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

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## Abstract

**Objective:** To test associations of pre-pregnancy BMI (BMI), gestational weight gain (GWG), oral glucose challenge test (OGCT) results, and post-partum weight loss (PPWL) as predictors of breastmilk 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 with insulin was stronger and with adiponectin was weaker at 3-months than at 1-month (time interaction $p= 0.017$ for insulin and $p= 0.045$ for adiponectin). GWG was positively

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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 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, EWD, LJL assisted with statistical analyses. EWD assisted with writing the manuscript. All authors critically reviewed the manuscript and had final approval of the submitted and published versions.

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and PPWL was negatively associated with milk leptin (both $p<0.001$) independent of BMI. OGCT was not associated with these milk hormone concentrations.

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

### Keywords

Maternal Obesity; Pregnancy; Breast feeding; Gestational Weight Gain; Hormones

## Introduction

Mothers' milk is the gold standard for human infant nutrition, and breastfeeding exclusively to six months is strongly recommended by the American Academy of Pediatrics,[1] American College of Obstetrics 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 provide 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, due to 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, that variation in levels of hormones, cytokines and other bioactive compounds present in breastmilk may have sustained effects on offspring's appetite and metabolic rate. Although 25% of women in the United Stated have BMI in the obese range prior to pregnancy, [8] increasing the risk of obesity in the offspring by 2-3 fold.[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] are less inconsistent. 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] Further, studies of milk hormone variation to date have not taken account of maternal metabolic status at different critical developmental windows. Growing evidence suggests that fetal, infant, and childhood adiposity and obesity risk are modified by pregnancy glucose dysregulation, gestational weight gain (GWG), and post-partum weight loss (PPWL), independent of pre-pregnancy 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 are current or pre-pregnancy BMI.

The present study aimed to test the relationship of maternal pre-pregnancy BMI, gestational weight gain (GWG), postpartum weight loss (PPWL), and oral glucose tolerance test (OGCT) results with breastmilk hormone concentrations (leptin, insulin, and adiponectin). We hypothesized that greater pre-pregnancy BMI, excessive GWG, higher OGCT, and lower PPWL would be associated with higher concentrations of insulin and leptin, and lower concentration of adiponectin in breastmilk, and that 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 Research Foundation in Minnesota. This study was approved by the Institutional Review Boards of University of Minnesota, University of Oklahoma Health Sciences Center, and HealthPartners Institute for Education and Research. Informed consent was obtained from all adult participants. The MILk study is registered with clinicaltrials.gov (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) 18.5-40 $kg/m^2$ BMI at first prenatal visit; and 4) self-reported to have social support for and intention to exclusively breastfeed for at least three months. Women were excluded if they had 1) alcohol consumption of 2 or more drinks per week during pregnancy and lactation; 2) tobacco use during pregnancy and lactation; 3) history of or current Type I, II, or gestational diabetes; 4) unable to speak or understand English; and 5) presence of a known infant congenital metabolic or endocrine disease, or other congenital illness affecting the infant's feeding/growth. Of these enrolled women, those who delivered a term infant (37-42 weeks' gestation) with a 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 Supplemental Figure 1. 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 post-partum phase of the study. Of these, 153 women provided 1 and 3 months milk samples, and 135 of these had sufficient milk for all measurements (>=25 ml) and their samples were sent to the laboratory for assay. After removing statistical outliers and missing covariate information, data were available for 130 women in the pre-pregnancy BMI and GWG analyses, and 129 women in the OGCT and postpartum weight loss analyses. To assess potential bias, these groups of participants were compared; differences in the means of important participant characteristics were tested using the Student's T-test and differences in frequency of categorical variables were tested using Fischer's Exact Test. Maternal BMI (26.1 vs. 28.2, P=0.0042) and infant birth weight (3541 vs. 3325 g, P=0.0118), but not maternal age differed between women that were eligible to participate in post-partum milk collection (N=170) as compared to women who

