The Impact of Maternal Obesity and Breast Milk Inflammation on Developmental Programming of Infant Growth

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Abstract

Background: Little is known about how maternal obesity impacts breast milk (BM) composition and how BM composition may impact growth. We sought to determine the role of maternal body mass index (BMI) on BM inflammatory and oxidative stress markers and to delineate the role of these BM markers on infant growth.

Methods: This was a secondary analysis of 40 mother-infant dyads. We first assessed the association between maternal BMI and BM marker (omega-6:omega-3 polyunsaturated fatty acid ratio (n-6:n-3 PUFA), leptin, interleukin (IL)-8, IL-6, IL-1β and malondialdehyde (MDA)) concentration at one (V1) and four (V4) months postpartum. We then examined the association between BM markers on infant growth trajectory from birth to seven months.
**Results:** Higher maternal BMI was associated with higher BM n-6:n-3 PUFA (V1 β=0.12, 95% CI 0.01, 0.2; V4 β=0.13, 95% CI 0.01, 0.3) and leptin (V1 β=107, 95% CI 29, 184; V4 β=254, 95% CI 105, 403) concentrations. Infants exposed to high BM n-6:n-3 PUFA had higher BMI z-scores over time (p=0.01). Higher BM leptin was associated with lower infant percent fat mass at V4 (β=−9, 95% CI −17, −0.6). Infants exposed to high BM IL-8, IL-6, or IL-1β had higher weight z-scores over time (IL-8 p<0.001; IL-6 p=0.001; IL-1β p=0.02). There was no association between BM MDA and maternal BMI or infant growth.

**Conclusions:** Higher maternal BMI is associated with higher BM n-6:n-3 PUFA and leptin concentrations. In addition, higher BM n-6:n-3 PUFA and inflammatory cytokines were associated with accelerated weight gain in infancy.

**INTRODUCTION**

Obesity is a growing epidemic worldwide. One third of women enter pregnancy obese. Children born to obese mothers are 3–5 times more likely to become obese as adults. Potential biomedical mechanisms that have been proposed include genetics, fetal metabolic programming, and lactational metabolic programming. Evidence from a murine model suggests that breast milk (BM) composition has a significant impact on offspring growth and cardiometabolic health. There is limited human evidence in this area, making investigation into the role of lactational programming in transgenerational obesity critically important, as some components of BM are potentially modifiable with maternal dietary and healthy lifestyle interventions.

We and others have reported that differences in BM composition in obese women may play a role in programming of offspring obesity. The BM of obese women has been found to have increased levels of CRP, inflammatory cytokines, leptin, and insulin. Our team has looked specifically at BM fatty acids and found that obese women have a higher ratio of the more pro-inflammatory omega-6 (n-6) to omega-3 (n-3) polyunsaturated fatty acids (PUFAs). A recent study evaluating BM PUFA concentrations did not find a difference between lean and obese women. However, in this cohort there was also no difference in the serum concentration of n-6 and n-3 PUFAs between the two groups. Additional studies have evaluated the impact of BM markers on infant growth with mixed findings. Some studies noted a positive association between BM marker and infant growth while others found a negative association at discrete timepoints in the first two years of life. Other studies have reported no association between BM markers and infant growth. To our knowledge, there have not been any studies examining the association between BM PUFA, cytokine, and adipokine concentration and their influence on the trajectory of infant growth during the critical period of early infancy. Rapid weight gain in infancy and early childhood has been shown to be a predictor of obesity in adulthood so identifying modifiable components of BM that influence growth has the potential to alter the overall lifetime obesity risk.

Given the paucity of human studies examining the link between BM markers and infant growth; the goal of our study was to determine the association between maternal body mass index (BMI), BM composition, and infant growth trajectories. We hypothesized that higher
maternal BMI would be associated with higher concentrations of BM inflammatory cytokines, adipokines, markers of oxidative stress, and proinflammatory fatty acids and that higher concentrations of these inflammatory markers would be associated with higher infant adiposity.

SUBJECTS AND METHODS

Participants

This study was a secondary analysis of participants who provided informed consent to complete a randomized trial of maternal vitamin D supplementation during lactation\textsuperscript{22}. This study was classified as exempt by the Institutional Review Board of Brigham and Women’s Hospital. Eligibility criteria for the parent study are reported in previous publications from this cohort\textsuperscript{22}.

