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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.

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AUTHOR CONTRIBUTIONS

All authors were involved in the conceptualization of the study and design. SE and SS carried out analysis of data. SC, RT, and RF carried out experiments. CRM, POG, and CLW provided feedback on the analysis and results. All authors were involved in writing the paper and had final approval of the submitted and published versions.

CONFLICTS OF INTEREST

No authors have conflicts of interest to disclose.

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[1]. Children born to obese mothers are 3–5 times more likely to become obese as adults[2]. Potential biomedical mechanisms that have been proposed include genetics, fetal metabolic programming, and lactational metabolic programming[3–5]. Evidence from a murine model suggests that breast milk (BM) composition has a significant impact on offspring growth and cardiometabolic health[6]. 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[7]. The BM of obese women has been found to have increased levels of CRP, inflammatory cytokines, leptin, and insulin[8–10]. 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)[11]. 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[12]. 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[12–17]. Other studies have reported no association between BM markers and infant growth[18–20]. 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[21] 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[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[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^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^2 and 20 had a BMI ≥30 kg/m^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 $-20^{O}C$ 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-1β, 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[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 μm (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[24,25]. with assay parameters listed in Supplemental Table S1.

MDA: BM whey was separated from whole BM, according to the method previously described[26] and modified by Vaidya and Cheema[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[22]. Birth weight *z*-scores were calculated using the 2010 Olsen growth charts[28]. Monthly weight, length, and BMI z-scores were calculated from WHO reference data[29] 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[30]. 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 (β=0.12, 95% CI 0.01, 0.2) and V4 (β=0.13, 95% CI 0.01, 0.3) (Table 2). Higher maternal BMI was also associated with higher BM leptin concentrations at V1 (β=107, 95% CI 29, 184) and V4 (β=254, 95% CI 105, 403). Maternal BMI was not associated with BM IL-8, IL-6, IL-1β, 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-1β were associated with higher length z-scores in infants at V4 (n-6:n-3 β=0.2, 95% CI 0.04, 0.4; IL-8 β=0.6, 95% CI 0.2, 1; IL-1β β=0.4 95% 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 β=0.3, 95% CI 0.1, 0.5; IL-8 β=0.6, 95% 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 (β fat=−9, 95% CI −17, −0.6; β lean=9.1, 95% 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-1β: 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-1β 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[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[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[32], other studies have investigated the correlation between serum and BM cytokine levels with no clear relationship identified[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[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[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[37]. Maternal DHA supplementation during lactation has been shown to increase maternal plasma DHA and BM DHA levels[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[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[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[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[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[45]. During lactation, leptin is produced by mammary epithelial cells and secreted into BM as a component of the milk fat globule[46]. Adipocytes have been shown to secrete factors that influence mammary epithelial cell differentiation[47,48], 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[39]. Weight loss via dietary alterations and exercise has been shown to decrease circulating leptin levels in obese individuals[49,50]. 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[18–20], 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[12–17]. Leptin primarily acts on the hypothalamus to regulate food intake[51]. Initial exposure to leptin suppresses appetite[52], but if leptin levels remain elevated, such as in obesity, leptin resistance can develop leading to increased appetite and food intake[53]. Animal studies have shown that exposure to elevated n-6 PUFA induces central cellular leptin resistance[54]. 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[13]. 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[55,56], resulting in elevated cytokine secretion which ultimately induces a chronic state of inflammation and metabolic dysregulation[32,57,58]. 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[59].

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[60]. 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.

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REFERENCES

1. Branum AM, Kirmeyer SE, Gregory EC. Prepregnancy body mass index by maternal characteristics and state: data from the birth certificate, 2014. Natl Vital Stat Rep. 2016;65:1–11.
2. Catalano PM, Farrell K, Thomas A, Huston-Presley L, Mencin P, de Mouzon SH et al. Perinatal risk factors for childhood obesity and metabolic dysregulation. Am J Clin Nutr. 2009;90(5):1303–1313. [PubMed: 19759171]
3. Gluckman PD, Hanson MA, Pina LC. The developmental origins of adult disease. Matern Child Nutr. 2005;1:130–41. [PubMed: 16881892]
4. Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. Int J Obes. 2008;32(9):1373–9.
5. Rooney K, Ozanne SE. Maternal over-nutrition and offspring obesity predisposition: targets for preventative e interventions. Int J Obes. 2011;35(7):883–90.
6. Gorski JN, Dunn-Meynell AA, Hartman TG, Levin BE. Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. Am J Physiol Regul Integr Comp Physiol. 2006;291(3):R768–78. [PubMed: 16614055]
7. Sen S, Carpenter AH, Hochstadt J, Huddleston JY, Kustanovich V, Reynolds AA et al. Nutrition, weight gain and eating behavior in pregnancy: a review of experimental evidence for long-term effects on the risk of obesity in offspring. Physiol Behav. 2012;107(1):138–145. [PubMed: 22546810]

