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The infant gut microbiota at 12 months of age is associated with human milk exposure but not with maternal pre-pregnancy body mass index or infant BMI-for-age z-scores

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ABSTRACT

Background: As obesity rates continue to rise, it is increasingly important to understand factors that can influence body weight and growth, especially from an early age. The infant gut microbiota has broad effects on a variety of bodily processes, but its relation to infant growth is not yet fully characterized. Since the infant gut microbiota is closely related to breastfeeding practices and maternal health, understanding the relationship between these factors and infant growth may provide insight into the origins of childhood obesity.

Objectives: Identify the relationship between human milk exposure, maternal pre-pregnancy body mass index (BMI), the infant gut microbiota, and 12-month-old BMI-for-age z-scores (12M BAZ) to identify key factors that shape infant growth.

Methods: Two Michigan cohorts (ARCHGUT and BABYGUT) comprised of a total of 33 mother-infant dyads provided infant fecal samples at 12M. After DNA extraction, amplification, and sequencing of the V4 16S rRNA region using Illumina MiSeq v2 Chemistry, gut bacterial diversity metrics were analyzed in relation to human milk exposure, maternal pre-pregnancy BMI, and infant growth parameters.

Results: Recent human milk exposure was inversely related to maternal pre-pregnancy BMI and most strongly associated with infant gut bacterial community membership and individual gut microbiota richness differences. Maternal pre-pregnancy BMI was not associated with the infant gut microbiota after adjusting for human milk exposure. However, maternal pre-pregnancy BMI was the only factor significantly associated with 12M BAZ.

Conclusions: Human milk exposure is one of the central influences on the infant gut microbiota at 12M of age. However, the lack of association between the infant gut microbiota and 12M-old infant BAZ suggests that genetic, physiological, dietary, and other environmental factors may play a more direct role than the gut microbiota in determining infant BAZ at 12M.

1. Introduction

Between 1980 and 2013, worldwide obesity rates for children rose nearly 50% (Ng et al., 2014). Obesity is strongly associated with a plethora of medical complications including type II diabetes, cancer, and cardiovascular disease (Guh et al., 2009) and produces significant global economic costs of around $2 trillion (Dobbs et al., 2014). It is therefore of utmost importance to understand the potential causes of obesity and to continue to develop novel, efficient solutions to reduce the prevalence of obesity. This is especially relevant for childhood obesity, which correlates with adult obesity/overweight status (Simmonds et al., 2016). Beginning at age five, the body mass index (BMI) category of a child is likely to continue on the same or similar trajectory throughout their development into adulthood (Geserick et al., 2018). Furthermore, there is a significant positive correlation between an individual's BMI at 4 months of age and their BMI at 5 years of age (Gittner et al., 2014). Therefore, understanding the causes and variables associated with the development of childhood obesity can provide important information to

Abbreviations: 6M, 6 months; 12M, 12 months; BAZ, BMI-for-age z-score; HM, Human Milk; LAZ, Length-for-age z-score; LWZ, Length-for-weight z-score; PCoA, Principal Coordinates Analysis; WAZ, Weight-for-age z-score.

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tackle the obesity epidemic from its roots.

From birth, an infant is inoculated with their mother's bacterial communities (Bäckhed et al., 2015). Several factors may play a role in altering the infant gut microbiota, with some of the most important being mode of delivery, human milk exposure/cessation, maternal health, and antibiotic exposure (Bäckhed et al., 2015; Koleva et al., 2015). Examining the relationship between maternal pre-pregnancy BMI and the gut microbiota can reveal important insights into the development of the infant gut microbiota, especially in light of the well-established energy-harvesting effects of the gut microbiota demonstrated in mice and humans (Backhed et al., 2004; Schwiertz et al., 2010).

Breastfeeding is associated with positive health outcomes in infants (Bashiardes et al., 2016; van den Elsen et al., 2019; Miliku and Azad, 2018), and has been shown to distinctly alter the infant gut microbiota (Bode et al., 2014; Wang et al., 2015). As such, understanding the ways in which human milk shapes the 12-month (12M) infant gut microbiota can have important public health implications. Furthermore, maternal BMI has been reported to influence bacterial communities in human milk (Cabrera-Rubio et al., 2012). Hence, by examining the gut microbiota of infants and important covariates such as human milk exposure and maternal pre-pregnancy BMI, valuable information may be gained concerning the origins of childhood obesity. The objective of this study is to define the ways that the 12M infant gut microbiota is associated with the interrelated factors of maternal BMI, infant human milk exposure, and infant BMI-for-age z-scores, a measure of infant growth.

2. Methods

2.1. Subjects (Fig. 1)

Two cohorts (ARCHGUT and BABYGUT) of Michigan women were enrolled for this study. ARCHGUT, a subset of the Archive for Research in Child Health, recruited pregnant women at a prenatal clinic in Traverse City and another in Lansing, Michigan. Meanwhile, BABYGUT recruitment occurred in several prenatal clinics in the greater Lansing area. All participants provided written, informed consent and study activities were approved by the Michigan State University Human Research Protection Program (IRB 15–1240 and 14–170M) (Sugino et al., 2019).