For this study, we included 40 of 460 mother-infant dyads from the original cohort who were a part of the control group (infants received 400 IU vitamin D daily per AAP recommendations and mothers received standard of care consisting of a prenatal vitamin containing 400 IU vitamin D). Maternal BMI (kg/m\textsuperscript{2}) was calculated using height and weight measured at the one-month postpartum visit (V1). From the total sample of 40 women included in the study, 20 had a BMI <30 kg/m\textsuperscript{2} and 20 had a BMI ≥30 kg/m\textsuperscript{2}. We excluded dyads who reported more than one formula feeding per day.

Measures

**BM Samples:** BM samples were obtained at two time points, V1 and four-months (V4). Samples were non-fasting and collected either directly prior to or during the study visit. BM samples were stored at −20\degree C as whole milk. We chose to focus on inflammation and oxidative stress by measuring fatty acids, leptin, the cytokines IL-8, IL-6, and IL-1\(\beta\), and malondialdehyde (MDA), given their association with obesity, inflammation, and metabolic dysregulation.

**Fatty Acids:** To measure PUFAs, total BM lipids were extracted and analyzed following the protocol previously described\textsuperscript{23}. Briefly, plasmalogens in the total lipid extract were converted to dimethyl acetals and fatty acids converted to fatty acid methyl esters (FAME). Methylated fatty acids were separated with a BPX70 high-resolution column 10 m × 0.1 mm ID × 0.2 \(\mu\)m (Canadian Life Science, ON, Canada) and analyzed via gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID).

**Leptin and Cytokines:** Adipokines and cytokines were measured using electrochemiluminescence (ECL) on the Meso Scale Discovery (MSD) Sector imager S600 platform (MSD, Gaithersburg, MD, USA) as previously described\textsuperscript{24},\textsuperscript{25}, with assay parameters listed in Supplemental Table S1.

**MDA:** BM whey was separated from whole BM, according to the method previously described\textsuperscript{26} and modified by Vaidya and Cheema\textsuperscript{27}. MDA concentrations were measured using a thiobarbituric acid reactive substance (TBARS) assay kit (cat. no. KGE013) following the manufacturer’s protocol (R&D Systems, Inc, Minneapolis, MN, USA). The
analytical range for MDA assay was 0.26 μM to 16.7 μM; the lower limit of detection for the MDA assay in samples was 0.61 μM.

**Anthropometrics:** Infant measurements were obtained monthly from 1 to 7 months of age as previously described. Birth weight z-scores were calculated using the 2010 Olsen growth charts. Monthly weight, length, and BMI z-scores were calculated from WHO reference data using a 2005 macro (the WHO Child Growth Standards SPSS Syntax File [igrowup.sps]).

**Body Composition:** Infants underwent whole-body dual-energy X-ray absorptiometry (DXA) scans at 1, 4, and 7 months of age (V7) as previously described. We obtained global mass, fat mass, and lean mass from DXA results. We were able to determine percent fat mass by dividing fat mass by global mass and percent lean mass by dividing lean mass by global mass.

**Statistical analysis**

**Maternal BMI and BM composition:** We used linear regression to analyze the association between maternal BMI and BM marker. We adjusted for maternal race and days postpartum at time of BM collection. The primary exposure was maternal BMI at one-month postpartum. The primary outcomes were n-6:n-3 PUFA, leptin, IL-8, IL-6, IL-1β, and MDA concentration in BM expressed at V1 and V4.

**BM composition and infant growth:** We then analyzed the association between BM markers and infant growth outcomes. BM marker concentration from V1 and V4 samples were highly correlated, so the mean value of the two samples was used for further analysis. We first used linear regression, adjusting for maternal race, infant age at the time of growth measurement, and baseline infant measurement (measured at birth or V1) to evaluate the association between mean BM marker concentration with infant BMI, weight, and length z-scores, percent fat mass, and percent lean mass at V4 and V7.