were excluded, respectively. There were also significant differences using Fisher's 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 pre-pregnancy BMI; 3) GWG calculated as body weight at admission for delivery minus the first recorded maternal weight as above; 4) mode of delivery coded as caesarean section or vaginal delivery; and 5) parity as number of times a woman has given birth to an infant over 24 weeks of gestational age. Subclinical glucose dysregulation was captured by the 1-hour blood glucose concentration after a 50g oral glucose challenge test (OGCT), administered between 26-28 weeks of gestation to screen for gestational diabetes (GDM). Women with OGCT levels greater than 140 g/dl received an oral glucose tolerance test to confirm and exclude those with GDM. Postpartum weight loss was calculated as the difference between maternal delivery weight and her body weight measured at the 1- and 3 months post-partum 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 8:00-10:00 am. The mother was instructed to be ready to breastfeed the infant upon arrival, and then fed the child *ad libitum* from one or both breasts as per her usual practice and 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 hind-milk within each sample.[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, stored at −80° C for up to 6 months. Leptin, insulin, total adiponectin was assayed using commercially available ELISA kits as previously described by Fields et al.[12] and in Supplemental File 1. 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 (Cary, NC, USA.). Dependent variables were examined using histograms and quantile-quantile (Q-Q) 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 pre-pregnancy BMI group (normal weight, overweight, and obese), with chi-square tests and one-way 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 pre-pregnancy BMI, and each of the other maternal metabolic factors, with each repeated milk hormone measure (leptin, insulin, adiponectin). Preliminary analyses by pre-pregnancy 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 non-linear associations were observed for GWG. Pre-pregnancy BMI, OGCT glucose concentration, and PPWL were therefore treated as continuous variables, while GWG was dichotomized as below/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 AIC 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 pre-pregnancy 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 breast feeding 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 pre-pregnancy BMI groups are shown in Table 1. Attained education level, gestational age of the infant at birth, the frequency of excessive GWG, and PPWL at 3 months were significantly different between normal weight, overweight, and obese women 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. This tended to be slightly lower in women with pre-pregnancy obesity and overweight than in women in the pre-pregnancy normal weight category.

### Hormone Concentrations from 1- to 3 Months Postpartum

Mean concentrations of the breastmilk hormones (raw and log transformed) at 1- and 3 months are reported in Table 2. Mean log transformed adiponectin and leptin significantly decreased (*p*-value = 0.0006 and < 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*-value < 0.0001), but no serial correlation was observed for adiponectin (r = 0.07; *p*-value = 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 concentrations. There was a positive association between pre-pregnancy BMI and leptin in both the crude and adjusted models (β = 0.525 and 0.494 respectively, *p*-value < 0.001). In addition, there was a statistically significant, independent, difference between GWG (below/within vs. exceed guidelines) and leptin levels (crude: β =0.488 *p*-value = 0.0001; adjusted: β= 0.298 *p*-value = 0.009). PPWL was inversely associated with leptin concentration in the crude model, and remained significant in the adjusted models (β = −0.235 *p*-value = 0.001, β = −0.184 *p*-value =0.003 respectively). OGCT results at 1- and 3 months were not associated with breastmilk 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 pre-pregnancy BMI was inversely associated with milk adiponectin in the crude model (β= −0.067, *p*-value = 0.02) and remained significant in the adjusted model (β= −0.070, *p*-value = 0.02). The pre-pregnancy BMI- time interaction was statistically significant (*p*-value = 0.001 and *p*-value = 0.045 in crude and adjusted models, respectively), indicating that the association between maternal BMI and adiponectin was different at 3-months as compared to 1 month (referent). The positive beta coefficient for the interaction term indicates that the negative main effect of maternal pre-pregnancy BMI with adiponectin was weaker (closer to zero) at 3 months as compared to 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 pre-pregnancy BMI and insulin was observed in both the crude and adjusted models (β= 0.177, *p*-value = 0.003; and β= 0.144, *p*-value = 0.030, respectively). In addition, the pre-pregnancy BMI-time interaction was statistically significant (*p*-value = 0.002 and *p*-value = 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 pre-pregnancy BMI on milk insulin was stronger (more positive) at 3 months as compared to 1 month. No other significant associations were observed between maternal factors and milk insulin concentration levels.