2.2. Sample collection

The pregnant women provided fecal samples in their third pregnancy trimester, and later from infants at 1 week, 6 months, 12 months and 24 months of age. Samples were sent to the lab by way of mail or collected from the participant's home. Stool was collected from the diaper and put into a Para-Pak Clean Vial collection tube (Meridian Biosciences, Cincinnati, OH). Fecal aliquots were stored at −80 °C upon arrival to the lab. The focus of this paper is the relationship between pre-pregnancy BMI, human milk exposure, and the 12M infant stool samples (n = 33). Results from the pregnancy, 1-week and 6-month timepoints have been reported elsewhere (Sugino et al., 2019, 2020; Sosa-Moreno et al., 2020). For the 12M infant stool samples, the mean time from sample collection to receipt by the laboratory was 3.7 ± 1.9 days (median of 3 days).

2.3. DNA extraction and rRNA gene amplification

DNA extraction, 16S rRNA gene amplification, and sequencing were as presented in (Sugino et al., 2019).

2.4. Processing and analysis of sequence data

Processing and analysis of sequence data also followed the same, previously-described protocol (Sugino et al., 2019). In this study, samples were each rarefied to 10,000 reads 999 times, followed by averaging and rounding of each OTU to the nearest integer prior to subsequent analysis.

2.5. Data analysis

The women who participated in this study were classified by maternal pre-pregnancy BMI (calculated from self-reported height and weight) as normal ($18.5 \leq BMI < 25$; n = 10), overweight ($25 \leq BMI < 30$; n = 11) or obese ($BMI \geq 30$; n = 12). Participants estimated the fraction of their infant's diet, in the past week, that was human breast milk as 100%, 80%, 50–80%, 50%, 20–50%, 20% or 0% (Bonuck et al., 2005). To increase power, we collapsed all breast milk categories from 20% or more (n = 13) and contrasted findings to the no human milk group (n = 20). Infant length and weight at 12M were reported by the mother. BMI-for-age (BAZ), length-for-age (LAZ), weight-for-age (WAZ), and length-for-weight (LWZ) z-scores were calculated using infant length, weight, sex and age using the WHO's Anthro Software (WHO, 2011). Comparison of population characteristics was done using a chi-square test for categorical variables or ANOVA for continuous variables. Post hoc analysis of chi-square values for HM exposure versus maternal pre-pregnancy BMI was done in R (R Core Team, 2011) using a pairwise comparison through the rcompanion package (Mangiafico, 2020).

Alpha (within-sample) diversity, measured with Chao1 and Shannon indices, was calculated using R via the vegan package (Oksanen et al., 2015). Normality of the alpha diversity was confirmed using the Shapiro-Wilk test and ANOVA was used to test for significant differences across groups. Post-hoc comparison between HM exposure categories was performed using a Tukey's Honest Significant Difference test. Sorensen, which pertains to community composition, and Bray-Curtis, which pertains to community structure, dissimilarities were calculated from the abundance data via the vegan package in R and plotted using principal coordinate analysis (PCoA). Using the adonis function, PERMANOVA was performed to identify significant beta-diversity differences, specifically with taxa driving Sorensen distance separation. The p-values for the separation driven by the taxa were adjusted for false positives by the Benjamini-Hochberg method. To identify differences in sample dispersion, PERMDISP (betadisper function in the vegan package) was utilized. For both alpha and beta diversity, multivariable linear regression models were used to test the associations between maternal BMI category and human milk exposure. Alpha diversity models were tested using a type II ANOVA. Analyses of beta diversity used PERMANOVA (adonis2 function in the vegan package) to fit the models. Individual taxa were compared by human milk exposure groups using a negative binomial model in the MASS package (Venables and Ripley, 2002). Post-hoc power analysis was done through the G*power version 3.1.9.2 (Faul et al., 2007) for alpha diversity and through the micropower package (Kelly et al., 2015) in R for beta diversity. Significance levels were set at $p < 0.05$.

3. Results

Participant (n = 33) characteristics did not differ by cohort (Table 1). Ten (30.3%) mothers were normal weight, 11 (33.3%) were overweight and 12 (36.4%) were obese prior to becoming pregnant. The majority of infants were male (n = 24; 72.7%), and 12 (36.4%) infants were born via C-section. Mode of delivery did not significantly associate with maternal BMI category (chi-square, p = 0.09). At 6M, 20 (62.5%) infants consumed at least some human milk. Whereas at 12M, 13 (39.4%) infants were still consuming at least some human milk. No infants were exposed to antibiotics within 30 days of providing the stool sample. Infants averaged 379.8±16.4 days of age, 76.0±3.3 cm in length, and 10.2±1.1 kg of weight. Infant anthropometrics were as follows: BAZ (0.7±1.4), LAZ (−0.04±1.33), LWZ (0.7±1.3), and WAZ (0.4±1.2).

3.1. Human milk exposure

Infant human milk exposure was inversely associated with maternal pre-pregnancy BMI (Fig. 2). At each set of time periods, a higher ratio of women with pre-pregnancy obesity did not give their infants any human milk compared to women of normal weight. In fact, infants with mothers

Table 1
Population characteristics. Abbreviations: 6M: 6 months, 12M: 12 months, BAZ: BMI-for-age z-score, HM: Human Milk, LAZ: Length-for-age z-score, LWZ: Length-for-weight z-score, WAZ: Weight-for-age z-score.

