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Obesity

Relationship of Maternal Weight Status Before, During, and After Pregnancy with Breast Milk Hormone Concentrations

Ghazaleh Sadr Dadres[1], Kara M. Whitaker [1], Jacob L. Haapala[1], Laurie Foster[1], Katy D. Smith[2], April M. Teague[2], David R. Jacobs Jr.[1], Elyse O. Kharbanda[3], Patricia M. McGovern[4], Tonya C. Schoenfuss[5], Laura J. Le[6], Lisa Harnack[1], David A. Fields[2], and Ellen W. Demerath [1]

Objective: The aim of this study was to test associations of prepregnancy BMI, gestational weight gain, oral glucose challenge test results, and postpartum weight loss as predictors of breast milk leptin, insulin, and adiponectin concentrations and whether these relationships vary over time.

Methods: Milk was collected at 1 and 3 months from 135 exclusively breastfeeding women from the longitudinal Mothers and Infants Linked for Healthy Growth (MILk) study. Hormones were assayed in skimmed samples using ELISA. Mixed-effects linear regression models were employed to assess main effects and effect-by-time interactions on hormone concentrations.

Results: In adjusted models, BMI was positively associated with milk leptin ($P < 0.001$) and insulin ($P = 0.03$) and negatively associated with milk adiponectin ($P = 0.02$); however, the association was stronger with insulin and weaker with adiponectin at 3 months than at 1 month (time interaction $P = 0.017$ for insulin and $P = 0.045$ for adiponectin). Gestational weight gain was positively associated and postpartum weight loss was negatively associated with milk leptin (both $P < 0.001$), independent of BMI. Oral glucose challenge test results were not associated with these milk hormone concentrations.

Conclusions: Maternal weight status before, during, and after pregnancy contributes to interindividual variation in human milk composition. Continuing work will assess the role of these and other milk bioactive factors in altering infant metabolic outcomes.

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Introduction

Mothers' milk is the gold standard for human infant nutrition, and breastfeeding exclusively to 6 months is strongly recommended by the American Academy of Pediatrics (1), American College of Obstetricians and Gynecologists, and World Health Organization (2). There is, however, evidence that human milk exhibits substantial individual variation in the concentration of appetite-regulating hormones, cytokine levels, fatty acid profiles, and other factors (3,4). As reviewed by Ellsworth et al. (5), animal models have provided intriguing evidence that maternal obesity may alter milk leptin, insulin, and other bioactive elements, which in turn are associated with diabetes, obesity, and hepatic steatosis in the adulthood offspring (5-7). As animal models do not always translate well into human studies because of species differences in growth rate, mammary gland physiology, and critical windows of development (5), more human research is needed to test the "lactational programming" hypothesis; that is, the variation in levels of hormones, cytokines, and other bioactive compounds present in breast milk may have sustained effects on the offspring's appetite and metabolic rate. Although 25% of women in the United States have BMI in

[1] Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, Minnesota, USA. *Correspondence: Ellen W. Demerath (ewd@umn.edu) [2] Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA [3] HealthPartners Institute, Minneapolis, Minnesota, USA [4] Division of Environmental Health Sciences, University of Minnesota, Minneapolis, Minnesota, USA [5] Department of Food Science and Nutrition, University of Minnesota, Minneapolis, Minnesota, USA [6] Division of Biostatistics, University of Minnesota, Minneapolis, Minnesota, USA.

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Author contributions: DAF and EWD conceived the project and were responsible for data collection. GSD completed the literature review, ran the statistical analyses, and wrote the manuscript. KMW and EWD assisted with manuscript writing and statistical analysis. LF and KDS assisted with data collection. AMT conducted all breast milk assays. JLH was responsible for data management. DRJ and LJL assisted with statistical analyses. EOK conducted data collection and management and assisted with manuscript writing. PMM helped to design the study, interpreted the statistical analyses, and assisted with manuscript writing. TCS collected data and assisted with manuscript writing. LH oversaw data collection and assisted with manuscript writing. All authors critically reviewed the manuscript and had final approval of the submitted and published versions.

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the obese range prior to pregnancy (8), increasing the risk of obesity in the offspring by two- to threefold (9,10), the role of human milk composition in the transmission of obesity risk from mother to child has only recently been examined as a potential mechanism.