Given that only education and gestational age at birth were statistically significant in the 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 breast feeding 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 postpartum, maternal pre-pregnancy 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 addition, we found a statistically higher milk leptin concentration in women whose GWG exceeded the IOM guidelines as compared to those whose GWG was either below or within the IOM guidelines, independent of time. Lastly, our study has shown a negative association between postpartum weight loss and concentration of milk leptin level, independent of time. Our findings on the association of pre-pregnancy BMI and increased leptin levels is largely consistent with other studies; however, our findings on the time-dependent relationship of pre-pregnancy BMI and higher concentration of milk insulin and lower concentration of milk adiponectin, as well as the relationships of excessive GWG and lower postpartum weight loss 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 has been found in much higher concentrations in human serum compared to breastmilk,[20] and in serum of obese than normal weight women.[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 phenomenon is 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 anti-atherogenic 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 pre-pregnancy BMI and milk adiponectin, with a stronger (negative) association observed at 1-month than at 3 months post-partum. Literature to date is 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 concentrations in

breastmilk in a study of 22 women with serial skimmed milk samples, but this finding was based on post-pregnancy, and not pre-pregnancy BMI, and was not confirmed after covariate adjustments. Other studies that reported no association between maternal BMI and milk adiponectin levels had lower precision of estimates due to 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 pre-pregnancy BMI and adiponectin levels in breastmilk at 3 to 4 months postpartum. 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 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 post-partum, in contrast to leptin and adiponectin. Indeed, the positive association between maternal pre-pregnancy 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 post-partum maternal BMI (measured at the time of breastmilk sample collection), to have no influence on insulin concentrations in transitional milk (days 3 and 10). Two other studies, which may have suffered from low statistical power due to low sample size[14,16] also showed no association between pre-pregnancy 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.

## Strengths and Limitations

This study had a rigorous milk collection protocol, where all the samples were expressed with the same equipment, collected in the morning and precisely two hours after the previous breast feeding, 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 overweight and obese women. There were also some limitations to this study. First, whether maternal weight changes relate specifically to milk hormone levels in foremilk versus hind milk 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 pre-pregnancy BMI; our finding that GWG and PPWL had relationships only with leptin level 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.

## Conclusions

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 breastmilk 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 pre-pregnancy BMI but also gestational weight gain and postpartum weight loss 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**What is Already Known About This Topic**

- Human milk is a highly complex fluid and varies between and within women over time, but the determinants of this variation remain understudied.
- Human milk leptin concentration increases in a stepwise fashion with increasing maternal BMI.

### What This Study Adds

- Concentrations of leptin and adiponectin in mature human milk decline from 1 to 3 months, while milk insulin does not change over this period of lactation.
- Milk leptin increases with greater gestational weight gain and lower post-partum weight loss, independent of its relationship with pre-pregnancy BMI
- Pre-pregnancy BMI is positively associated with milk insulin and negatively associated with milk adiponectin, and the strength of these relationships change from 1 to 3 months post-partum.