	ARCHGUT (n = 20)	BABYGUT (n = 13)	p-value
Pre-pregnancy BMI category			0.35
Normal	6 (30.0)	4 (30.8)	
Overweight	5 (25.0)	6 (46.2)	
Obese	9 (45.0)	3 (23.1)	
Girls	7 (35.0)	2 (15.4)	0.4
Vaginal delivery	14 (70.0)	7 (53.8)	0.57
Any HM – 6M	10 (52.6)[a]	10 (76.9)	0.31
Any HM – 12M	9 (45.0)	4 (30.8)	0.65
Antibiotic exposure in the past month[b]	0 (0.0)	0 (0.0)	–
Infant age, days	379.4 ± 15.7	380.3 ± 18.0	0.51
Infant length, cm	74.4 ± 3.5	75.4 ± 3.1	0.54
Infant weight, kg	10.5 ± 1.0	9.8 ± 1.2	0.43
BAZ	0.9 ± 1.3	0.4 ± 1.6	0.46
LAZ	0.1 ± 1.3	−0.3 ± 1.4	0.46
LWZ	1.0 ± 12	0.4 ± 1.4	0.52
WAZ	0.7 ± 0.9	0.04 ± 1.04	0.47

[a] One value missing.
[b] Reported antibiotic exposure is for infants; maternal antibiotic use was not collected at 6M or 12M.

of normal BMI all received some exposure to human milk, whereas four of the infants with overweight mothers and eight of the infants with obese mothers were never exposed to human milk at 6M or 12M.

Human milk exposure at 6M and 12M was associated with 12M infants' gut microbiota beta diversity as determined by Sorensen distances (Fig. 3) but not with Bray-Curtis distances (Supplementary Fig. 1). Gut microbiota membership in infants who were not exposed to human milk at 6M or 12M differed significantly from that of infants who were exposed to some human milk at 6M and 12M. Infants exposed to human milk at only 6M had specific members of the gut microbiota that resembled those found in infants who were never exposed to human milk and others similar to those found in infants exposed to human milk at both 6M and 12M. Not all genera found in the infant gut microbiota differed by human milk exposure. The taxa whose presence/absence drove Sorensen distance separation are included (Supplementary Table 1).

Infants exposed to human milk at both 6M and 12M of age had significantly lower alpha diversity, as measured by the Chao1 index, than infants who were not exposed at 6M or 12M (Fig. 4). However, overall bacterial alpha diversity represented by Shannon index values did not differ by human milk exposure.

3.2. Maternal pre-pregnancy BMI

Infants with mothers who had higher pre-pregnancy BMIs also had a greater richness of gut microbes at 12M ($r = 0.50$, $p = 0.01$) (Fig. 5a), but this significant association between maternal pre-pregnancy BMI and alpha diversity richness (Chao1) disappeared ($p = 0.49$) when adjusting for human milk exposure through a bivariate analysis. Indeed, human milk exposure ($p = 0.03$) drove the association with the Chao1 index. Similarly, maternal pre-pregnancy BMI was positively associated with 12M infant alpha diversity represented through the Shannon index ($r = 0.43$, $p = 0.04$) (Fig. 5b), which factors richness and evenness, but this significant association between pre-pregnancy BMI and Shannon scores became a trend ($p = 0.07$) when adjusting for human milk exposure. In this case, human milk exposure was not associated with infant Shannon index scores ($p = 0.54$). Maternal pre-pregnancy BMI was not associated with infant 12M beta diversity measures (Supplementary Fig. 2).

Infant BAZ was positively associated with maternal pre-pregnancy BMI (Fig. 6) but not associated with gut microbial alpha/beta diversity measures (Supplementary Figs. 3 and 4) or human milk exposure (Supplementary Fig. 5). Additionally, neither 1-week nor 6M infant gut microbial diversities were associated with 12M infant BAZ (Supplementary Figs. 6 and 7). Effect sizes and power for all analyses are included (Supplementary Table 2).

4. Discussion

This study examined the associations between the infant gut microbiota, maternal pre-pregnancy BMI, infant human milk exposure, and infant BAZ at 12M of age. Mothers with higher pre-pregnancy BMI did not provide as much human milk at both 6M and 12M as did women with BMIs ≤25. Human milk exposure was associated with decreased alpha diversity richness within infant fecal samples at 12M. We observed a positive relationship between maternal pre-pregnancy BMI and infant gut microbial alpha diversity. However, this association was driven by differences in human milk exposure. When assessing 12M infant growth, as characterized by BAZ scores, both the infant gut microbiota and human milk exposure were unassociated with BAZ. Mothers with higher BMIs were more likely to have infants with a greater BAZ at 12M of age. These results underscore the importance of variables apart from the gut microbiota in shaping infant growth at 12M of age. These factors are postulated to be environmental, dietary, physiological, and genetic in origin.

Infant human milk exposure was inversely associated with maternal pre-pregnancy BMI (Fig. 2). Mothers with a pre-pregnancy BMI falling in the normal range provided their infants with more breastmilk at 6M and at 12M than mothers with pre-pregnancy obesity. This is consistent with previous research, which has repeatedly found that mothers with a BMI over 25 are less likely to breastfeed than mothers with lower BMI (Lucas et al., 2015; Donath and Amir, 2008; Guelinckx et al., 2012). This is likely due to a combination of physical, physiological and psychological obstacles that decrease the probability that an overweight or obese mother will attempt to initiate or continue breastfeeding their infant (Garner et al., 2014; Jevitt et al., 2007). Some of these obstacles include delayed lactogenesis, edema in breasts, hormonal imbalances, and body-image insecurities (Bever Babendure et al., 2015).