In line with this hypothesis, we and others have demonstrated positive associations between milk leptin levels and maternal BMI (11-13), but findings on the relationship of maternal BMI to milk insulin (11,12,14,15) and adiponectin (13,14,16-19) have been less consistent. One factor that may explain disparate findings is that while milk composition is known to change over the course of lactation, with a general finding of declines in milk peptides over lactation (20,21), the current literature is characterized by within- and between-study heterogeneity in the timing of milk sample collection for analysis (16,19,22). Furthermore, studies of milk hormone variation to date have not taken account of maternal metabolic status at different critical developmental windows. Growing evidence has suggested that fetal, infant, and childhood adiposity and obesity risk are modified by pregnancy glucose dysregulation, gestational weight gain (GWG), and postpartum weight loss (PPWL), independent of prepregnancy BMI (5,23), and also that metabolic status during these periods could be involved in mammary gland development and lactogenesis I and II (24). This is important because GWG, PPWL, and subclinical glucose dysregulation may be more easily modifiable aspects of maternal metabolic status than current or prepregnancy BMI.

The present study aimed to test the relationship of maternal prepregnancy BMI, GWG, PPWL, and oral glucose challenge test (OGCT) results with breast milk hormone concentrations (leptin, insulin, and adiponectin). We hypothesized that greater prepregnancy BMI, excessive GWG, higher OGCT, and lower PPWL would be associated with higher concentrations of insulin and leptin and a lower concentration of adiponectin in breast milk, and these associations would vary from earlier in lactation (1 month) to later in lactation (3 months).

Methods

Study sample

The present analysis used data from the Mothers and Infants Linked for Healthy Growth (MILk) study. The MILk study is a prospective cohort study taking place at the University of Oklahoma Health Sciences Center and University of Minnesota in collaboration with HealthPartners Institute in Minnesota. This study was approved by the Institutional Review Boards of University of Minnesota, University of Oklahoma Health Sciences Center, and HealthPartners Institute. Informed consent was obtained from all adult participants. The MILk study is registered with ClinicalTrials.gov (identifier NCT03301753).

At enrollment, study participants were (1) women in their second trimester of a singleton pregnancy, (2) 21 to 45 years old at time of delivery, (3) with BMI of 18.5 to 40 kg/m^2 at first prenatal visit, and (4) self-reported to have social support for and intention to exclusively breastfeed for at least 3 months. Women were excluded if they (1) had alcohol consumption of two or more drinks per week during pregnancy and lactation; (2) had tobacco use during pregnancy and lactation; (3) had history of or current type I, II, or gestational diabetes; (4) were unable to speak or understand English; or (5) had an infant with a known infant congenital metabolic or endocrine disease or other congenital illness affecting the infant's feeding and/or growth. Of these enrolled women, those who delivered a term infant (37-42 weeks gestation) with birth weight ≥2,500 g and ≤4,500 g and who were exclusively breastfeeding at 1 month post partum were requested to provide milk samples at the study center.

A flow diagram of MILk study participants included in this analysis is found in Supporting Information Figure S1. At the time of this analysis, a total of 253 pregnant women had been enrolled in the study and had given birth to an infant who was at least 3 months old, of whom 170 met all of the criteria for continuation in the postpartum phase of the study. Of these, 153 women provided 1- and 3-month milk samples, and 135 of these had sufficient milk for all measurements (≥25 mL) and had their samples sent to the laboratory for assay. After removing statistical outliers and missing covariate information, data were available for 130 women in the prepregnancy BMI and GWG analyses and 129 women in the OGCT and PPWL analyses. To assess potential bias, these groups of participants were compared; differences in the means of important participant characteristics were tested using the *t* test, and differences in frequency of categorical variables were tested using the Fisher exact test. Maternal BMI (26.1 vs. 28.2; $P = 0.0042$) and infant birth weight (3,541 vs. 3,325 g; $P = 0.0118$), but not maternal age, differed between women who were eligible to participate in postpartum milk collection ($n = 170$) compared with women who were excluded, respectively. There were also significant differences using the Fisher exact test in maternal race ($P = 0.0014$), maternal ethnicity ($P = 0.0081$), and type of insurance ($P = 0.0070$), but not infant sex ($P = 0.12$) or delivery mode ($P = 0.11$).

