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Associations of Maternal Weight Status Before, During, and After Pregnancy with Inflammatory Markers in Breast Milk

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Abstract

Objective—To examine the associations of maternal weight status before, during, and after pregnancy with breast milk C-Reactive Protein (CRP) and Interleukin-6 (IL-6), two bioactive markers of inflammation, measured at one and three months postpartum.

Methods—Participants were 134 exclusively breastfeeding mother-infant dyads taking part in the Mothers and Infants LinKed for Health (MILK) study, who provided breast milk samples. Pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) were assessed by chart abstraction; postpartum weight loss was measured at the one and three month study visits. Linear regression was used to examine the associations of maternal weight status with repeated measures of breast milk CRP and IL-6 at one and three months, after adjustment for potential confounders.

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Disclosures:

No other conflicts of interest were reported.

Author Contributions: DAF and EWD conceived the project and were responsible for data collection. KMW ran the statistical analyses and wrote the manuscript. LF and KDS assisted with data collection. AMT conducted all breast milk assays. JLH was responsible for data management. DRJ and EWD assisted with statistical analyses. RM completed the literature review. All authors critically reviewed the manuscript and had final approval of the submitted and published versions.

Results—Pre-pregnancy BMI and excessive GWG, but not total GWG or postpartum weight loss, were independently associated with breast milk CRP after adjustment ($\beta=0.49$, $p<0.001$ and $\beta=0.51$, $p=0.011$, respectively). No associations were observed for IL-6.

Conclusions—High pre-pregnancy BMI and excessive GWG are associated with elevated levels of breast milk CRP. The consequences of infants receiving varying concentrations of breast milk inflammatory markers are unknown; however, we speculate that there are implications for the intergenerational transmission of disease risk.

Keywords

breast feeding; maternal obesity; gestational weight gain; C-Reactive Protein; Interleukin-6

Introduction

It is recommended that infants be exclusively breastfed for the first six months of life, given the documented short- and long-term health benefits for the child.¹ Until recently, breast milk composition was thought to be fairly uniform among women. However, emerging evidence shows that breast milk is highly complex with significant variation between women in concentrations of bioactive compounds beyond that of basic macro nutrients.²⁻⁶ Little is known about the association between maternal obesity and non-nutritive components of breast milk. Given that nearly 60% of women enter pregnancy with overweight or obesity in the U.S.,⁷ and approximately 50% exceed the Institute of Medicine (IOM) gestational weight gain (GWG) recommendations,⁸ it is important to examine the effect of maternal weight status on breast milk composition, and in turn, whether this affects the offspring's risk of future obesity and other adverse outcomes.

Obesity is associated with elevated serum levels of two bioactive markers of inflammation: C-reactive protein (CRP), an acute-phase protein synthesized by the liver, and interleukin-6 (IL-6), a cytokine produced by leukocytes, endothelial cells, and adipocytes.⁹⁻¹⁴ Obesity is considered a condition of low-grade systemic inflammation;^{9,10} therefore, maternal weight status before, during, and after pregnancy could affect the concentrations of CRP and IL-6 found in breast milk. This may have important implications for the infant's health, as elevated levels of serum CRP and IL-6 are associated with cardiovascular disease, type 2 diabetes, and metabolic syndrome.^{14,15} There is abundant evidence that many non-nutritive factors found in breast milk can remain bioactive in the infant.¹⁶ The presence of protease inhibitors in milk,^{17,18} the immaturity of the infant gut and high gut pH that favors absorption of bioactive compounds,¹⁸⁻²⁰ and the existence of gut receptors for numerous milk hormones and cytokines,^{21,22} all support the notion that proteins in breast milk remain bioactive.

However, few studies have examined whether breast milk inflammatory markers, including CRP and IL-6, are altered by maternal obesity or weight gain patterns, or are related to infant outcomes.^{2,3,23,24} In previous work, we reported no association between maternal pre-pregnancy body mass index (BMI) with 1 and 6 month breast milk IL-6 levels in 37 exclusively breastfeeding women.³ Fujimori and colleagues also found no associations between maternal pre-pregnancy BMI category and colostrum IL-6 or CRP in 45 Brazilian

women.²³ However, we did observe an inverse association between breast milk IL-6 and infant weight-for-length z-score, bmi-for-age z-score, weight gain from birth to one month, percent fat, and fat mass.² Thus, while it is established that IL-6 and CRP are found in breast milk, and may play an active role in infant development, existing studies on a connection between maternal obesity or weight patterns on breast milk inflammatory markers are limited by small sample sizes. In addition, no studies to our knowledge have examined the associations of maternal weight status during and after pregnancy with breast milk CRP or IL-6. There is a need to better understand the biochemical impact of maternal weight status on breast milk inflammatory markers, which may have important implications for the intergenerational transmission of disease risk while potentially representing a nexus for intervention.²⁵