8. Whitaker KM, Marino RC, Haapala JL, Foster L, Smith KD, Teague AM et al. Associations of Maternal Weight Status Before, During, and After Pregnancy with Inflammatory Markers in Breast Milk. Obesity (Silver Spring). 2017;25:2092–9. [PubMed: 28985033]
9. Collado MC, Laitinen K, Salminen S, Isolauri E. Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. Pediatr Res. 2012;72:77–85. [PubMed: 22453296]
10. Fields DA, George B, Williams M, Whitaker K, Allison DB, Teague A et al. Associations between human breast milk hormones and adipocytokines and infant growth and body composition in the first 6 months of life. Pediatr Obes. 2017;12(Suppl. 1):78–85. [PubMed: 28160457]
11. Panagos PG, Vishwanathan R, Penfield-Cyr A, Matthan NR, Shivappa N, Wirth MD et al. Breastmilk from obese mothers has pro-inflammatory properties and decreased neuroprotective factors. J. Perinatol 2016;36:284–290. [PubMed: 26741571]
12. Nuss H, Altazan A, Zabaleta J, Sothern M, Redman L. Maternal pre-pregnancy weight status modifies the influence of PUFAs and inflammatory biomarkers in breastmilk on infant growth. PLoS One. 2019;14(5):e0217085. [PubMed: 31141526]
13. Fields DA, Demerath EW. Relationship of insulin, glucose, leptin, IL-6 and TNF-α in human breast milk with infant growth and body composition. Pediatr Obes. 2012;7(4):304–312. [PubMed: 22577092]
14. Miralles O, Sanchez J, Palou A, Pico C. A physiological role of breast milk leptin in body weight control in developing infants. Obesity (Silver Spring). 2006;14:1371–1377. [PubMed: 16988079]
15. Schuster S, Hechler C, Gebauer C, Kiess W, Kratzsch J. Leptin in maternal serum and breast milk: association with infants' body weight gain in a longitudinal study over 6 months of lactation. Pediatr Res. 2011;70:633–637. [PubMed: 21857386]
16. Doneray H, Orbak Z, Yildiz L. The relationship between breast milk leptin and neonatal weight gain. Acta Paediatr. 2009;98(4):643–7. [PubMed: 19141141]
17. Brunner S, Schmid D, Zang K, Much D, Knoeferl B, Kratzsch J et al. Breast milk leptin and adiponectin in relation to infant body composition up to 2 years. Pediatr Obes. 2015;10(1):67–73. [PubMed: 24729519]
18. Uysal FK, Onal EE, Aral YZ, Adam B, Dilmen U, Ardicolu Y. Breast milk leptin: its relationship to maternal and infant adiposity. Clin Nutr. 2002;21(2):157–60. [PubMed: 12056789]
19. Weyermann M, Brenner H, Rothenbacher D. Adipokines in human milk and risk of overweight in early childhood: a prospective cohort study. Epidemiology. 2007;18(6):722–9. [PubMed: 18062063]
20. Ucar B, Kirel B, Bor O, Kiliç FS, Doğruel N, Aydoğdu SD et al. Breast milk leptin concentrations in initial and terminal milk samples: relationships to maternal and infant plasma leptin concentrations, adiposity, serum glucose, insulin, lipid and lipoprotein levels. J Pediatr Endocrinol Metab. 2000;13(2):149–56. [PubMed: 10711659]
21. Druet C, Ong KK. Early childhood predictors of adult body composition. Best Pract Res Clin Endocrinol Metab. 2008;22:489–502. [PubMed: 18538288]
22. Hollis BW, Wagner CL, Howard CR, Ebeling M, Shary JR, Smith PG et al. Maternal versus infant vitamin D supplementation during lactation: a randomized controlled trial. Pediatrics. 2015;136:625–34. [PubMed: 26416936]
23. Vidal NP, Pham HT, Manful C, Pumphrey R, Nadeem M, Cheema M et al. The use of natural media amendments to produce kale enhanced with functional lipids in controlled environment production system. Sci Rep. 2018;8(1):14771. [PubMed: 30282974]
24. Fichorova RN, Richardson-Harman N, Alfano M, Belec L, Carbonneil C, Chen S et al. Biological and technical variables affecting immunoassay recovery of cytokines from human serum and simulated vaginal fluid: a multicenter study. Anal Chem. 2008;80(12):4741–4751. [PubMed: 18484740]
25. Fichorova RN et al. Maternal microbe-specific modulation of inflammatory response in extremely low-gestational-age newborns. MBio 2, e00280–00210, doi:10.1128/mBio.00280-10 (2011).
26. Yuksel S, Yigit AA, Cinar M, Atmaca N, Onaran Y. Oxidant and antioxidant status of human breast milk during lactation period. Dairy Sci. & Technol 2015;95(3):295–302.