Maternal characteristics

Pregnancy history data were collected from the electronic health records, including (1) maternal and gestational age at time of delivery; (2) first recorded maternal weight and height in the medical record within 6 weeks from conception (dated using last menstrual period), which was used to calculate prepregnancy BMI; (3) GWG, calculated as body weight at admission for delivery minus the first recorded maternal weight; (4) mode of delivery, coded as cesarean section or vaginal delivery; and (5) parity, defined as the number of times a woman has given birth to an infant more than 24 weeks of gestational age. Subclinical glucose dysregulation was captured by the 1-hour blood glucose concentration after a 50-g OGCT administered between 26 and 28 weeks of gestation to screen for gestational diabetes. Women with OGCT levels greater than 140 g/dL received an oral glucose tolerance test to confirm and exclude those with gestational diabetes. PPWL was calculated as the difference between maternal delivery weight and body weight measured at the 1- and 3-month postpartum visits.

Human milk collection

Mothers and infants were scheduled and seen at the study centers within 5 days of the 1-month and 3-month time points between 8:00 and 10:00 AM. The mother was instructed to be ready to breastfeed the infant upon arrival and then asked to feed the child *ad libitum* from one or both breasts as per her usual practice; whether the infant was fed from the left, right, or both breasts was recorded. Two hours after feeding, the mother provided a single complete breast expression sample (until the flow of milk stopped) from the right breast using a hospital-grade electric breast pump (Medela Symphony; Medela, Inc., Zug, Switzerland), ensuring the collection of fore-, mid-, and hindmilk within each sample, as described (12,25). The volume and weight of the milk collected from the single breast expression were recorded. Breast milk was gently mixed, aliquoted, and stored at −80°C within 20 minutes of collection.

Milk hormone assays

Skimmed milk samples were prepared and stored at −80°C for up to 6 months. Leptin, insulin, and total adiponectin were assayed using commercially available enzyme-linked immunosorbent assay (ELISA) kits as previously described by Fields et al. (12) and in the online Supporting Information. The inter- and intra-assay coefficients of reliability were <6% for all hormones, and limits of quantitation were 0.24 μU/mL for insulin, 1 ng/mL for total adiponectin, and 7.86 pg/mL for leptin.

Statistical analysis

Data were analyzed using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina). Dependent variables were examined using histograms and quantile-quantile plots to determine normality. Milk leptin, adiponectin, and insulin concentrations were positively skewed and were log transformed for all subsequent analyses.

Descriptive statistics were presented by prepregnancy BMI group (normal weight, overweight, and obesity), with χ^2 tests and one-way analysis of variance (ANOVA) applied to identify potential confounding variables for the primary exposure, maternal BMI. Differences in milk hormone concentrations from 1 to 3 months were tested using paired *t* tests.

Separate mixed-effects linear regression models were used to test the association of maternal prepregnancy BMI and each of the other maternal metabolic factors with each repeated milk hormone measure (leptin, insulin, adiponectin). Preliminary analyses by prepregnancy BMI category and tertiles of OGCT, GWG, and PPWL showed a stepwise dose-response relationship with milk hormones across groups for BMI, OGCT, and PPWL, suggesting linearity, but nonlinear associations were observed for GWG. Prepregnancy BMI, OGCT glucose concentration, and PPWL were therefore treated as continuous variables, while GWG was dichotomized as below or within versus above the Institute of Medicine (IOM) 2009 guidelines (26). The compound symmetry covariance structure for the repeated measures was selected as best by comparing the Akaike Information Criterion across models. Crude models included only the main effect of the metabolic factor, time (1 and 3 months), and their interaction. Adjusted models additionally included variables that differed across maternal BMI categories ($P < 0.15$) or were identified from the literature to be associated with milk composition. These included maternal age, parity, education level, gestational age at birth, infant sex, mode of delivery (vaginal or cesarean section), exclusive breast milk feeding at 3 months (yes/no), and breast milk volume. Adjusted regression models testing GWG, OGCT, and PPWL also included prepregnancy BMI as a covariate. Significance was set at $\alpha = 0.05$. Sensitivity analyses examined the influence of the following on regression estimates of the exposure variables: infant birth weight *z* score, which breast (right or left) was used during the test feeding, restriction of the analysis to exclusively breastfeeding dyads at 3 months, removal of three women with GWG below IOM guidelines, and reduction of covariates to those with $P < 0.15$ in bivariate analysis.