**Table 1:**

Maternal Characteristics at Baseline and One and Three Months Postpartum Stratified by Pre-pregnancy Body Mass Index (N=135)

| | Pre-Pregnancy BMI Category | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Normal Weight | | | Overweight | | | Obese | | | |
| **Maternal Characteristics** | **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/Associates | 10 | 15.6 | | 10 | 26.3 | | 15 | 51.7 | | |
| Bachelors 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 | | |
| >$90,000 | 26 | 40.6 | | 17 | 44.7 | | 9 | 31.0 | | |
| Baseline Parity | | | | | | | | | | 0.92 |
| None | 27 | 41.5 | | 16 | 42.1 | | 11 | 40.7 | | |
| 1 child | 27 | 41.5 | | 13 | 34.2 | | 11 | 40.7 | | |
| ≥2 children | 11 | 16.9 | | 9 | 23.7 | | 5 | 18.5 | | |
| Pre-pregnancy BMI, kg/m$^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 | | |
| Oral Glucose Challenge Test (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 |

| | Pre-Pregnancy BMI Category | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Normal Weight | | | Overweight | | | Obese | | | |
| **Maternal Characteristics** | **N** | **%** | **Mean (SD)** | **N** | **%** | **Mean (SD)** | **N** | **%** | **Mean (SD)** | **P-value** |
| Infant Sex | | | | | | | | | | 0.12 |
| Male | 38 | 56.7 | | 21 | 53.9 | | 10 | 34.5 | | |
| Female | 29 | 43.3 | | 18 | 46.2 | | 19 | 65.6 | | |
| Breastmilk 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 |
| Postpartum weight loss, 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** |

*p*-value testing for differences in participant characteristics by pre-pregnancy BMI category using chi-square or one-way ANOVA, as appropriate. Data presented as column percentages. Sample size varies across covariates due to missing data.

IOM: Institute of Medicine

GWG: Gestational weight gain

**Table 2:**

Concentration changes in human milk hormones from 1 to 3 months

| | 1 Month Postpartum | | | 3 Months Postpartum | | | |
|---|---|---|---|---|---|---|---|
| | **N** | **Mean (SD)** | **Range** | **N** | **Mean (SD)** | **Range** | **p** |
| Leptin (pg/ml) | 135 | 640 (606) | 75, 4318 | 125 | 484 (672) | 55, 6576 | |
| 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.

**Table 3:**

Associations of maternal factors with log-transformed breastmilk leptin at 1- and 3 months postpartum[a]

| | | Crude Model | | | | Adjusted Model[b] | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Models** | **Maternal Factors** | **N** | **β** | **SE** | **P** | **N** | **β** | **SE** | **p** |
| Model 1 | Pre-pregnancy BMI, (kg/m$^2$) | 135 | 0.52 | 0.05 | **<0.001** | 130 | 0.49 | 0.06 | **<0.001** |
| | Pre-pregnancy BMI, (kg/m$^2$) *Time | 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 | 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 * Time | 134 | −0.01 | 0.05 | 0.850 | 129 | 0.01 | 0.06 | 0.792 |
| Model 4 | Post-partum weight loss (kg)[d,f] | 132 | −0.24 | 0.07 | **0.001** | 129 | −0.18 | 0.06 | **0.003** |
| | Post-partum weight loss (kg) * Time | 132 | 0.02 | 0.06 | 0.696 | 129 | 0.04 | 0.06 | 0.528 |

[a] All continuous independent variables (pre-pregnancy BMI, Oral glucose challenge test and post-partum weight loss) were standardized to a mean of 0 and a standard deviation of 1. Bolded values are statistically significant at p-value < 0.05. Time was the time point for measurement of milk hormones (1 and 3 months, with 1 month as the referent)

[b] Adjusts for maternal age, maternal education, mode of delivery, breast milk feeding exclusivity, infant sex, gestational age at delivery, and breast milk volume.

[c] Gestational Weight Gain; excessive gain based on Institute of Medicine 2009 guidelines for gestational weight gain

[d] Models additionally adjusted for pre-pregnancy BMI

[e] Oral Glucose Challenge Test, 50 g glucose challenge administered between 24-28 weeks of gestation to screen for gestational diabetes

[f] Post-partum weight loss was calculated as maternal weight at delivery minus maternal weight at 1 or 3 months post-partum, entered into the mixed models as a time varying independent variable.