27. Vaidya H, Cheema SK. Breastmilk with a high omega-6 to omega-3 fatty acid ratio induced cellular events similar to insulin resistance and obesity in 3T3-LI adipocytes. Pediatr Obes. 2018;13(5):285–291. [PubMed: 28335075]

28. Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS. New intrauterine growth curves based on United States data. Pediatrics. 2010;125:e214–24. [PubMed: 20100760]

29. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl. 2006;450:76–85. [PubMed: 16817681]

30. Sen S, Penfield-Cyr A, Hollis BW, Wagner CL. Maternal Obesity, 25-Hydroxy Vitamin D Concentration, and Bone Density in Breastfeeding Dyads. J Pediatr. 2017;187:147–152. [PubMed: 28549637]

31. Young BE, Patinkin Z, Palmer C, de la Houssaye B, Barbour LA, Hernandez T et al. Human milk insulin is related to maternal plasma insulin and BMI: but other components of human milk do not differ by BMI. Eur J Clin Nutr. 2017;71(9):1094–1100. [PubMed: 28513622]

32. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA. 1999;282(22):2131-2135. [PubMed: 10591334]

33. Hawkes JS, Bryan DL, Gibson RA. Cytokine production by human milk cells and peripheral blood mononuclear cells from the same mothers. J Clin Immunol. 2002;22(6):338-344. [PubMed: 12462333]

34. Hassiotou F, Hepworth AR, Metzger P, Tat Lai C, Trengove N, Hartmann PE, et al. Maternal and infant infections stimulate a rapid leukocyte response in breastmilk. Clin Transl Immunology. 2013;2(4):e3. [PubMed: 25505951]

35. McCrory MA, Fuss PJ, McCallum JE, Yao M, Vinken AG, Hays NP et al. Dietary variety within food groups: association with energy intake and body fatness in men and women. Am J Clin Nutr. 1999;69:440–470. [PubMed: 10075328]

36. Simopoulos AP. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. Nutrients. 2016;8(3):128. [PubMed: 26950145]

37. Innis SM. Human milk and formula fatty acids. J Pediatr. 1992;120:S56–S61. [PubMed: 1343204]

38. Jensen CL, Maude M, Anderson RE, Heird WC. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. Am J Clin Nutr. 2000;71:292S–299S. [PubMed: 10617985]

39. Tian HM, Wu YX, Lin YQ, Chen XY, Yu M, Lu T et al. Dietary patterns affect maternal macronutrient intake levels and the fatty acid profile of breast milk in lactating Chinese mothers. Nutrition. 2018;58:83–8. [PubMed: 30391695]

40. Amri EZ, Ailhaud G, Grimaldi PA. Fatty acids as signal transducing molecules: Involvement in the differentiation of preadipose to adipose cells. J. Lipid Res 1994;35:930–937. [PubMed: 8071615]

41. Jump DB, Clarke SD, Thelen A, Liimatta M. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. J. Lipid Res 1994;35:1076–1084. [PubMed: 8077846]

42. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. Am. J. Clin. Nutr 2000;71:343S–348S. [PubMed: 10617994]

43. Gaillard D, Negrel R, Lagarde M, Ailhaud G. Requirement and role of arachidonic acid in the differentiation of preadipose cells. Biochem. J 1989;257:389–397. [PubMed: 2539084]

44. Mirnikjoo B, Brown SE, Kim HF, Marangell LB, Sweatt JD, Weeber EJ. Protein kinase inhibition by omega-3 fatty a Sánchez J, Oliver P, Miralles O, Ceresi E, Picó C, Palou A 2005 Leptin orally supplied to neonate rats is directly uptaken by the immature stomach and may regulate short-term feeding. Endocrinology 146: 2575–2582cids. J. Biol. Chem 2001;276:10888–10896.

45. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med. 1995;1:950–953. [PubMed: 7585223]

46. Smith-Kirwin SM, O'Connor DM, De Johnston J, Lancey ED, Hassink SG, Funanage VL. Leptin expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab 1998;83:1810–1813. Smith-Kirwin SM, O'Connor DM, De Johnston J, Smith-Kirwin SM, O'Connor DM, De Johnston J, Lancey ED, Hassink SG, Funanage VL 1998 Leptin expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab 83: 1810–1813 Smith-Kirwin SM, O'Connor DM, De Johnston J, Lancey ED, Hassink SG, Funanage VL 1998 Leptin

expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab 83: 1810–1813 Smith-Kirwin SM, O'Connor DM, De Johnston J, Lancey ED, Hassink SG, Funanage VL 1998 Leptin expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab 83: 1810–1813 Smith-Kirwin SM, O'Connor DM, De Johnston J, Lancey ED, Hassink SG, Funanage VL 1998 Leptin expression in human mammary epithelial cells and breast milk. J Clin Endocrinol Metab 83: 1810–1813 [PubMed: 9589698]

47. Wiens D, Park CS, Sotckdale FE. Milk protein expression and ductal morphogenesis in the mammary gland in vitro: hormone-dependent and -independent phases of adipocyte-mammary epithelial cell interaction. J. Cell Biol 1985;100:1415–1422. [PubMed: 3886667]
48. Hosick HL, Beck JC. Growth of mouse mammary epithelium in response to serum-free media conditioned by mammary adipose tissue. Cell Biol Int Rep. 1988;12:85–97. [PubMed: 3396081]
49. Eriksson J, Valle T, Lindstrom J, Haffner S, Louheranta A, Uusitupa M et al. Leptin concentrations and their relation to body fat distribution and weight loss: a prospective study in individuals with impaired glucose tolerance. DPS-study group. Horm Metab Res. 1999; 31(11): 616–9. [PubMed: 10598830]
50. Reseland JE, Anderssen SA, Solvoll K, Anderssen SA, Jacobs DR Jr, Urdal P et al. Effect of long-term changes in diet and exercise on plasma leptin concentrations. Am J Clin Nutr. 2001;73(2): 240–245. [PubMed: 11157319]
51. Masuzaki H, Ogawa Y, Isse N, Satoh N, Okazaki T, Shigemoto M et al. Human obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. Diabetes 1995;44:855–858. [PubMed: 7789654]
52. Meister B Control of food intake via leptin receptors in the hypothalamus. Vitam Horm 2000;59:265–304. [PubMed: 10714243]
53. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab. 1996;81:4162–4165. [PubMed: 8923877]
54. Cheng L, Yu Y, Zhang Q, Szabo A, Wang H, Huang XF. Arachidonic acid impairs hypothalamic leptin signaling and hepatic energy homeostasis in mice. Mol. Cell. Endocrinol 2015;412(5):12–18. [PubMed: 25986657]
55. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest. 2004;114:1752–1761. [PubMed: 15599400]
56. Gustafson B, Gogg S, Hedjazifar S, Jenndahl L, Hammarstedt A, Smith U. Inflammation and impaired adipogenesis in hypertrophic obesity in man. Am J Physiol Endocrinol Metab. 2009;297:E999–E1003. [PubMed: 19622783]
57. McArdle MA, Finucane OM, Connaughton RM, McMorrow AM, Roche HM. Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies. Front Endocrinol (Lausanne). 2013;4:52. [PubMed: 23675368]
58. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112(12):1796. [PubMed: 14679176]
59. Huh JY, Park YJ, Ham M, and Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. Mol. Cells 2014; 37:365–371. [PubMed: 24781408]
60. Rigo J Body composition during the first year of life. Nestle Nutr Workshop Ser Pediatr Program. 2006;58:65–67.