The aim of this study was to examine the associations between maternal weight status (pre-pregnancy BMI, total GWG, excessive GWG as defined by the IOM guidelines, and postpartum weight loss) with breast milk CRP and IL-6 measured at one and three months postpartum. Given that the existing literature shows a positive association between obesity and inflammatory markers, we hypothesize that high pre-pregnancy BMI and GWG, and low postpartum weight loss will be positively associated with breast milk CRP and IL-6 concentrations. Additionally, as our previous work and other studies have observed differences in breast milk composition by infant sex, we also tested for sex interactions.^{3,26,27}

Methods

Study Participants

The Mothers and Infants LinKed for Health (MILK) study is an ongoing prospective cohort study of exclusively breastfeeding mother-infant dyads recruited from Minneapolis, Minnesota and Oklahoma City, Oklahoma. The primary objective of the study is to assess the “lactational programming hypothesis,” or whether the recently documented variation in breast milk composition is related to both maternal adiposity and infant metabolic status. Women were eligible to participate if they met the following criteria: pregnant, 21–45 years of age at delivery, pre-pregnancy BMI between 18.5–40.0 kg/m², and reported an intention to exclusively breastfeed for at least three months. Women were excluded from participating if they reported tobacco or alcohol use (≥ 1/week) during pregnancy or lactation; had a history or current presence of diabetes mellitus (Type I, Type II, or gestational); presumed or known congenital metabolic, endocrine disease, or congenital illness affecting infant feeding or growth; or were unable to speak or understand English. Women who gave birth to singleton infants between 37–42 weeks gestation who were normal birth weight (2,500–4,500 grams), and reported exclusive breastfeeding were asked to provide milk samples at the study center at one month postpartum. Women who attended the one month visit were asked to return at three months postpartum and provide a second milk sample, regardless of whether they were exclusively breastfeeding or not. Written informed consent was obtained at baseline, and the institutional review boards at the University of Minnesota, Health Partners Institute for Education and Research, and the University of Oklahoma Health

Sciences Center approved all study protocols. Participants received stipends for completion of each measurement visit.

At the time the analyses were conducted for the present study, a total of 492 mother-infant dyads were enrolled in the MILK study, N=261 of whom were exclusively breastfeeding at 1 month and therefore eligible for milk analysis. Breast milk concentrations have been assayed for women who either attended both a one- and three-month postpartum visit, or had completed the study as of September 1, 2016, resulting in a final study sample for this analysis of 134 women. Eight women who provided a breast milk sample at the one month visit did not attend the three month visit (n=4) or provide adequate breast milk samples for analyses (n=4), resulting in a sample of 126 at three months.

Maternal Anthropometrics

Pre-pregnancy BMI was calculated using available weight and height from the participant's electronic medical records, within 6 weeks of conception. Pre-pregnancy weight was subtracted from weight at delivery (abstracted from electronic medical records), to calculate total GWG. Based on their pre-pregnancy BMI, women were classified as below, within or exceeding the 2009 Institute of Medicine GWG guidelines.⁸ Postpartum weight loss was calculated as maternal weight at delivery minus maternal weight measured at the 1 and 3 month postpartum study visits.

Breast milk Collection and Assays

Mother-infant dyads came to the study centers at one and three months postpartum (± 5 days), between 8:00–10:00am. The mothers were asked upon arrival to feed their infants *ad libitum* from one or both breasts until their infants were satisfied. Two hours after this feeding, they were asked to provide a complete expression from the right breast using a hospital grade electric pump (Medela, Inc., McHenry, IL), ensuring the collection of fore-, mid- and hind-milk within each sample using similar procedures performed by others.^{13,25,26} The volume of milk collected was recorded, and milk was gently mixed, aliquoted and stored at -80°C within 20 minutes of collection.