We next analyzed infant growth trajectories between V1 and V7 to understand the role of BM components on longitudinal changes in infant body habitus. We performed linear mixed model regression adjusting for maternal race and infant sex. We used mean BM marker concentration from V1 and V4 samples as our exposure. For each BM marker, we divided the cohort into two groups, infants exposed to high BM marker (above the median) and infants exposed to low BM marker (below the median). We analyzed trajectories of infant BMI, weight, and length z-scores, percent fat mass, and percent lean mass by group of BM marker concentration (high vs. low). We also compared each outcome by group (high vs. low BM marker) at each timepoint cross-sectionally. We performed these analyses both with and without maternal BMI as a confounder and did not see a difference in the strength of the associations. All statistical analysis was performed using SPSS 24 and STATA 15.
RESULTS

Demographics

Characteristics of the mothers and their infants are shown in Table 1. The characteristics of our sub-study cohort were similar to the original cohort. The mothers had an average BMI of 29 kg/m$^2$ and were predominantly Caucasian. The infants were predominately male with an average gestational age of 39 weeks.

Maternal BMI and BM composition

Higher maternal BMI was associated with a higher BM n-6:n-3 PUFA ratio at V1 ($\beta=0.12, 95\% \text{ CI } 0.01, 0.2$) and V4 ($\beta=0.13, 95\% \text{ CI } 0.01, 0.3$) (Table 2). Higher maternal BMI was also associated with higher BM leptin concentrations at V1 ($\beta=107, 95\% \text{ CI } 29, 184$) and V4 ($\beta=254, 95\% \text{ CI } 105, 403$). Maternal BMI was not associated with BM IL-8, IL-6, IL-1$\beta$, or MDA at V1 or V4 (Table 2).

BM composition and infant growth at four-months and seven-months

Higher mean BM n-6:n-3 PUFA, IL-8, and IL-1$\beta$ were associated with higher length z-scores in infants at V4 (n-6:n-3 $\beta=0.2, 95\% \text{ CI } 0.04, 0.4$; IL-8 $\beta=0.6, 95\% \text{ CI } 0.2, 1$; IL-1$\beta$ $\beta=0.4 95\% \text{ CI } 0.02, 0.8$, Table 3). Higher BM n-6:n-3 PUFA and IL-8 were associated with higher infant BMI z-score at V7 (n-6:n-3 $\beta=0.3, 95\% \text{ CI } 0.1, 0.5$; IL-8 $\beta=0.6, 95\% \text{ CI } 0.02, 1.1$). In an unadjusted model, higher BM IL-8 was also associated with higher infant weight z-score at seven-months, but this association was attenuated after adjustment. Higher BM leptin was associated with lower infant percent fat mass and higher infant percent lean mass at V4 ($\beta_{\text{fat}}=-9, 95\% \text{ CI } -17, -0.6$; $\beta_{\text{lean}}=9.1, 95\% \text{ CI } 0.6, 17.5$) but not V7.

High vs. Low BM markers and infant growth trajectories

**BM n-6:n-3 PUFAs:** BMI, weight, and length z-scores all had significantly different trajectories between infants exposed to high vs. low BM n-6:n-3 PUFA (p=0.01, p<0.001, p=0.03, Table 4 and Figure 1). Specifically, BMI z-scores of infants exposed to high BM n-6:n-3 PUFA increased over seven months by 0.1 U/mo on average, while BMI z-score of infants exposed to low BM n-6:n-3 PUFA decreased by 0.08 U/mo.

**BM Leptin:** Infants exposed to high BM leptin had lower BMI z-scores at V1 and remained lower compared to infants exposed to low BM leptin (Supplemental Figure S1). Infants exposed to high BM leptin also had higher percent lean mass and lower percent fat mass at V1 and V4 (p=0.04 and p=0.004), but did not have significantly different trajectories over the study period.

**BM IL-8, IL-6, IL-1$\beta$:** Infants exposed to high BM cytokines had higher weight z-scores at birth with an initial decrease during the first 1–2 months of life followed by an increase over the next six months compared to the infants exposed to low BM cytokines (IL-8 p<0.001; IL-6 p<0.001; IL-1$\beta$ p=0.02, Supplemental Figures S2, S3, S4).