Results

Baseline characteristics

Descriptive statistics stratified by prepregnancy BMI groups are shown in Table 1. Attained education level, gestational age of the infant at birth, frequency of excessive GWG, and PPWL at 3 months were significantly different between women with normal weight, overweight, and obesity in the sample ($P < 0.05$). All the women were exclusively breastfeeding at 1 month by design, and 93% were still exclusively breastfeeding at 3 months. The frequency of exclusive breastfeeding tended to be slightly lower in women with prepregnancy obesity and overweight than in women in the prepregnancy normal weight category.

Hormone concentrations from 1 to 3 months post partum

Mean concentrations of the breast milk hormones (raw and log transformed) at 1 and 3 months are reported in Table 2. Mean log-transformed adiponectin and leptin significantly decreased ($P = 0.0006$ and $P < 0.0001$, respectively) from 1 to 3 months, with no such change observed in insulin. The serial measures of milk leptin and insulin were highly correlated from 1 to 3 months ($r = 0.75$ and 0.70, respectively; $P < 0.0001$), but no serial correlation was observed for adiponectin ($r = 0.07$; $P = 0.41$).

Hormone concentrations and relationships to maternal metabolic characteristics

Table 3 provides results from the unadjusted and adjusted models assessing the association of maternal metabolic characteristics with milk leptin concentration. There was a positive association between prepregnancy BMI and leptin in both the crude and adjusted models ($\beta = 0.525$ and 0.494, respectively; $P < 0.001$). In addition, there was a statistically significant, independent difference between GWG (below/within vs. exceed guidelines) and leptin levels (crude: $\beta = 0.488$, $P = 0.0001$; adjusted: $\beta = 0.298$, $P = 0.009$). PPWL was inversely associated with leptin concentration in the crude model and remained significant in the adjusted models ($\beta = -0.235$, $P = 0.001$; $\beta = -0.184$, $P = 0.003$, respectively). OGCT results at 1 and 3 months were not associated with breast milk leptin in any of the tested models. The exposure-time interaction was not significant in any of the tested leptin models.

As seen in Table 4, prepregnancy BMI was inversely associated with milk adiponectin in the crude model ($\beta = -0.067$; $P = 0.02$) and remained significant in the adjusted model ($\beta = -0.070$; $P = 0.02$). The prepregnancy BMI-time interaction was statistically significant ($P = 0.001$ and $P = 0.045$ in crude and adjusted models, respectively), indicating that the association between maternal BMI and adiponectin was different at 3 months compared with 1 month (referent). The positive beta coefficient for the interaction term indicates that the negative main effect of maternal prepregnancy BMI with adiponectin was weaker (closer to zero) at 3 months compared with 1 month. The other three maternal metabolic characteristics were not associated with milk adiponectin concentration.

For the insulin models shown in Table 5, a positive association between prepregnancy BMI and insulin was observed in both the crude and adjusted models ($\beta = 0.177$, $P = 0.003$; $\beta = 0.144$, $P = 0.030$, respectively). In addition, the prepregnancy BMI-time interaction was statistically significant ($P = 0.002$ and $P = 0.017$), marking a difference in the association at 1 and 3 months. Here, the positive beta coefficient for the interaction term indicates that the positive main effect of maternal prepregnancy BMI on milk insulin was stronger (more positive) at 3 months compared with 1 month. No other significant associations were observed between maternal factors and milk insulin concentration levels.