Milk fat was separated from the aqueous phase by centrifugation, and the resulting skimmed milk was assayed using commercially available immunoassay kits for CRP and IL-6. Based on the literature,^{28,29} we expected breastmilk CRP and IL-6 to be low relative to serum. Thus, assays were chosen based on sensitivity and performance in validation experiments using skimmed milk as the test matrix (spike recovery and linearity 80–100% of expected values). CRP was assayed by ELISA (Abcam, Cambridge, MA; catalog number ab99995) in milk that was diluted 1000-fold prior to analysis. IL-6 was measured in undiluted milk using a chemiluminescent ELISA from R&D Systems (Minneapolis, MN; catalog number Q6000B) according to the manufacturer's protocol, except that the primary incubation was overnight at 4°C . For both assays, samples with values outside the assay range were reanalyzed at a higher or lower dilution, as appropriate. Inter-assay variability for CRP and IL-6 was 5.5% and 12.8%, and intra-assay variability was 4.7% and 9.1%, respectively. All breast milk samples were analyzed at the University of Oklahoma Health Sciences Center Metabolic Research Program laboratory.

Covariates

Maternal race, educational attainment, household income, and breastfeeding status (exclusive, partial, or none at one and three months) were self-reported. Additional information abstracted from medical records included maternal age, parity, mode of delivery (caesarean section or vaginal birth), and gestational age at delivery (weeks). The volume of milk from the single breast expression used for CRP and IL-6 analyses was recorded in milliliters.

Statistical Analyses

Descriptive statistics (means and frequencies) were calculated for participant characteristics, after stratifying by pre-pregnancy BMI category. Dependent variables (CRP and IL-6) were examined for normality, both were skewed and therefore log transformations were used for subsequent analyses. A variety of potential confounders were explored by examining the bivariate associations with maternal pre-pregnancy BMI category, including: maternal age, race, education, income, baseline parity, mode of delivery, gestational age at delivery, infant sex, breast milk volume, and breastfeeding status at 3 months (all participants exclusively breastfed at 1 month). Significant associations were observed for maternal education and gestational age at delivery; therefore these variables were included as potential confounders in adjusted models.

Repeated measures linear regression was used to test the associations between each of the four exposure variables: pre-pregnancy BMI (continuous), total GWG (continuous), GWG category (below/within or exceeds guidelines), and repeated within subject measures of postpartum weight loss at one and three months (continuous), with the two outcome variables: repeated within subject measures of breast milk CRP and IL-6 at one and three months postpartum, for a total of eight primary models (PROC MIXED). For analyses, individuals who gained weight below or within guidelines were combined because there were no significant differences in the outcomes between the two groups. All continuous independent variables were standardized to a mean of 0 and a standard deviation of 1 to facilitate comparisons between exposures. In all instances, the exposure was included as a main effect and as an interaction with time. We first examined in separate models, the crude associations of the four exposures with each of the two outcome variables, including time and an exposure by time interaction. Next, we controlled for maternal education and gestational age at delivery. Finally, the models for total GWG, excessive GWG, and postpartum weight loss also controlled for pre-pregnancy BMI. All potential confounders were included as a main effect, but only retained as an interaction with time if the corresponding estimate achieved $p < 0.1$. We also tested for interactions by infant sex by including cross-product terms in all models; however none were significant and therefore not included in the final models. All statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

Results

Maternal Characteristics

As seen in Table 1, the average maternal age was 30.9 years, with a range of 21.0–41.0 years. Of the mothers recruited, 50%, 28%, and 22% had a normal weight ($<25.0\text{kg/m}^2$), overweight ($25.0\text{--}29.9\text{kg/m}^2$), or obese pre-pregnancy BMI ($\geq 30.0\text{kg/m}^2$), respectively. Across pre-pregnancy BMI categories, significant differences were observed in maternal education and gestational age at delivery. Normal weight women were more likely to have a bachelor's degree or higher compared to women with overweight or obesity, and women with obesity delivered earlier on average than normal weight or overweight women. Women with overweight or obesity were also more likely to exceed the IOM weight gain guidelines as compared to normal weight women. Women with obesity also had lower average postpartum weight loss, and further, their mean postpartum weight loss at three months was less than their mean postpartum weight loss at 1 month, indicating average weight gain from 1 to 3 months in women with obesity.