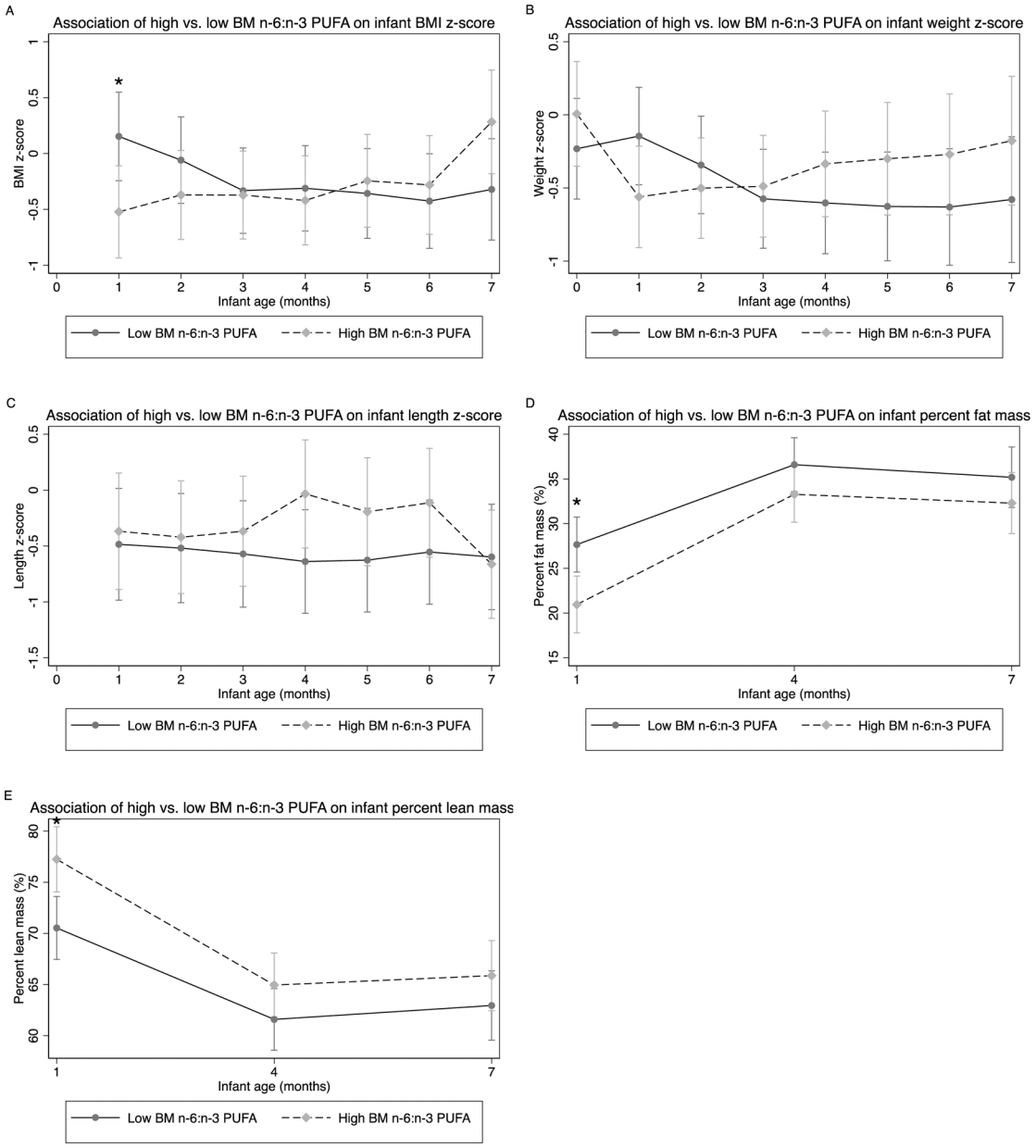

Figure 1:

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/m^2), 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 (β, 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)	**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)	**−9 (−17, −0.6)**	**9.1 (0.6, 17.5)**
IL-8 (pg/mL)	0.4 (−0.05, 0.8)	0.4 (−0.06, 0.8)	**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)	**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	**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)	**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**	p=0.2	p=0.2
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=0.5	p=0.4	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<0.001**	p=0.3	p=0.1	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<0.001**	p=0.2	p=0.6	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=0.02**	p=0.5	p=0.2	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=0.9	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