TABLE 1 Maternal characteristics at baseline and 1 and 3 months post partum stratified by prepregnancy BMI (n = 135)

	Prepregnancy BMI category									
	Normal weight			Overweight			Obesity			
	n	%	Mean (SD)	*n*	%	Mean (SD)	*n*	%	Mean (SD)	*P value*
Age	67		30.5 (3.9)	39		32.0 (4.1)	29		30.2 (4.3)	0.12
Race										0.99
White	59	88.1		35	89.7		24	88.9		
Other	8	11.9		4	10.3		3	11.1		
Education										0.01
High school/GED/associate's	10	15.6		10	26.3		15	51.7		
Bachelor's degree	30	46.9		15	39.5		10	34.5		
Graduate degree	24	37.5		13	34.2		4	13.8		
Annual household income										0.37
<$60,000	18	28.1		12	31.6		14	48.3		
$60,000-$90,000	20	31.3		9	23.7		6	20.7		
>$90,000	26	40.6		17	44.7		9	31.0		
Baseline parity										0.92
None	27	41.5		16	42.1		11	40.7		
1 child	27	41.5		13	34.2		11	40.7		
≥2 children	11	16.9		9	23.7		5	18.5		
Prepregnancy BMI, kg/m^2	67		22.0 (1.7)	39		27.1 (1.4)	29		34.3 (3.7)	<0.001
IOM GWG guidelines										<0.001
Below or within	49	73.1		14	35.9		13	44.8		
Exceed	18	26.9		25	64.1		16	55.2		
OGCT	67		100.0 (18.0)	39		98.2 (15.7)	29		108.0 (16.7)	0.05
Gestational age at birth	67		39.8 (1.1)	39		40.1 (0.9)	29		39.1 (1.1)	<0.001
Mode of delivery										0.11
Vaginal	55	82.1		29	76.3		18	62.1		
Cesarean	12	17.9		9	23.7		11	37.9		
Infant birth weight for age *z* score	67		0.4 (0.7)	39		0.5 (0.9)	29		0.5 (0.9)	0.19
Infant sex										0.12
Male	38	56.7		21	53.9		10	34.5		
Female	29	43.3		18	46.2		19	65.6		
Breast milk volume, mL										
1 month	64		66.9 (42.1)	39		71 (36.6)	29		60.4 (40.8)	0.56
3 months	57		79.7 (44.2)	36		64.1 (32.3)	22		65.4 (39.1)	0.13
Exclusively breastfeeding at 3 months	58	93.6		33	94.3		24	85.7		0.38
PPWL, kg										
1 month	65		8.6 (3.3)	37		9.4 (3.8)	29		9.1 (4.0)	0.54
3 months	61		10.8 (4.1)	35		10.5 (4.9)	27		8.3 (4.4)	0.04

Data presented as column percentages. Sample size varies across covariates because of missing data. *P* value testing for differences in participant characteristics by prepregnancy BMI category using χ^2 or one-way ANOVA, as appropriate.

Given that only education and gestational age at birth were statistically significant in mixed-effect models, more parsimonious mixed-effect linear regression models adjusting for education and gestational age only were performed. These models resulted in very similar findings, and, therefore, these results were not reported. In additional analyses, we found no significant association of birth weight z score or which breast the infant fed from during the test feed on any of the outcomes ($P > 0.15$), and the estimates of BMI, GWG, PPWL, and OGCT were not influenced significantly (greater than 10% difference in regression coefficient) by their inclusion (results not shown). Restriction to dyads that were exclusively breastfeeding at 3 months or who had GWG greater than or equal to IOM guidelines likewise did not alter the results.

Discussion

In this longitudinal analysis from birth to 3 months post partum, maternal prepregnancy BMI was positively associated with elevated concentrations of leptin and insulin and lower concentrations of adiponectin in mature human milk after adjusting for potential confounders. In

TABLE 2 Concentration changes in human milk hormones from 1 to 3 months post partum

	1 month post partum			3 months post partum			
	n	Mean (SD)	Range	*n*	Mean (SD)	Range	*P*
Leptin (pg/mL)	135	640 (606)	75-4,318	125	484 (672)	55-6,576	
Log leptin	135	6.1 (0.8)	4.3-8.4	125	5.8 (0.9)	4.0-8.8	**<0.001**
Adiponection (ng/mL)	135	16.8 (9.6)	6.8-110.1	125	15.6 (15.2)	5.2-172.1	
Log adiponectin	135	2.7 (0.4)	1.9-4.7	125	2.6 (0.4)	1.7-5.2	**<0.001**
Insulin (μIU/mL)	135	29.7 (22.4)	4.4-116.5	125	30.9 (28.0)	1.5-172.1	
Log insulin	135	3.2 (0.7)	1.5-4.8	125	3.13 (0.8)	0.4-5.2	0.973

P value testing for differences between 1 and 3 months log-transformed breast milk analytes using paired *t* tests. Bolded values are statistically significant at $P < 0.05$.