C-Reactive Protein and Interleukin-6 Concentrations and Correlations

Average concentrations of raw and log transformed CRP and IL-6 at one and three months postpartum are shown in Table 2. There was a significant decrease in CRP and IL-6 levels from 1 to 3 months postpartum for both raw (median CRP difference = -6.0 , $p=0.01$; median IL-6 difference = -2.4 , $p<0.001$) and transformed data (mean CRP difference = -0.3 , $p<0.001$; mean IL-6 difference = -0.9 , $p<0.001$). Log-transformed CRP and IL-6 were correlated over time (CRP: $r = 0.76$, $p<0.001$; IL-6: $r=0.29$, $p=0.001$). The Pearson correlations between log-transformed CRP and IL-6 were $r=0.19$, $p=0.03$ at 1 month and $r=0.00$, $p=0.96$ at 3 months, respectively.

Associations of Maternal Anthropometrics with Breast Milk C-Reactive Protein

As seen in Table 3, for each one standard deviation increment in BMI, log transformed breast milk CRP was greater by 0.43 and 0.49 units ($p<0.001$ for all) in crude and adjusted analyses, respectively. Excessive GWG was also positively associated with breast milk CRP ($\beta=0.68$, $p<0.001$) and this association remained significant after adjustment for education and gestational age at birth ($\beta=0.67$, $p<0.01$), as well as pre-pregnancy BMI ($\beta=0.51$, $p=0.01$). Total GWG and postpartum weight loss at one and three months were not associated with breast milk CRP in any of the tested models. The exposure-time interaction was not significant across all models, indicating that the slope of the association between the exposure and breast milk CRP was similar at both one and three months postpartum.

Due to the observed associations of pre-pregnancy BMI and GWG category with breast milk CRP, we additionally present the adjusted means of back-transformed CRP at one and three months postpartum, stratified by pre-pregnancy BMI (normal weight, overweight, obese) and GWG categories (below/within or exceeds guidelines) (Figure 1). Values for CRP are presented for one and three months separately because there was a significant interaction between time and the six BMI-GWG categories in the repeated measures model ($p=0.03$). CRP levels generally increased with pre-pregnancy BMI category at both time points. When examining differences in the adjusted means across the six pre-pregnancy BMI and GWG

categories, we found that at one month postpartum, women who were normal weight and gained weight below/within the guidelines had significantly lower breast milk CRP values than all other groups of women. Normal weight women who exceeded GWG guidelines also had significantly lower CRP values than women with obesity, regardless of gestational weight gain category. At three months postpartum, we observed a similar pattern, where normal weight women who gained below/within guidelines had significantly lower CRP values than all other groups. Additionally, women with obesity who experienced excessive weight gain had significantly higher CRP values than all other groups ($p < 0.05$ for all).

Associations of Maternal Anthropometrics with Breast Milk Interleukin-6

There were no significant associations observed between maternal anthropometrics and IL-6 in any of the tested models (see Table 4).

Discussion

In this cohort study of breastfeeding mother-infant dyads, we found maternal pre-pregnancy BMI and excessive GWG were positively associated with breast milk CRP at one and three months postpartum. Normal weight women who gained weight below or within the IOM guidelines had the lowest breast milk CRP values, while women with obesity who gained excessive weight had the highest values. There were no associations between maternal pre-pregnancy BMI, GWG, or postpartum weight loss with breast milk IL-6. Our findings add to the existing literature by being the first to examine the association between multiple measures of maternal weight status and breast milk CRP and IL-6, in one of the largest cohort studies to date examining inflammatory markers in human breast milk. These findings may have important implications for intergenerational transmission of disease risk.

While the analysis of breastmilk inflammatory markers in epidemiological studies is still in its infancy and there are relatively few studies to which to compare our results, the mean levels of IL-6 are similar to what has been previously reported in breast milk.^{2,3} Previously reported values for breast milk CRP are higher than observed in this study; however, one study included women with mastitis,²⁸ and the second measured CRP levels in colostrum rather than mature breast milk,²³ therefore we would expect to observe higher values. Further, both of these studies used a less sensitive assay method than used in the current study.

There is limited research on the relationship of maternal weight and weight changes to breast milk cytokines; however, other studies have reported associations between maternal BMI and various other non-nutritive factors in breast milk. For example, Andreas and colleagues published a systematic review examining the associations between maternal BMI with various appetite regulating hormones found in breast milk, including: leptin, adiponectin, insulin, ghrelin, resistin, obestatin, Peptide YY, and Glucagon-Like Peptide 1.³⁰ The authors found positive associations between maternal BMI and breast milk leptin in 11 of the 15 identified studies. The evidence for an association between maternal BMI with adiponectin or insulin was more mixed, and the data was lacking or inconclusive for the other hormones examined.^{6,30} Inverse relationships have been observed between breast milk leptin, adiponectin, and insulin with measures of infant growth in the early postnatal

period,¹⁶ which suggests that certain bioactive compounds in breast milk may enter the infant's circulation, thereby having an effect on the health of that infant.