**BM MDA:** There was no significant difference in trajectories for infants exposed to high vs. low BM MDA (Supplemental Figure S5).
DISCUSSION

We used data from a longitudinal birth cohort to delineate the role of maternal BMI and BM markers on infant growth and adiposity outcomes. Expanding beyond previous literature, we were able to assess the associations of BM composition at two timepoints with longitudinal growth trajectories, as well as assess the difference in body composition at multiple time points.

We found a positive association between maternal BMI and BM n-6:n-3 PUFA and leptin concentrations, consistent with previous literature\textsuperscript{11–15}. The importance of this relationship lies in the potentially modifiable nature of these BM components. We did not find an association between maternal BMI and BM cytokines or oxidative stress. This is consistent with prior studies that assessed the relationship between maternal BMI and BM IL-6 and IL-8\textsuperscript{8,13,31}, while the association with BM IL-1β and MDA has not previously been explored. Given the known association between obesity and systemic inflammation\textsuperscript{32}, other studies have investigated the correlation between serum and BM cytokine levels with no clear relationship identified\textsuperscript{12,33}. This difference in concentration is likely related to the multiple factors stimulating or inhibiting expression of these markers in BM such as maternal and neonatal infection and the role immune cells play in the neonatal immune system\textsuperscript{34}. Additional studies are needed to further explore how maternal obesity might alter this balance of inflammation and immune modulation.

Obesity is highly associated with differences in dietary intake, including an unbalanced ratio of n-6:n-3 PUFA consumption\textsuperscript{35,36}. N-6 and n-3 fatty acids are not synthesized de novo in mammary glands or other parts of the human body; therefore, the BM concentration of these essential fatty acids is dependent on maternal dietary intake\textsuperscript{37}. Maternal DHA supplementation during lactation has been shown to increase maternal plasma DHA and BM DHA levels\textsuperscript{38}. Women who eat a diet rich in foods containing n-6 PUFAs, such as meat and poultry, have been found to have higher levels of n-6 PUFAs in their BM compared to women who eat a diet with lower n-6 PUFA intake\textsuperscript{39}. We found that BM n-6:n-3 PUFAs were associated with higher infant BMI z-score at seven months of age and even more strikingly, were associated with BMI and weight z-score gain compared to a BMI z-score loss in infants exposed to low BM n-6:n-3 PUFAs. Exposure to n-3 and n-6 fatty acids may have differing effects on body fat gain through mechanisms of adipogenesis, lipid homeostasis, and systemic inflammation\textsuperscript{40–42}. Metabolites of n-6 PUFAs have been shown to play an important role in the differentiation of pre-adipocytes to mature adipocytes, while n-3 PUFAs can inhibit this maturation\textsuperscript{43,44}. Previous in vitro work analyzing the impact of BM PUFAs on adipose tissue metabolism found that BM with a higher ratio of n-6:n-3 PUFA increased the expression of genes involved in lipogenesis\textsuperscript{27}. Future studies should investigate how adipogenesis may be impacted by BM n-6:n-3 composition and whether altering this ratio during critical periods of development, such as early infancy, can impact growth trajectories. Given the association between maternal diet and BM fatty acid concentrations, a change in diet may affect BM PUFA composition, which could potentially impact infant weight gain. Randomized controlled trials are needed to definitively understand the impact of maternal fat intake on BM n-6:n-3 PUFA composition and subsequent infant growth patterns.
In addition to differences in diet, obesity is also associated with a unique metabolic profile. Obese individuals have elevated leptin concentrations due to increased secretion by adipocytes. During lactation, leptin is produced by mammary epithelial cells and secreted into BM as a component of the milk fat globule. Adipocytes have been shown to secrete factors that influence mammary epithelial cell differentiation, potentially increasing the concentration of leptin in BM. Perhaps mediating the association between maternal BMI and BM leptin concentration is the higher maternal serum leptin concentrations in obesity. Weight loss via dietary alterations and exercise has been shown to decrease circulating leptin levels in obese individuals. How this reduction in serum leptin would impact BM leptin concentration has not been investigated. Our study found higher BM leptin to be associated with lower infant percent fat mass and higher infant percent lean mass at one and four months, but not at seven months of age. Prior studies have investigated the association between BM leptin and infant growth outcomes. Several studies did not identify an association between BM leptin and infant growth, while others found an inverse relationship between BM leptin and infant weight gain, length, and lean body mass over the first two years of life. Leptin primarily acts on the hypothalamus to regulate food intake. Initial exposure to leptin suppresses appetite, but if leptin levels remain elevated, such as in obesity, leptin resistance can develop leading to increased appetite and food intake. Animal studies have shown that exposure to elevated n-6 PUFA induces central cellular leptin resistance. Our findings that BM from obese mothers has higher concentrations of both n-6:n-3 PUFA and leptin raises the question of how this interaction might impact offspring appetite, subsequent feeding behaviors, and long-term growth. Future studies assessing growth outcomes over a longer duration are imperative to further characterize the role of BM leptin on offspring growth.