TABLE 3 Associations of maternal factors with log-transformed breast milk leptin at 1 and 3 months post partum[a]

		Crude model				Adjusted model[b]			
Model	Maternal factors	*n*	β	SE	*P*	*n*	β	SE	*P*
Model 1	Prepregnancy BMI (kg/m^2)	135	0.52	0.05	**<0.001**	130	0.49	0.06	**<0.001**
	Prepregnancy BMI (kg/m^2) × time (3 months)	135	0.08	0.05	0.113	130	0.07	0.06	0.249
Model 2	Excessive GWG[c,d]	135	0.49	0.14	**0.001**	130	0.30	0.11	**0.009**
	Excessive GWG × time (3 months)	135	0.04	0.10	0.710	130	0.07	0.11	0.530
Model 3	OGCT (g/dL)[d,e]	134	0.14	0.07	0.056	129	0.04	0.06	0.521
	OGCT (g/dL) × time (3 months)	134	−0.01	0.05	0.850	129	0.01	0.06	0.792
Model 4	PPWL (kg)[d,f]	132	−0.24	0.07	**0.001**	129	−0.18	0.06	**0.003**
	PPWL (kg) × time (3 months)	132	0.02	0.06	0.696	129	0.04	0.06	0.528

[a]All continuous independent variables (prepregnancy BMI, OGCT, and PPWL) standardized to mean of 0 and SD of 1. Bolded values statistically significant at $P < 0.05$. Time was the time point for measurement of milk hormones (1 and 3 months, with 1 month as the referent).
[b]Adjusted for maternal age, maternal education, mode of delivery, breast milk feeding exclusivity, infant sex, gestational age at delivery, and breast milk volume.
[c]Excessive GWG based on IOM 2009 guidelines.
[d]Models additionally adjusted for prepregnancy BMI.
[e]OGCT: 50-g glucose challenge administered between 24 and 28 weeks of gestation to screen for gestational diabetes.
[f]PPWL calculated as maternal weight at delivery minus maternal weight at 1 or 3 months post partum, entered into mixed models as time-varying independent variable.

TABLE 4 Associations of maternal factors with log-transformed breast milk adiponectin at 1 and 3 months post partum[a]

		Crude model[b]				Adjusted model[c]			
Model	Maternal factors	*n*	β	SE	*P*	*n*	β	SE	*P*
Model 1	Prepregnancy BMI (kg/m^2)	135	−0.067	0.028	**0.020**	130	−0.070	0.030	**0.020**
	Prepregnancy BMI (kg/m^2) × time (3 months)	135	0.052	0.020	**0.011**	130	0.040	0.020	**0.045**
Model 2	Excessive GWG[c,d]	135	0.033	0.057	0.564	130	0.078	0.057	0.171
	Excessive GWG × time (3 months)	135	−0.024	0.040	0.546	130	−0.001	0.038	0.720
Model 3	OGCT (g/dL)[d,e]	134	−0.051	0.028	0.073	129	−0.044	0.029	0.131
	OGCT (g/dL) × time (3 months)	134	−0.007	0.020	0.719	129	−0.016	0.019	0.391
Model 4	PPWL (kg)[d,f]	132	0.007	0.027	0.792	129	0.009	0.026	0.733
	PPWL (kg) × time (3 months)	132	−0.019	0.022	0.387	129	−0.006	0.020	0.773

[a]All continuous independent variables (prepregnancy BMI, OGCT, and PPWL) standardized to mean of 0 and SD of 1. Bolded values statistically significant at $P < 0.05$. Time was the time point for measurement of milk hormones (1 and 3 months, with 1 month as the referent).
[b]Adjusted for maternal age, maternal education, mode of delivery, breast milk feeding exclusivity, infant sex, gestational age at delivery, and breast milk volume.
[c]Excessive GWG based on IOM 2009 guidelines.
[d]Models additionally adjusted for prepregnancy BMI.
[e]OGCT: 50-g glucose challenge administered between 24 and 28 weeks of gestation to screen for gestational diabetes.
[f]PPWL calculated as maternal weight at delivery minus maternal weight at 1 or 3 months post partum, entered into mixed models as time-varying independent variable.