Of all factors analyzed in this study, human milk exposure was most consistently associated with the gut microbiota composition (Bäckhed et al., 2015; Stewart et al., 2018). Infants not exposed to human milk at or beyond 6M had very distinct microbiotas—characterized by Sorensen distance which accounts for presence/absence—from infants who continued breastfeeding (Fig. 3). This result is consistent with a previous meta-analysis that concluded that breastfed infants do indeed have a significantly distinct gut microbiota from non-breastfed infants that is duration/exposure-dependent, with significant differences persisting from 6M to two years of age (Ho et al., 2018). The presence of a wide variety of taxa drove separation of gut microbial communities of infants with little to no recent human milk exposure (Supplementary Table 1), which is in line with the well-established notion that infants with lesser degrees of exposure to human milk exhibit more diverse gut microbial compositions (Bäckhed et al., 2015; O'Sullivan et al., 2015). However, community differences were not significant when analyzed on the basis of Bray-Curtis distance (Supplementary Fig. 1), which takes bacterial abundance, as well as presence/absence, into account.

Increased breastmilk exposure was associated with a reduction in microbial richness in this sample of infants (Fig. 4a). Although it is not yet clear which bacteria are necessary and/or sufficient to assemble a healthy gut microbiota (McBurney et al., 2019), high microbial diversity has classically been thought to indicate a healthier gut in adults, whose gut microbiota is relatively stable (Valdes et al., 2018; Yassour et al., 2016; Lloyd-Price et al., 2016; Lozupone et al., 2012; Turnbaugh et al., 2009; Caporaso et al., 2011). The infant gut microbiota, in contrast, is highly variable, especially in the first three years of life (Yatsunenko et al., 2012). Nonetheless, it is commonly reported that HM-fed infants have less diverse gut bacterial communities (Ho et al., 2018; Azad et al., 2013), and this aligns with the results herein. Maturation of the infant gut

