PLASTIC EXPOSURE AND THE INFANT MICROBIOTA, SCFAs, AND GROWTH TRAJECTORY

by

Heather Jianbo Zhao

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ABSTRACT

Animal models have demonstrated that exposure to plastic and its chemical constituents is associated with altered metabolism and negative health outcomes, including obesity. However, there have been few human studies on the association of plastic exposure during infancy with growth and adiposity, nor have there been any studies to examine whether plastic exposure is associated with altered gut microbiome or microbial metabolites. This question is particularly relevant to infants because prior research has demonstrated that fecal plastic particles are highest among infants. This study assesses the impacts of plastic bottle feeding (every feeding vs. less than every feeding) on the infant microbiome and gut metabolites short-chain fatty acids (SCFA) via fecal samples, and anthropometric measurements, including skinfolds, length for age, and weight-for-length until 1 year of age. Methods: A total of 461 infants from the prospective Nurture pre-birth cohort study were included to examine frequency of plastic bottle feeding at 3 months with anthropometric outcomes at 1 year of age. In addition, a total of 64 and 67 infants were included in analyses on the impact on the gut microbiome and fecal SCFAs, respectively. Microbial taxa were measured by 16S rRNA gene sequencing of the V4 region and SCFA concentrations was quantified using gas chromatography. Results: Infants with less than every feeding plastic bottle at 3 months had a significantly lower alpha diversity at 3 months of age (mean difference= -0.53, 95% CI: -0.90, -0.17) compared to infants with every feeding plastic bottle. Infants with less than every feeding plastic bottle at 3 months had a significantly lower propionic acid concentration at 3 months (mean log difference= -0.53, 95% CI: -1.00, -0.06) compared to infants with every feeding. Infants who were plastic bottle fed 1-3 times/day at 3 months had a significantly lower length for age z-scores at 12 months (mean difference= -0.40, 95% CI: -0.72, -0.07) compared to infants with every feeding plastic bottle. However, such

significant differences in alpha diversity, SCFAs, and anthropometric growth variables were not observed at 12 months nor for the change from 3 to 12 months. Conclusion: Findings from the present study suggest that plastic exposure may impact the infant gut microbiome and SCFAs, potentially affecting growth.

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Dedication

This thesis is dedicated to my parents, Dr. Guangzhi Zhao and Ms. Wei Xia, for nurturing my curiosities and passions, and for cheering me on always.

CONTENTS

INTRODUCTION

In the last decade, exposure to plastics has been recognized as a public health concern (1), especially during vulnerable windows of development, such as infancy. Notably, ingested plastic particles are bioavailable for uptake in the human blood (2). The major sources of plastic particles are postulated to come from diet. Interestingly, recent data suggests that infants have significantly higher fecal concentrations of plastic materials, with 83,000 ng/kg body weight per day in infants compared to 5,800ng/kg body weight per day in adults (3). The disproportionately high plastic exposure among infants is thought to be related to plastic-based products during feeding, such as bottle-feeding and sippy cups (3, 4). Although exposure to plastics has been previously linked with several health outcomes such as obesity, inflammatory bowel disease, and respiratory illnesses (5-8), there is still limited data on how plastics affect metabolic health outcomes like obesity. Furthermore, there is limited data on potential mechanisms by which plastic exposure might alter metabolic health outcomes.

Examination of the human gut microbiome—the ensemble of microorganisms living in the intestinal track—is largely determined by environmental factors, rather than genetics (9) and thus provides an opportunity to examine whether the provenance health effects for environmental factors, like plastic, starts in the intestine. The human microbiome is purported to affect health outcomes by production of metabolites. In particular, the microbial production of short chain fatty acid (SCFA) metabolites is thought to mediate microbiome-health outcome associations. SCFAs are products of the colonic microbiota from fibre and resistant starch. Specific SCFAs, such as acetate, propionate, and butyrate, play a key role in influencing host metabolism (10). To our knowledge, no studies have examined the association of exposure to plastics, particularly

during infancy, with the gut microbiome or microbial metabolites (11). Additionally, fetal and childhood exposure to some plastics has been shown to be associated with altered development such as gestational length, infant weight at birth, and infant growth (12). However, the direct relation between infant plastic exposure and anthropometric growth variables in the first year of life have not been extensively analyzed.

The primary aim of this study was to examine plastic exposure frequency, measured by frequent exposure to plastic bottle feeding, with the infant gut microbiota composition and diversity at 3 and 12 months of age in a longitudinal birth cohort study. We hypothesize that high plastic bottle usage frequency is associated with higher gut microbiome diversity and composition. The secondary objectives of this study are to examine the relation between plastic exposure frequency and SCFA concentration in the stool, and to examine the relation between plastic exposure frequency and anthropometric growth variables in the first year of life.

METHODS

Study population

Our study used data from the prospective Nurture birth cohort (13) located at Durham, North Carolina. From 2013 to 2015, we recruited women with a singleton pregnancy at 20-36 weeks of gestation from county health departments or private prenatal clinics. The cohort required mothers to be at least 18 years of age, live at their current address a minimum of one year, and be able to speak and read English. We excluded mother-infant pairs if the infant was delivered less than 28 weeks of gestation, had congenital abnormalities, or required 3 or more weeks of hospitalization postnatal. We received maternal written informed consent at recruitment and reconfirmed via

phone at delivery. Data collectors conducted home visits when infants were approximately 3, 6, 9, and 12 months of age, supplemented by monthly automated interactive voice response (IVR) calls in between visits. We asked a subsample of mothers to collect stool samples for microbiome analysis from infants at 3 and 12 months of age. This study followed the guidelines of the Declaration of Helsinki and procedures involving human subjects were approved by Duke University Medical Center Institutional Review Board (human subjects committee) (PRO0036342). Although not a clinical trial, the study is registered at clinicaltrials.gov (NCT01788644).