TABLE 5 Associations of maternal factors with log-transformed breast milk insulin at 1 and 3 months post partum[a]

		Crude model[b]				Adjusted model[c]			
Model	**Maternal factors**	***n***	**β**	**SE**	***P***	***n***	**β**	**SE**	***p***
Model 1	Prepregnancy BMI (kg/m^2)	135	0.18	0.06	**0.003**	130	0.14	0.07	**0.03**
	Prepregnancy BMI (kg/m^2) × time (3 months)	135	0.16	0.05	**0.002**	130	0.14	0.06	**0.02**
Model 2	Excessive GWG[c,d]	135	0.02	0.13	0.91	130	−0.05	0.13	0.69
	Excessive GWG × time (3 months)	135	0.14	0.10	0.16	130	0.15	0.11	0.17
Model 3	OGCT (g/dl)[d,e]	134	0.11	0.06	0.09	129	0.08	0.06	0.23
	OGCT × time (3 months)	134	−0.02	0.05	0.67	129	−0.06	0.05	0.25
Model 4	PPWL (kg)[d,f]	132	−0.12	0.06	0.06	129	−0.09	0.06	0.15
	PPWL (kg) × time (3 months)	132	0.040	0.055	0.47	129	0.07	0.06	0.24

[a]All continuous independent variables (prepregnancy BMI, OGCT, and PPWL) standardized to mean of 0 and SD of 1. Bolded values statistically significant at $P < 0.05$. Time was the time point for measurement of milk hormones (1 and 3 months, with 1 month as the referent).
[b]Adjusted for maternal age, maternal education, mode of delivery, breast milk feeding exclusivity, infant sex, gestational age at delivery, and breast milk volume.
[c]Excessive GWG based on IOM 2009 guidelines.
[d]Models additionally adjusted for prepregnancy BMI.
[e]OGCT: 50-g glucose challenge administered between 24 and 28 weeks of gestation to screen for gestational diabetes.
[f]PPWL calculated as maternal weight at delivery minus maternal weight at 1 or 3 months post partum, entered into mixed models as time-varying independent variable.

addition, we found a statistically higher milk leptin concentration in women whose GWG exceeded the IOM guidelines compared with those whose GWG was either below or within the IOM guidelines, independent of time. Lastly, our study has shown a negative association between PPWL and concentration of milk leptin level, independent of time. Our findings on the association of prepregnancy BMI and increased leptin levels is largely consistent with other studies; however, our findings on the time-dependent relationship of prepregnancy BMI and higher concentration of milk insulin and lower concentration of milk adiponectin, as well as the relationships of excessive GWG and lower PPWL with milk leptin, are novel to the field.

Leptin is an appetite-regulating hormone produced in the adipose tissue (27) as well as epithelial cells of the mammary gland (28), and it has been found in much higher concentrations in human serum compared with breast milk (20) and in serum of women with obesity than those with normal weight (29). Milk leptin's positive association with maternal BMI (with a small number of exceptions) (19,30) has been well established (11,13,16,31). Our results confirmed this association in exclusively breastfeeding women and extended it by documenting that milk leptin concentration is responsive to dynamic aspects of weight change in pregnancy and lactation. Maternal serum leptin increases during late gestation and declines after parturition (the so-called "leptin surge"), which is a phenomenon thought to be critical for maturation of appetite-regulating centers in the fetal brain (32) and which we saw reflected in a significant decline in milk leptin from 1 to 3 months. Milk leptin is thought to derive in large part from maternal serum, the level of which tracks changes in adiposity; this may explain why we found it to associate with maternal GWG and PPWL as well as BMI. It is not clear why the same results were not found for insulin or adiponectin, but it may relate to differences across hormones in the degree to which their appearance in milk occurs via active expression and/or transport by the mammary epithelial cells rather than passive paracellular diffusion (21,32). Serial measurement of maternal serum would help to address this point in future. Randomized weight-management trials during these critical periods could also better assess whether the relationships we have observed are causal.