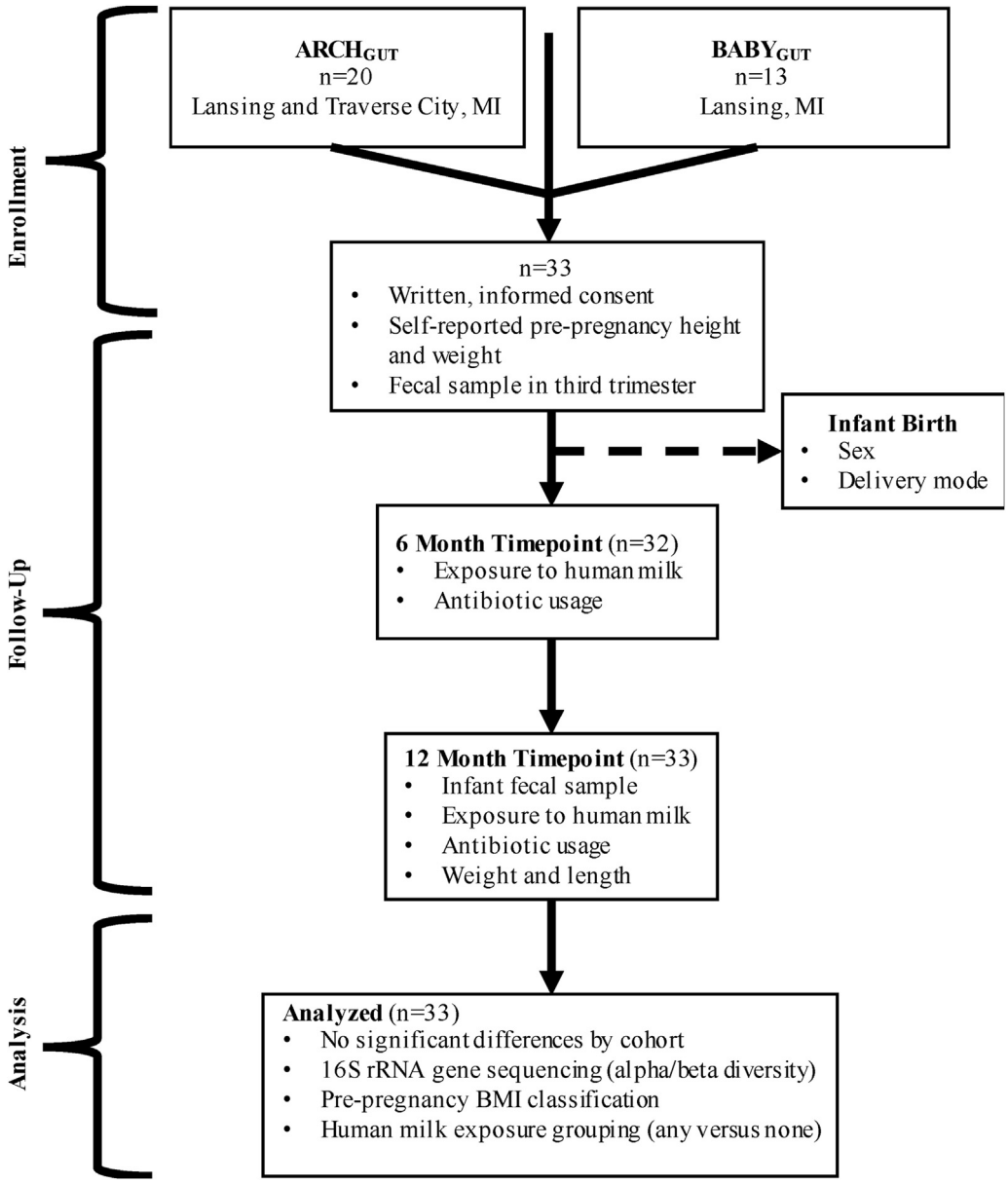

Fig. 1. Participant flowchart showing enrollment, follow-up, and analysis of data from all participants who provided a 12-month infant fecal sample in addition to 6 month and pre-pregnancy data.

through an increase in microbial diversity to result in an adult-like gut microbiota is brought about by the cessation of breastfeeding (Bäckhed et al., 2015; De Filippo et al., 2010). Lower gut microbial richness at 12M in association with HM exposure is likely due to the selective nature of human milk oligosaccharides which promote the growth of a limited number of beneficial microbes such as *Bifidobacterium longum* subsp. *infantis* and some *Bacteroides* species (Marcobal et al., 2010). Although the bacterial gut microbiota of 12M breastfed infants in this study had decreased richness, their overall community alpha diversity was not significantly different from infants not exposed to HM or to those only exposed at 6M, as shown by the Shannon scores (Fig. 4b). This is potentially due to the greatly increased counts of *Bifidobacteria*, and to a lesser degree *Bacteroides*, that are present in breastfed infant guts (Bezirtzoglou et al., 2011). The elevated abundances of these genera may serve to counterbalance overall community diversity changes in spite of a richness reduction.

Maternal pre-pregnancy BMI was significantly correlated with alpha diversity in the microbiota of 12M infants, but once human milk exposure was included in the bivariate model, the significant positive association disappeared (Fig. 5). Instead, human milk exposure became the main factor accounting for the differences in richness (Fig. 5a), but not Shannon diversity scores (Fig. 5b), which suggests that the influence of maternal pre-pregnancy BMI on the infant gut microbiota at 12M was likely indirect and driven by other complex factors, especially breastfeeding. This possibility is supported by the lack of association between maternal pre-pregnancy BMI and infant gut microbial beta diversity (Supplementary Fig. 2). Considering the environmental and genetic factors that influence development within an infant's first year, it is plausible that maternal pre-pregnancy BMI no longer plays as important of a role in modulating the infant gut microbiota at 12M. In early infancy, however, maternal pre-pregnancy BMI is significantly associated with several parameters of the gut microbiota (Sugino et al., 2019).

Infant 12M BAZ scores were significantly associated with maternal pre-pregnancy BMI (Fig. 6), but not infant gut microbial alpha and beta diversity (Supplementary Figs. 3 and 4) or human milk exposure (Supplementary Fig. 5). Previous studies have demonstrated a positive

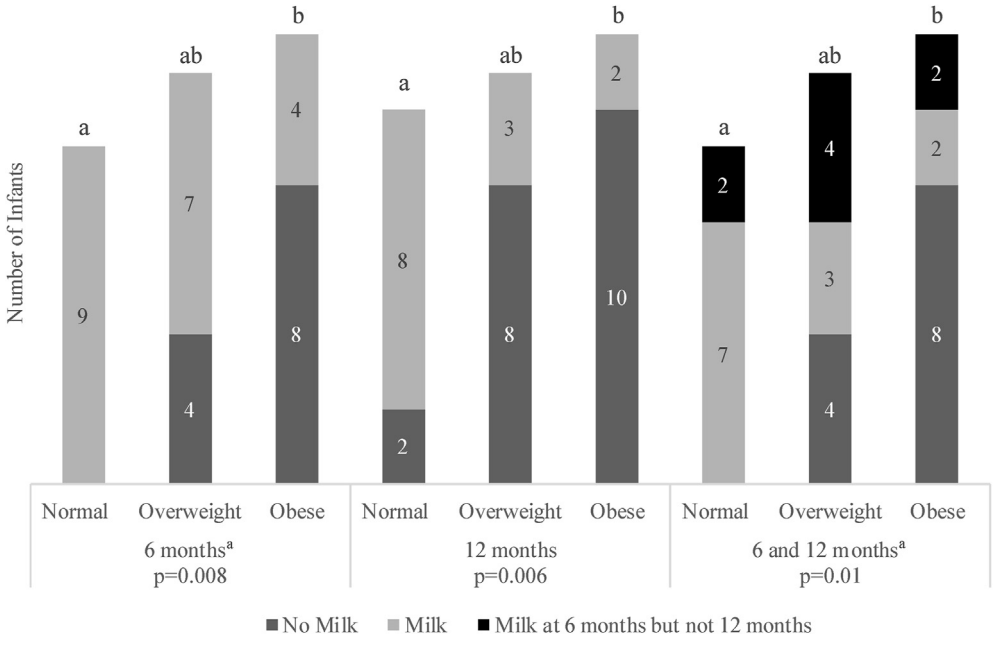

Fig. 2. Stacked bar chart demonstrating the number of infants (n = 33) that were exposed to human milk at 6 months, 12 months, and both timepoints together versus maternal pre-pregnancy BMI category. Bars within each age grouping that share a superscript letter do not differ from each other.
[a] 6 months and 6 + 12 months data exclude one value in the normal pre-pregnancy BMI category due to incomplete data collection.

