gut microbiome and metabolome. Last, because our definition of breastfeeding only restricted the use of infant formulas, solid food introduction may have occurred in some infants and contributed to growth and body composition. Despite these limitations, results from this study provide preliminary evidence that the specific makeup and diversity of HMOs found in breast milk may contribute to infant growth and body composition.

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