Secreted by adipose tissue, serum adiponectin is inversely associated with adiposity, plays a role in modulation of glucose and lipid metabolism in insulin sensitive tissues, and has antiatherogenic and anti-inflammatory effects (17,33-35). Adiponectin is one of the most abundant milk hormones, the concentration of which is thought to be driven by maternal circulating levels. Milk levels decline over the period of lactation (36), as we have also shown here from 1 to 3 months. We found a time-dependent inverse relationship between maternal prepregnancy BMI and milk adiponectin, with a stronger (negative) association observed at 1 month than at 3 months post partum. Literature to date has been inconsistent on the association of maternal BMI and milk adiponectin (14,16-19), with no clear association between maternal BMI and adiponectin levels in colostrum, hindmilk, foremilk, or whole milk. Martin et al. (18) first showed a positive association of maternal BMI with adiponectin concentration in breast milk in a study of 22 women with serial skimmed milk samples, but this finding was based on postpregnancy and not prepregnancy BMI and was not confirmed after covariate adjustments. Other studies that have reported no association between maternal BMI and milk adiponectin levels had lower precision of estimates because of fewer women with elevated BMI, self-reported maternal weights, and wide infant age ranges for milk sampling (14,17,18). Our results of a diminished association over time supports findings by Chan et al. (16), who reported no association between maternal prepregnancy BMI and adiponectin levels in breast milk at 3 to 4 months post partum. The finding points out the importance of timing of milk collection. The relative benefits of hormones in early versus later milk for infant growth remain to be determined.

Produced in the pancreas, insulin plays a major role in maintaining glucose homeostasis and metabolic function, but it also has less-recognized roles in appetite suppression through receptors binding in hypothalamic neurons (37,38) and as a potent growth-regulating hormone in early life (39). Milk insulin concentration is found at equimolar concentrations, as in maternal serum, and is thought to be actively transported into milk by the mammary epithelial cell rather than diffusing through the paracellular pathway (21). This active transport may explain why we found that milk insulin did not decline on average from 1 to 3 months after delivery, in contrast to leptin and adiponectin. Indeed, the positive association between maternal prepregnancy BMI and milk insulin not only remained significant from 1 to 3 months but was stronger at the later time point. Previous findings

on the relationship of maternal BMI to milk insulin have been mixed. Our results are consistent with several other studies reporting a positive correlation with maternal pregnancy BMI (21,22). In contrast, Shehadeh et al. (15) reported postpartum maternal BMI (measured at the time of breast milk sample collection) to have no influence on insulin concentration in transitional milk (days 3 and 10). Two other studies, which may have suffered from low statistical power because of low sample size (14,16), also showed no association between prepregnancy BMI and milk insulin levels. These results warrant further investigation on the potential role of milk insulin in infant appetite, metabolic rate, and obesity risk.

This study had several strengths, including a rigorous milk collection protocol in which all the samples were expressed with the same equipment, collected in the morning and precisely 2 hours after the previous breastfeeding, and within narrow age-specific collection windows. This allowed for control of diurnal and lactation stage variations in milk, which have been well recorded (40). Numerous confounding and technical factors were assessed, and our sample included a relatively large proportion of women with overweight and obesity. There were also some limitations to this study. First, whether maternal weight changes relate specifically to milk hormone levels in foremilk versus hindmilk was not assessed (11). Second, study population was relatively homogenous in ethnic and racial composition, making the results less generalizable to non-white or Hispanic populations. Third, the effect sizes for the relationship of milk hormones with GWG, PPWL, and OGCT appear to be weaker than for prepregnancy BMI; our finding that GWG and PPWL had relationships only with leptin levels may be a function of statistical power. Fourth, potential confounding effects of maternal physical activity level and dietary intake were unavailable for analysis. Finally, lack of maternal serum hormone concentrations limited the ability to explain observed differences in results across the different milk hormones.

Conclusion

Numerous benefits of breastfeeding to the health of the infant and the mother have been established (1,2); however, much is to be learned regarding the causes and consequences of individual variation in breast milk composition. Our study advances this body of knowledge by (1) showing that milk hormone concentrations and their relationship with maternal metabolic status change over time and (2) illustrating that not only prepregnancy BMI but also GWG and PPWL are associated with variation in milk leptin. Future work will test the relationship of these and other milk bioactive components with infant growth and metabolic health.O

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