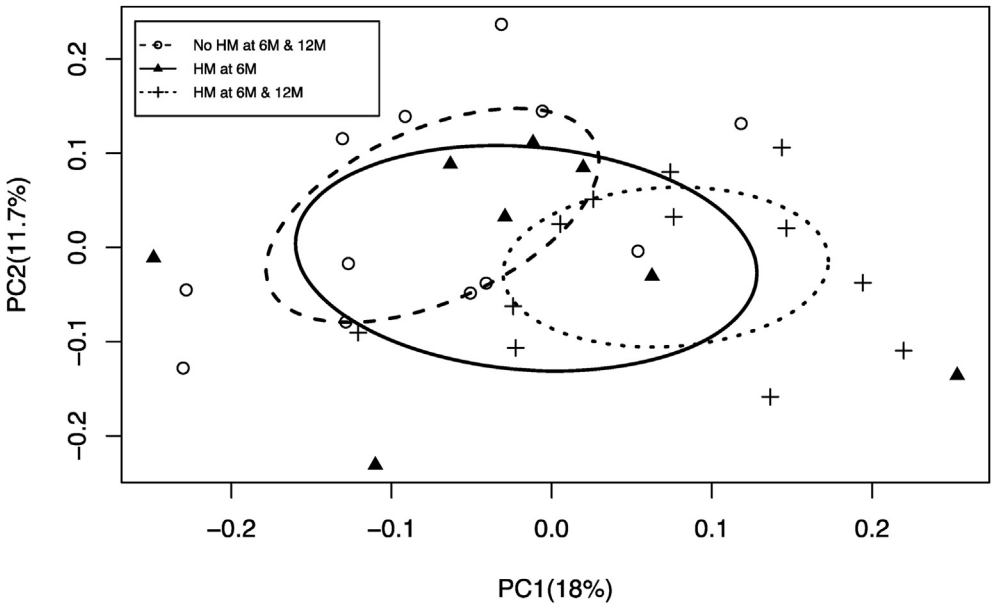

Fig. 3. PCoA of the Sorensen distances for the 12M-old microbiota (p = 0.003). The comparison is across infants without human milk exposure, with human milk exposure at 6M timepoint only, and human milk exposure at both 6M and 12M. A principal coordinates analysis plot represents each microbial community from a given sample as a single point on a plot with axes that account for the major difference-driving factors. Sorensen distance accounts for the presence or absence of bacterial types between samples. The farther apart the points on the plot, the more dissimilar the samples. Abbreviations: 6M: 6 months, 12M: 12 months, HM: Human Milk, PCoA: Principal Coordinates Analysis.

association between maternal BMI and infant BMI at birth and throughout later development (Williams et al., 2014; Voerman et al., 2019; Taveras et al., 2009; Kjaer et al., 2019; Bonakdar et al., 2019; Heslehurst et al., 2019). In adults, the gut microbiota has been shown to be significantly associated with obesity (Davis, 2016; Muscogiuri et al., 2019; Turnbaugh et al., 2006; Huttenhower et al., 2012), but associations with 12M infant risk for obesity are less clear (Stanislawski et al., 2018; Moossavi and Azad, 2019; Gohir et al., 2015; Mohammadkhah et al., 2018). Although one previous study identified a negative correlation between 12M *Staphylococcus* counts and BAZ at 1–3 years of age (Vael et al., 2011), our results suggest that, overall, 12M-old infant BAZ is most correlated with maternal BMI, rather than the gut microbiota. We postulate that genetic, physiological, dietary, and environmental factors mediate this observed association between maternal BMI and 12M infant BAZ, but future investigations accounting for an extensive range of confounders including socioeconomic status, physical activity, and health history are required to truly ascertain this. Additionally, 12M of age is a time of significant dietary changes and developmental strides (Dwyer, 2018), which may limit the ability to detect a signal in the noise. When testing for possible associations of the gut microbiota at 1 week or 6M with 12M BAZ, no associations were found (Supplementary Figs. 6 and 7). Previous research has indicated that the gut microbiota at 3–4 months but not 12M is significantly associated with 12M growth parameters (Forbes et al., 2018), and our study confirms the lack of association at timepoints before or after this 3–4 month "key" timeframe.