Exposure to high vs. low BM IL-8, IL-6, and IL-1β was associated with a difference in infant weight z-score trajectory over the study period. A previous study investigating the impact of BM IL-6 on infant growth found that infants exposed to higher BM IL-6 had lower weight for length z-scores, BMI z-scores, weight gain, percent fat mass, and total fat mass at one month of age. This is consistent with our findings at one month, but our longitudinal data shows a higher weight z-score over the first few months of life when exposed to high BM IL-6. Obesity is associated with adipocyte hypertrophy and dysfunction, resulting in elevated cytokine secretion which ultimately induces a chronic state of inflammation and metabolic dysregulation. The association of elevated levels of BM cytokines and infant weight z-scores may be related to the inflammatory exposure causing metabolic dysregulation and adiposity accrual as is seen in obese individuals.

**Strengths and Limitations**

The strengths of this study include its longitudinal design and compartmental infant body composition measurements. Infant body composition was assessed at three timepoints using DXA which is precise and accurate in infants. The repeated measures in this dataset enabled us to assess trajectory of growth allowing for a more thorough assessment of the growing infant over time. We adjusted for several variables that could confound the relation of BM composition and infant growth, including maternal race, infant age at the time of measurement, infant sex, and baseline infant measurement. However, given the observational...
nature of this study, the possibility of residual confounding remains present. The limitations of this study include the small cohort, which could likely impute low power for some associations. We were underpowered for mediation analysis for assessing the association of maternal BMI on BM composition or on infant growth. This study consisted of predominantly exclusively breastfed infants, however there was some formula supplementation limited to less than daily, but this exposure could have impacted infant growth outcomes. BM samples were non-fasting and collected either before or during a study visit which could impact BM composition.

Conclusion

This study demonstrates that higher maternal BMI was associated with higher BM n-6:n-3 PUFA and leptin concentrations. In addition, higher BM n-6:n-3 PUFA and inflammatory cytokines were associated with accelerated weight gain in infancy. These findings and the association between childhood obesity and medical comorbidities reiterate the importance of future studies investigating the role of maternal obesity on lactational programming and offspring obesity risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We would like to thank Dr. Thu Huong Pham and Peter O. Iselele for their expertise in breast milk analyte analysis.

FUNDING

National Institutes of Health (NIH) 5R01HD043921, NIH RR01070, NIH P30 DK040561, NIH/National Center for Advancing Translational Sciences UL1 TR000062.

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Figure 1:
Trajectories of infant body mass index (BMI) z-score (A), weight z-score (B), length z-score (C), percent fat mass (D), and percent lean mass (E) in infants exposed to high vs. low breast milk (BM) n-6:n-3 PUFA. Results are displayed as estimated means ± SEM from mixed model regression adjusted for maternal race and infant sex. *p-value <0.05.
Table 1:

Demographics of study participants.

|                         | Study cohort |
|-------------------------|--------------|
| **N**                   | 40           |
| **Maternal**            |              |
| BMI (kg/m²), mean (SD)  | 29 (5.1)     |
| Age (years), mean (SD)  | 31 (6)       |
| Race/Ethnicity, n (%)   |              |
| Caucasian, non-Hispanic | 24 (60%)     |
| Hispanic                | 10 (25%)     |
| African-American, non-Hispanic | 4 (10%) |
| Asian or Pacific Islander | 2 (5%)     |
| **Infant**              |              |
| Sex, n (%)              |              |
| Male                    | 27 (67.5%)   |
| Gestational age (wk), mean (SD) | 39 (1.5) |
| Birth weight (g), mean (SD) | 3361 (507) |