We observed no associations with maternal weight status and breast milk IL-6. This is surprising, as IL-6 is a precursor molecule for CRP,¹⁴ and is consistently found at increased levels in both adipose tissue and serum of individuals with obesity.³¹ In addition, a previous report showed that cesarean section delivery (which is more common in women with obesity compared to women of normal weight) is associated with elevated breastmilk IL-6 as well as TNF-alpha concentrations.³² There are multiple potential explanations for these discrepancies in findings. First, the correlation between breast milk IL-6 and CRP was small to null at one and three months; therefore, the differential associations may be due in part to the weak relationships between the two inflammatory markers. This is interesting, as IL-6 and CRP measured in serum are moderately correlated.³³ Second, evidence suggests that IL-6 levels in serum are not associated with IL-6 levels in human breast milk,³⁴ while a strong correlation between CRP in serum and breast milk was reported in a study of canine mastitis ($r=0.69$).³⁵ Furthermore, while lactocytes obtain substrates for milk production from the maternal circulation, including circulating IL-6 and CRP,³⁶ these cells also have the ability to spontaneously produce IL-6 in the absence of stimulation,³⁷ while it is unknown whether lactocytes produce CRP.

Given their naïve and immature adaptive immune systems, newborns must rely upon the innate immune system to defend against pathogenic infections.³⁸ Breast milk has an abundance of immune factors including immunoglobulin A, lactoferrin, epidermal growth factor, and many others that act as a part of the innate immune system of the infant gastrointestinal tract. Human milk interleukins and TNF-alpha, among others constitute another class of bioactive compounds with local anti-microbial effects on the infant gut mucosa,³⁸ altering the risk of gastrointestinal infection and possibly also the risk of infant allergies.³⁹ CRP has antimicrobial properties and clears bacterial infections.⁴⁰ If breast milk CRP survives digestion, it could thereby alter the diversity and relative abundance of different intestinal microbiota in early development. Our finding that CRP is elevated in the milk of women with obesity and excessive gestational weight gain suggests the need for mechanistic research to delineate the links between maternal nutrition, the wide variety of milk immune factor concentrations, and infant outcomes.

To our knowledge, this is the largest study to date examining associations of maternal anthropometrics with breast milk inflammatory markers. Other major strengths include the rigorous standardized protocol for breast milk collection, longitudinal collection of breast milk samples, multiple measures of maternal weight status, and examination of many potential confounders. However, multiple limitations must be noted. We did not examine CRP and IL-6 concentrations separately in breast milk foremilk and hindmilk. A study examining breast milk appetite-regulating hormones found differences in hormone concentrations and associations with maternal BMI depending on whether the measure was derived from foremilk or hindmilk;⁶ therefore it is possible that observed differences in breast milk inflammatory markers would depend on the stage of nursing. While the multiple measures of maternal weight status are a notable strength of this paper, they are not true measures of adiposity, but rather surrogate markers. Future studies should use more accurate

measures of body composition, such as skin-fold measures or dual x-ray absorptiometry. Additionally, our sample was largely limited to White women, and study findings may not be generalizable to women of other races or ethnicities.

In conclusion, we found that pre-pregnancy BMI and excessive GWG were positively associated with breast milk CRP, while no associations were observed between maternal weight status and breast milk IL-6. The consequences of infants receiving various concentrations of breast milk inflammatory markers are unknown. Given our findings, which may have important implications for the offspring's risk of future health outcomes, our next step is to examine the associations of breast milk inflammatory markers with infant growth.

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What is already known about this subject?

- Obesity is associated with elevated serum levels of inflammatory markers, including C-Reactive Protein and Interleukin-6.
- Prior studies have reported associations of maternal obesity with altered levels of breast milk hormones, but little is known about the associations between maternal weight status and breast milk inflammatory markers.

What does your study add?

- This is the first study to examine associations of maternal weight status before, during, and after pregnancy with two important markers of inflammation found in breast milk.
- Our study found an independent and positive association of maternal pre-pregnancy body mass index and excessive gestational weight gain with breast milk C-Reactive Protein, but not Interleukin-6.
- Study findings may have important implications for the intergenerational transmission of disease risk.