This study has some limitations to consider. BMI is a flawed indicator of fat mass and even health, but it is an acceptable metric due to its practicality and cost-efficiency (Gurunathan and Myles, 2016; Stefan

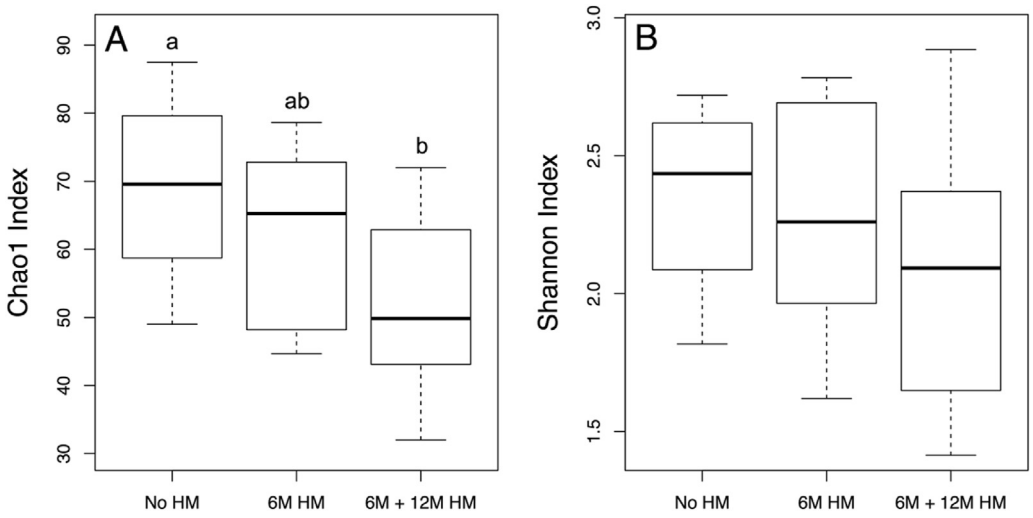

Fig. 4. Boxplots of A) Chao1 indices (p = 0.01) and B) Shannon indices (p = 0.22) for 12M infant gut microbial samples stratified based on timing of human milk exposure. Chao1 is an estimator of the species richness in a community, and the Shannon index considers both evenness and abundance to produce a measure of alpha diversity. Boxplots with shared superscript letters are not significantly different from one another. Abbreviations: 6M: 6 months, 12M: 12 months, HM: Human Milk.

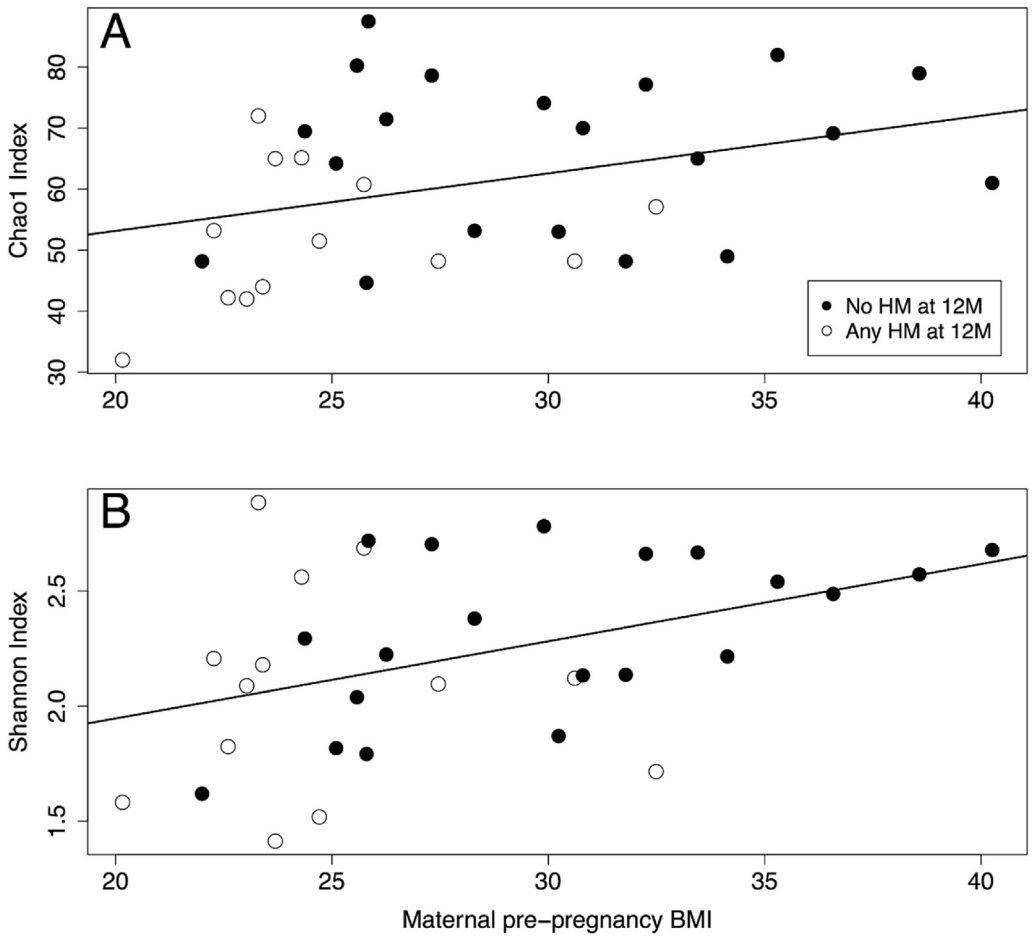

Fig. 5. A) Chao1 index values for each infant versus maternal pre-pregnancy BMI (r = 0.50, p = 0.01). B) Shannon index versus maternal pre-pregnancy BMI (r = 0.43, p = 0.04). Abbreviations: 6M: 6 months, 12M: 12 months, HM: Human Milk.