BMI=body mass index
Table 2:

Adjusted* association ($\beta$, 95% CI) of maternal body mass index with breast milk marker concentration at one (V1) and four (V4) months postpartum.

|                  | V1            | V4            |
|------------------|---------------|---------------|
| n-6:n-3 PUFA     | 0.12 (0.01, 0.2) | 0.13 (0.01, 0.3) |
| Leptin (pg/mL)   | 107 (29, 184)  | 254 (105, 403) |
| IL-8 (pg/mL)     | -0.02 (-0.07, 0.02) | -0.04 (-0.08, 0.01) |
| IL-6 (pg/mL)     | -0.02 (-0.06, 0.01) | -0.01 (-0.05, 0.03) |
| IL-1β (pg/mL)    | 0.02 (-0.03, 0.07) | 0.06 (-0.5, 0.6)  |
| MDA (μmol/L)     | 0.03 (-0.03, 0.09) | -0.02 (-0.09, 0.05) |

* Adjusted for maternal race and days postpartum at time of breast milk collection n-6:n-3 PUFA=omega-6:omega-3 polyunsaturated fatty acid ratio, MDA=malondialdehyde
Table 3:

Adjusted $^*$ association (β, 95% CI) of average breast milk marker concentration with infant growth at four (V4) and seven (V7) months.

|                  | V4 BMI z-score | V4 weight z-score | V4 length z-score | V4 percent fat mass (%) | V4 percent lean mass (%) |
|------------------|----------------|------------------|-------------------|------------------------|-------------------------|
| n-6:n-3 PUFA     | 0.05 (−0.1, 0.2) | 0.1 (−0.07, 0.3) | $\bf{0.2}$ (0.04, 0.4) | 0.8 (−0.8, 2.5) | −0.8 (−2.5, 0.8) |
| Leptin (pg/mL)   | −0.9 (−2, 0.1)  | −0.5 (−1.5, 0.5) | 0.6 (−0.5, 1.6) | $\bf{−9}$ (−17, −0.6) | $\bf{9.1}$ (0.6, 17.5) |
| IL-8 (pg/mL)     | 0.4 (−0.05, 0.8) | 0.4 (−0.06, 0.8) | $\bf{0.6}$ (0.2, 1) | 2.6 (−1.5, 6.8) | −2.6 (−6.8, 1.5) |
| IL-6 (pg/mL)     | 0.2 (−0.3, 0.8) | 0.2 (−0.3, 0.7) | 0.5 (−0.02, 0.9) | 0.3 (−4.2, 4.8) | −0.3 (−4.8, 4.2) |
| IL-1β (pg/mL)    | 0.3 (−0.2, 0.7) | 0.2 (−0.2, 0.7) | $\bf{0.4}$ (0.02, 0.8) | 1.7 (−1.8, 5.2) | −1.7 (−5.2, 1.9) |
| MDA (μmol/L)     | −0.1 (−0.5, 0.2) | −0.05 (−0.4, 0.3) | 0.04 (−0.3, 0.4) | −0.6 (−3.6, 2.5) | 0.6 (−2.5, 3.6) |

|                  | V7 BMI z-score | V7 weight z-score | V7 length z-score | V7 percent fat mass (%) | V7 percent lean mass (%) |
|------------------|----------------|------------------|-------------------|------------------------|-------------------------|
| n-6:n-3 PUFA     | $\bf{0.3}$ (0.1, 0.5) | 0.2 (−0.01, 0.4) | −0.01 (−0.2, 0.2) | −0.01 (−1.7, 1.6) | 0.1 (−1.6, 1.8) |
| Leptin (pg/mL)   | −0.7 (−1.9, 0.6) | −0.5 (−1.7, 0.6) | −0.04 (−1.1, 1.0) | −6 (−15.2, 3.1) | 6.2 (−3, 15.4) |
| IL-8 (pg/mL)     | $\bf{0.6}$ (0.02, 1.1) | 0.4 (−0.1, 0.9) | 0.3 (−0.2, 0.8) | 2.9 (−1.4, 7.2) | −1.9 (−6.3, 2.4) |
| IL-6 (pg/mL)     | 0.3 (−0.3, 1)   | 0.2 (−0.3, 0.8) | 0.3 (−0.3, 0.8) | 3.3 (−1.2, 7.8) | −2.3 (−6.9, 2.2) |
| IL-1β (pg/mL)    | 0.4 (−0.1, 0.9) | 0.4 (−0.1, 0.8) | 0.2 (−0.2, 0.7) | 2.3 (−1.3, 5.9) | −1.7 (−5.3, 2) |
| MDA (μmol/L)     | −0.2 (−0.7, 0.2) | −0.1 (−0.5, 0.3) | −0.1 (−0.5, 0.3) | 0.3 (−3, 3.5) | 0.05 (−3.2, 3.3) |