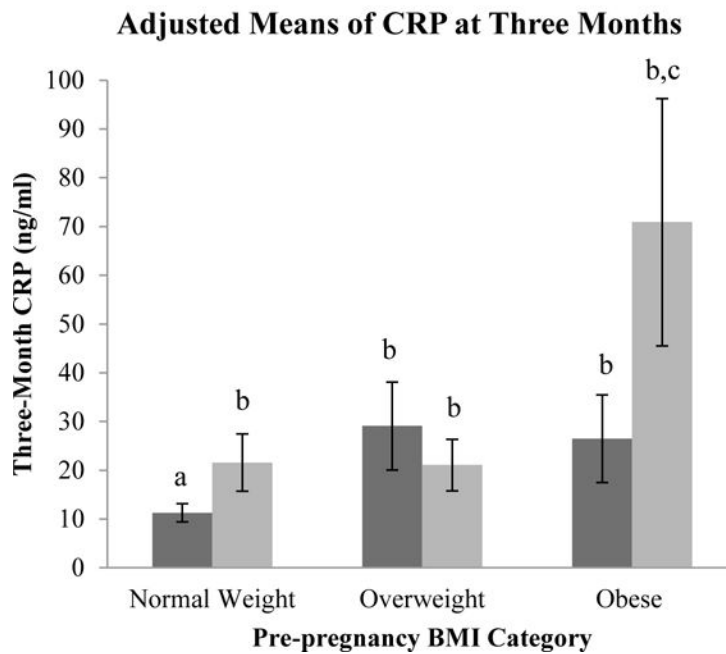
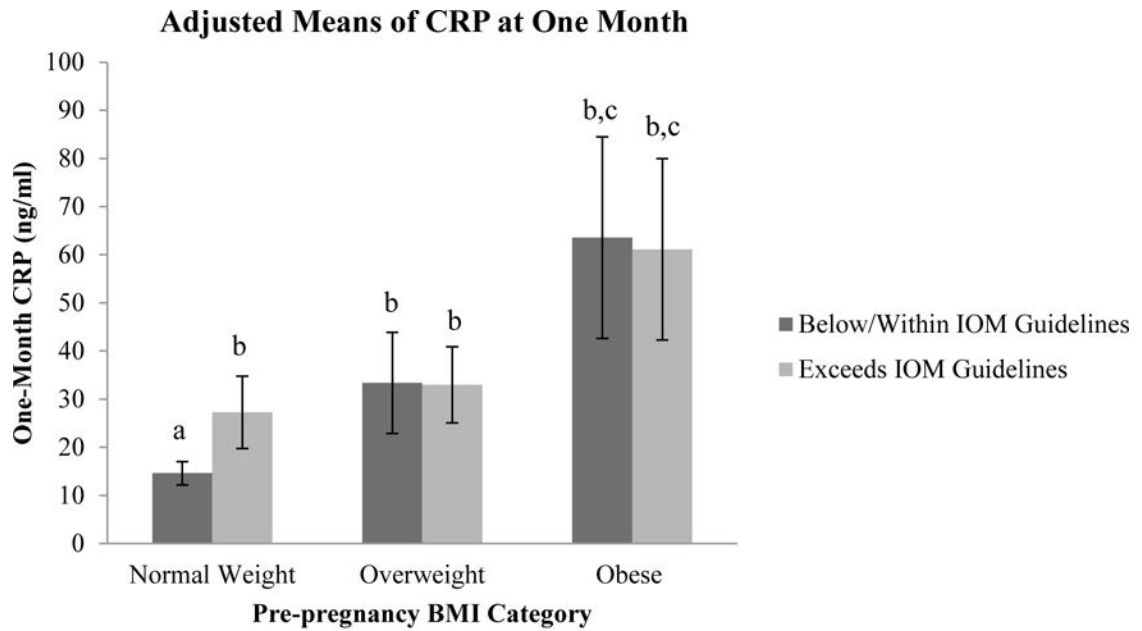


Figure 1. Adjusted means of back-transformed C-reactive Protein (CRP) at one and three months postpartum by pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) category

Differences between the mean values for each pre-pregnancy BMI and GWG categories are denoted by different superscript letters (a, b, or c). In the top panel, at one month postpartum, normal weight women who gained weight below/within the guidelines (a) had significantly lower breast milk CRP than all other groups (b). Normal weight women who exceeded GWG guidelines also had significantly lower CRP than women with obesity (c), regardless of gestational weight gain category. In the bottom panel, at three months

postpartum, normal weight women who gained weight below/within guidelines (a) had significantly lower CRP than all other groups (b). Women with obesity who experienced excessive weight gain (c) had significantly higher CRP than all other groups ($p < 0.05$ for all).