et al., 2013; Daniels, 2009). Furthermore, the weight and height measurements for both mothers and infants, as well as breastfeeding status, were self-reported, but this has been shown to be accurate in similar populations (Shin et al., 2014; Li et al., 2005). Since gut microbial changes are most affected by only a few days' dietary history (Johnson et al., 2019), recent reports of intake at 6M and 12M were assumed to be sufficient for the analyses described in this paper. Nonetheless, historical breastfeeding and other dietary patterns for both the infant and mother may be valuable in providing a more granular understanding of the associations of human milk exposure and diet with the gut microbiota in

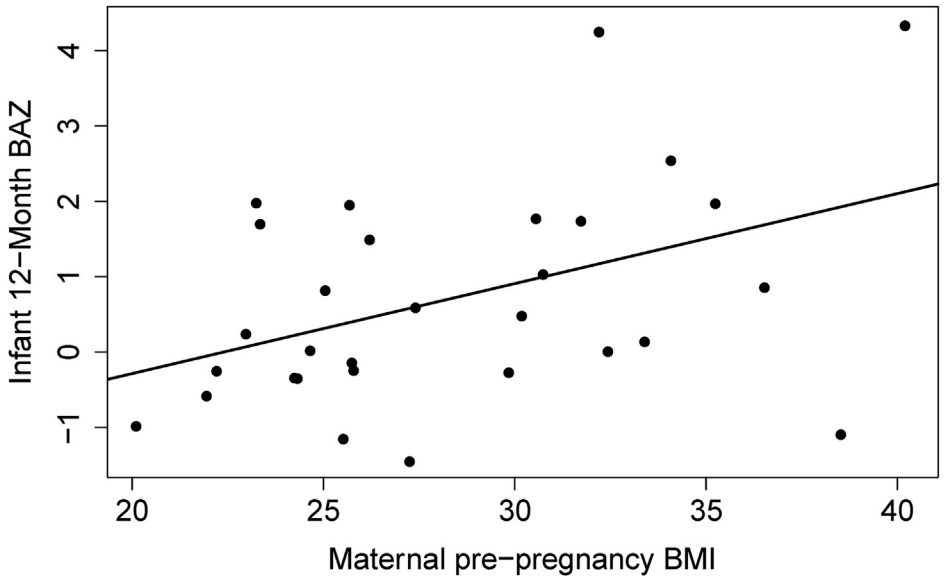

Fig. 6. 12-month infant BAZ is positively associated with maternal pre-pregnancy BMI (r = 0.43, p = 0.01). BAZ is a statistical measure of the number of standard deviations an infant's BMI is away from the mean for that age. Abbreviations: BAZ: BMI-for-age z-score.

future studies. For example, it may be useful for such considerations as whether the weaning diet of infants differs by maternal BMI status and whether this also potentiates infant growth at 12M. Infant size was only assessed at 12M of age, which may not be fully representative of growth over time. The infant gut microbiota can be assessed at a greater range of time points to more accurately determine whether a potential relationship exists and whether the relationship is time dependent. Additionally, the power for some statistical analyses was low (Supplementary Table 2), which may be due to small effect sizes or the number of participants. However, this does not diminish the value of the overarching results since associations between human milk with the infant gut microbiota and BAZ with pre-pregnancy BMI were sufficiently powered. Finally, samples were collected by the women in their homes and mailed to the laboratory, an approach which may be liable to error since the microbial composition of the samples can change during shipment. Nonetheless, the analyses and results can still be considered valid since differences in microbial communities between samples are preserved when all samples are collected and processed under the same conditions (Song et al., 2016; Lauber et al., 2010; Tedjo et al., 2015).

In conclusion, human milk exposure is associated with the infant gut microbiota whereas infant 12M BAZ is correlated with maternal pre-pregnancy BMI but not the infant gut microbiota, suggesting that genetics, physiology, and the environment play a more pronounced role in shaping 12M BAZ. These results underscore the importance of maternal wellbeing and healthy child-rearing practices on the establishment of the gut microbial community and the influence on infant BAZ, which have implications on health outcomes in later life and public health in general.

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CRediT authorship contribution statement

Eliot N. Haddad: interpreted results and, Writing – original draft, wrote the paper, Writing – review & editing, All authors have edited, read and approved the final manuscript. **Kameron Y. Sugino:** conducted the research, Formal analysis, analyzed the, Data curation, data and interpreted the results, Writing – review & editing, All authors have edited, read and approved the final manuscript. **Jean M. Kerver:** designed the research, Writing – review & editing, All authors have edited, read and approved the final manuscript. **Nigel Paneth:** designed the research, Writing – review & editing, All authors have edited, read and approved the final manuscript. **Sarah S. Comstock:** designed the research, conducted the research, Formal analysis, analyzed the, Data curation, data, and interpreted the results, had primary responsibility for final content, Writing – review & editing, All authors have edited, read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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JMK, NP, and SSC designed the research; KYS and SSC conducted the research, analyzed the data and interpreted the results; and ENH interpreted results and wrote the paper. SSC had primary responsibility for final content. All authors have edited, read and approved the final manuscript. Graphical abstract created with BioRender.com.

Appendix A. Supplementary data

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