* Adjusted for maternal race, infant age at the time of measurement, and baseline infant measurement

BMI=body mass index, n-6:n-3 PUFA=omega-6:omega-3 polyunsaturated fatty acid ratio, MDA=malondialdehyde
Table 4:
Trajectory differences of infant growth exposed to breast milk containing high vs. low breast milk (BM) marker concentration. Trajectories determined by mixed model regression adjusting for maternal race and infant sex. Slope of each group shown as change in growth per month. P-value demonstrates difference between the slopes of both groups.

|                  | BMI z-score | Weight z-score | Length z-score | Percent fat mass (%) | Percent lean mass (%) |
|------------------|-------------|----------------|---------------|----------------------|-----------------------|
| **Low BM n-6:n-3 PUFA slope** | -0.08 U/mo  | -0.07 U/mo     | -0.02 U/mo    | +1.3 %/mo            | -1.3 %/mo             |
| **High BM n-6:n-3 PUFA slope** | +0.1 U/mo   | +0.01 U/mo     | -0.01 U/mo    | +1.9 %/mo            | -1.9 %/mo             |
| **p=0.01**       | **p<0.001** | **p=0.03**     |               |                       |                       |
| **Low BM leptin slope** | +0.03 U/mo  | -0.01 U/mo     | +0.01 U/mo    | +1.9 %/mo            | -1.9 %/mo             |
| **High BM leptin slope** | +0.07 U/mo  | -0.01 U/mo     | +0.02 U/mo    | +1.8 %/mo            | -1.8 %/mo             |
| **p=0.5**        | **p=0.4**   | **p=0.4**      |               |                       |                       |
| **Low BM IL-8 slope** | -0.05 U/mo  | -0.06 U/mo     | -0.04 U/mo    | +1.4 %/mo            | -1.4 %/mo             |
| **High BM IL-8 slope** | +0.07 U/mo  | +0.01 U/mo     | +0.02 U/mo    | +2 %/mo              | -2 %/mo               |
| **p=0.3**        | **p=0.3**   | **p=0.1**      |               |                       |                       |
| **Low BM IL-6 slope** | -0.04 U/mo  | -0.05 U/mo     | -0.03 U/mo    | +1.4 %/mo            | -1.4 %/mo             |
| **High BM IL-6 slope** | +0.06 U/mo  | -0.01 U/mo     | +0.01 U/mo    | +1.9 %/mo            | -1.9 %/mo             |
| **p=0.5**        | **p=0.2**   | **p=0.6**      |               |                       |                       |
| **Low BM IL-1β slope** | -0.03 U/mo  | -0.05 U/mo     | -0.03 U/mo    | +1.3 %/mo            | -1.3 %/mo             |
| **High BM IL-1β slope** | +0.05 U/mo  | -0.01 U/mo     | +0.01 U/mo    | +2 %/mo              | -2 %/mo               |
| **p=0.1**        | **p=0.5**   | **p=0.2**      |               |                       |                       |
| **Low BM MDA slope** | +0.03 U/mo  | -0.02 U/mo     | +0.02 U/mo    | +1.5 %/mo            | -1.5 %/mo             |
| **High BM MDA slope** | -0.01 U/mo  | -0.04 U/mo     | -0.03 U/mo    | +1.8 %/mo            | -1.8 %/mo             |
| **p=0.7**        | **p=0.3**   | **p=0.3**      |               |                       |                       |

BMI=body mass index, n-6:n-3 PUFA=omega-6:omega-3 polyunsaturated fatty acid ratio, MDA=malondialdehyde