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Participant characteristics at baseline and one and three months postpartum, stratified by pre-pregnancy body mass index (BMI)

Table 1

Characteristics	Pre-pregnancy BMI Category				P-value
	Total (N=134)	Normal (N=67)	Overweight (N=38)	Obese (N=29)	
Age	30.9 ± 4.1	30.5 ± 3.9	32.1 ± 4.2	30.2 ± 4.3	0.114
Race					0.870
White	118 (89.4)	59 (88.1)	35 (92.1)	24 (88.9)	
Other	14 (10.6)	8 (11.9)	3 (7.9)	3 (11.1)	
Education					0.007
High school/GED or Associates Degree	35 (26.5)	10 (15.4)	10 (26.3)	15 (51.7)	
Bachelor's Degree	56 (42.4)	31 (47.7)	15 (39.5)	10 (34.5)	
Graduate Degree	41 (31.1)	24 (36.9)	13 (34.2)	4 (13.8)	
Annual household income					0.326
< \$60,000	44 (33.3)	18 (27.7)	12 (31.6)	14 (48.3)	
\$60,000-\$90,000	36 (27.3)	21 (32.3)	9 (23.7)	6 (20.7)	
> \$90,000	52 (39.4)	26 (40.0)	17 (44.7)	9 (31.0)	
Baseline Parity					0.918
None	53 (41.1)	27 (41.5)	15 (44.5)	11 (40.7)	
1 child	51 (39.5)	27 (41.5)	13 (35.1)	11 (40.7)	
2 children	25 (19.4)	11 (16.9)	9 (24.3)	5 (18.5)	
Mode of Delivery					0.109
Vaginal	68 (50.8)	55 (82.1)	28 (76.3)	18 (62.1)	
Cesarean	66 (49.3)	12 (17.9)	9 (23.7)	11 (37.9)	
Gestational Age at Delivery		39.8 (1.1)	40.1 ± 0.9	39.1 ± 1.1	<0.001
Infant Sex					0.130
Female	68 (50.8)	29 (43.3)	18 (47.4)	19 (65.5)	
Male	66 (49.3)	38 (56.7)	20 (52.6)	10 (34.5)	
Breast milk Volume, ml					

Pre-pregnancy BMI Category					
Characteristics	Total (N=134)	N (%) or Mean \pm SD			P-value
		Normal (N=67)	Overweight (N=38)	Obese (N=29)	
1 month	67.0 \pm 39.8	66.5 \pm 41.9	72.9 \pm 35.1	60.4 \pm 40.8	0.443
3 months	72.1 \pm 40.2	78.7 \pm 44.4	65.4 \pm 31.8	65.4 \pm 39.1	0.206
Exclusive Breastfeeding at 3 Months		58 (93.6)	33 (94.3)	24 (85.7)	0.428
Pre-pregnancy BMI, kg/m ²	26.1 \pm 5.3	22.0 \pm 1.7	27.1 \pm 1.4	34.3 \pm 3.7	<0.001
Gestational weight gain, kg	13.1 \pm 6.8	13.4 \pm 5.3	13.9 \pm 6.6	11.4 \pm 9.5	0.279
Institute of Medicine Gestational Weight Gain Guidelines					0.002
Below	34 (25.4)	20 (29.9)	6 (15.8)	8 (27.6)	
Within	42 (31.3)	29 (43.3)	7 (18.4)	6 (20.7)	
Exceeds	58 (43.3)	18 (26.9)	25 (65.8)	15 (51.7)	
Postpartum weight loss, kg					
At 1 Month	8.9 \pm 3.6	8.5 \pm 3.4	9.4 \pm 3.8	9.1 \pm 4.0	0.465
At 3 Months	10.1 \pm 4.5	10.8 \pm 4.1	10.3 \pm 4.8	8.3 \pm 4.3	0.046

P-value testing for differences in participant characteristics by pre-pregnancy BMI category using chi-square, fishers exact test, or one-way ANOVA, as appropriate. Bolded values are statistically significant (p<0.05). Data presented as column percentages. Small variation in sample sizes across covariates due to missing data.