**Table 4:**

Associations of maternal factors with log-transformed breastmilk adiponectin at 1- and 3 months postpartum[a]

| | | Crude Model[b] | | | | Adjusted Model[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Models** | **Maternal Factors** | **N** | **β** | **SE** | **P** | **N** | **β** | **SE** | **p** |
| Model 1 | Pre-pregnancy BMI, (kg/m$^2$) | 135 | −0.067 | 0.028 | **0.020** | 130 | −0.070 | 0.030 | **0.020** |
| | Pre-pregnancy BMI, (kg/m$^2$) *Time | 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 | 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 * Time | 134 | −0.007 | 0.020 | 0.719 | 129 | −0.016 | 0.019 | 0.391 |
| Model 4 | Post-partum weight loss (kg)[d,f] | 132 | 0.007 | 0.027 | 0.792 | 129 | 0.009 | 0.026 | 0.733 |
| | Post-partum weight loss (kg) * Time | 132 | −0.019 | 0.022 | 0.387 | 129 | −0.006 | 0.020 | 0.773 |

[a] All continuous independent variables (pre-pregnancy BMI, Oral glucose challenge test and post-partum weight loss) were standardized to a mean of 0 and a standard deviation of 1. Bolded values are statistically significant at p-value < 0.05. Time was the time point for measurement of milk hormones (1 and 3 months, with 1 month as the referent)

[b] Adjusts for maternal age, maternal education, mode of delivery, breast milk feeding exclusivity, infant sex, gestational age at delivery, and breastmilk volume

[c] Gestational Weight Gain; excessive gain based on Institute of Medicine 2009 guidelines for gestational weight gain

[d] Models additionally adjusted for pre-pregnancy BMI

[e] Oral Glucose Challenge Test, 50 g glucose challenge administered between 24-28 weeks of gestation to screen for gestational diabetes

[f] Post-partum weight loss was calculated as maternal weight at delivery minus maternal weight at 1 or 3 months post-partum, entered into the mixed models as a time varying independent variable.

**Table 5:**

Associations of maternal factors with log-transformed breastmilk insulin at 1- and 3 months postpartum[a]

| | | Crude Model[b] | | | | Adjusted Model[c] | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Models** | **Maternal Factors** | **N** | **β** | **SE** | **p** | **N** | **β** | **SE** | **P** |
| Model 1 | Pre-pregnancy BMI, (kg/m$^2$) | 135 | 0.18 | 0.06 | **0.003** | 130 | 0.14 | 0.07 | **0.03** |
| | Pre-pregnancy BMI, (kg/m$^2$) *Time | 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 | 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 | 134 | −0.02 | 0.05 | 0.67 | 129 | −0.06 | 0.05 | 0.25 |
| Model 4 | Post-partum weight loss (kg)[d,f] | 132 | −0.12 | 0.06 | 0.06 | 129 | −0.09 | 0.06 | 0.15 |
| | Post-partum weight loss (kg) *Time[d] | 132 | 0.040 | 0.055 | 0.47 | 129 | 0.07 | 0.06 | 0.24 |

[a] All continuous independent variables (pre-pregnancy BMI, Oral glucose challenge test and post-partum weight loss) were standardized to a mean of 0 and a standard deviation of 1. Bolded values are statistically significant at p-value < 0.05. Time was the time point for measurement of milk hormones (1 and 3 months, with 1 month as the referent)

[b] Adjusts for maternal age, maternal education, mode of delivery, breast milk feeding exclusivity, infant sex, gestational age at delivery, and breastmilk volume

[c] Gestational Weight Gain; excessive gain based on Institute of Medicine 2009 guidelines for gestational weight gain

[d] Models additionally adjusted for pre-pregnancy BMI,

[e] Oral Glucose Challenge Test, 50 g glucose challenge administered between 24-28 weeks of gestation to screen for gestational diabetes

[f] Post-partum weight loss was calculated as maternal weight at delivery minus maternal weight at 1 or 3 months post-partum, entered into the mixed models as a time varying independent variable.