Table 2

Summary statistics for breast milk inflammatory markers at one and three months postpartum

Breast milk Inflammatory Markers	1 Month (N=134)	3 Months (N=126)	P-value^a
C-reactive Protein, ng/ml	24.5 (11.2, 67.1)	18.5 (8.0, 43.9)	0.007
Log C-reactive Protein	3.3 ± 1.2	3.0 ± 1.2	<0.001
Interleukin-6, pg/ml	4.4 (2.0, 12.2)	2.0 (0.9, 5.2)	<0.001
Log Interleukin-6 ^b	2.0 ± 1.2	1.4 ± 0.9	<0.001

Data presented as Median (interquartile range) or Mean ± SD.

^aP-value testing for differences between 1 and 3 month summary statistics using Wilcoxon signed rank sum test for non-transformed data and paired t-tests for log-transformed data, bolded values are statistically significant (p<0.05).

^bThe value of one was added to the raw Interleukin-6 values before log-transformations were performed to avoid negative values on the log scale.

Associations of maternal anthropometrics with log-transformed breast milk C-Reactive Protein at one and three months postpartum^a

Table 3

Maternal Characteristics	Model 1 ^b				Model 2 ^c				Model 3 ^d			
	N	β	SE	p-value	N	β	SE	p-value	N	β	SE	p-value
Pre-pregnancy body mass index, kg/m ²	134	0.430	0.099	<0.001	132	0.488	0.105	<0.001	–	–	–	–
Gestational weight gain, kg	134	0.035	0.108	0.748	132	0.026	0.110	0.816	132	0.102	0.103	0.327
Exceeds gestational weight gain guidelines ^e	134	0.682	0.205	0.001	132	0.667	0.209	0.002	132	0.513	0.198	0.011
Postpartum weight loss at 1 and 3 months, kg ^f	133	-0.102	0.082	0.215	132	-0.117	0.082	0.157	132	-0.117	0.079	0.138

^aAll continuous independent variables (pre-pregnancy body mass index, gestational weight gain, and postpartum weight loss) were standardized to a mean of 0 and a standard deviation of 1. Bolded values are statistically significant ($p < 0.05$).

^bModel 1 is the crude model.

^cModel 2 adjusts for maternal education and gestational age at delivery.

^dModel 3 adjusts for covariates in Model 2, as well as pre-pregnancy BMI.

^eCalculated using the 2009 Institute of Medicine gestational weight gain guidelines, based on pre-pregnancy BMI.

^fPostpartum weight loss was calculated as maternal weight at delivery minus maternal weight at one or three months postpartum, and was entered into the mixed effects models as a time-varying independent variable.

Associations of maternal anthropometrics with log-transformed breast milk Interleukin-6 at one and three months postpartum^a

Table 4

Maternal Characteristics	Model 1 ^b				Model 2 ^c				Model 3 ^d			
	N	β	SE	p-value	N	β	SE	p-value	N	β	SE	p-value
Pre-pregnancy body mass index, kg/m ²	134	0.073	0.103	0.480	132	0.026	0.106	0.809	–	–	–	–
Gestational weight gain, kg	134	0.056	0.105	0.593	132	0.088	0.105	0.402	132	0.099	0.106	0.353
Exceeds gestational weight gain guidelines ^e	134	0.183	0.202	0.364	132	0.190	0.199	0.342	132	0.180	0.202	0.373
Postpartum weight loss at 1 and 3 months, kg ^f	133	0.015	0.091	0.874	132	0.045	0.091	0.626	132	0.045	0.092	0.625

^aAll continuous independent variables (pre-pregnancy body mass index, gestational weight gain, and postpartum weight loss) were standardized to a mean of 0 and a standard deviation of 1. Bolded values are statistically significant ($p < 0.05$).

^bModel 1 is the crude model.

^cModel 2 adjusts for maternal education and gestational age at delivery.

^dModel 3 adjusts for covariates in Model 2, as well as pre-pregnancy BMI.

^eCalculated using the 2009 Institute of Medicine gestational weight gain guidelines, based on pre-pregnancy BMI.

^fPostpartum weight loss was calculated as maternal weight at delivery minus maternal weight at one or three months postpartum, and was entered into the mixed effects models as a time-varying independent variable.