Primary Exposure – plastic bottle frequency use

Mothers reported the daily frequency of plastic bottle feeding among their infants every month up until 12 months during IVR calls. We grouped infant's plastic bottle usage in six categories: <1 times a day, 1/day, 2-3 times a day, 4-5 times a day, 5+ times a day but not at every feeding, or at every feeding. In the analysis for the growth trajectories, we classified plastic bottle usage into four groups: <1 times/day, 1-3 times/day, 4+ times/day, every feeding. In the analysis for microbiota and SCFA, because of our limited sample size, we classified plastic bottle usage into two groups: less than every feeding and every feeding. Less than every feeding plastic bottle usage included infants who used plastic bottle 0-5 times a day, and every feeding plastic bottle usage included infants who used plastic bottle at every feeding.

Covariate measurement

We abstracted data on delivery mode (C-section vs. vaginal delivery, with additional information on type of C-section), birth weight (kg), infant sex, and gestational age (weeks) from medical

records. We collected maternal age (year), ethnicity, and race, number of people per household, pre-pregnancy weight kg and height (meters), highest education obtained, household income, and smoking status at the time of birth. We used self-reported maternal pre-pregnancy weight and height to calculate the pre-pregnancy body mass index (BMI measured in kg/m^2) and categorized those with BMI 25 to 29.9 as overweight and BMI ≥30 as obese.

Sample collection, SCFA, and microbial 6S rRNA gene extraction

We collected stool at 3- and 12-month home visits from diapers and transferred the stool to a 2 ml cryogenic vial (ThermoFisher). Subsequently, we froze the vial at − 80 °C for later processing. The specimens were then thawed and extracted its DNA using the QIAgen MagAttract PowerSoil for KingFisher. We placed 0.5 g of stool in each bead plate well of the PowerSoil kit and extracted DNA per the manufacturer's instructions. Then, we quantified DNA with the Quant-iT dsDNA high sensitivity kit (ThermoFisher). We measured SCFA concentrations using gas chromatography (Thermo Trace 1310) paired to a flame ionization detector (Thermo).

Growth variables

We measured infant weight, height, and skinfold thickness at 3, 6, 9, and 12 months. Trained data collectors measured infant weight in light clothing without shoes via a ShorrBoard Portable Length Board to nearest 1/8 inch; we measured infant height using a Seca Infant Scale to the nearest 0.1 pound. We rounded infant abdomen, subscapular, and triceps skinfold thicknesses measurements to the nearest millimetre using standard techniques (14). We repeated all measurements three times to reduce measurement error, with the final measurement recorded

from the average value. We calculated the age and sex specific weight-for-length z-score, the BMI for age z- score, and length for age z-score using the World Health Organization Child Growth Standards (15). We summed subscapular and triceps skinfolds thickness measures as a proxy for overall fatness.

Data Analysis

Model adjustments and statistical significance thresholds

In all the regression models, we considered associations before and after adjustment for potential confounding factors. We defined a confounder as a variable that has been previously associated with our exposure (plastic bottle use) and our outcomes (gut microbiome or growth parameters), but not in the causal pathway. In our final multivariable model, we controlled for birth weight (kg), gestational age (weeks), maternal age (years), and household income (<$20,000 vs. >= $20,000). We performed additional covariate adjusted analyses to verify our findings were robust to model adjustments for alternative categorizations to plastic bottle exposure.

Alpha diversity

We conducted several linear regression analyses to estimate the association of plastic bottle frequency at 3 months with Shannon index at 3 months and 12 months (Table 2). First, we examined plastic bottle exposure at 3 months with Shannon index at 3 months. Then, we ran a regression with plastic bottle exposure at 3 months and Shannon index at 12 months. Finally, we ran a regression modelling plastic bottle exposure at 3 months with change in Shannon index between 3 and 12 months.

We then examined whether breastfeeding status modified the association of plastic bottle use with Shannon index by repeating the above analyses after stratifying by any breastfeeding at 3 months (Table 3).

We conducted sensitivity analyses to determine the robustness of our analyses. We repeated the above analyses in infants whose mothers were not smokers; who did not consume antibiotics during pregnancy; or who had higher educational status (i.e. had post-secondary education) [Supplemental Table 2]. We conducted further analysis for threats to validity among infants who were plastic bottle-fed formula compared to those who were plastic bottle-fed breastmilk. We further examined our associations by other categories of bottle use frequency and formula feeding status (breastmilk vs. formula) [Supplemental Table 1].

Beta diversity analysis

We used the *R* package phloseq to estimate weighted UniFrac distances, a measure of pairwise community composition, and performed a Principal Coordinate Analysis (PCoA) to graphically assess clustering by variables of interest (16). We further employed permutational multivariate analysis of variance (PERMANOVA) from the *R* package began with 9999 permutations to test for differences in weighted UniFrac distances before and after adjusting for covariates (17).

Microbial community differential abundance analysis

We used beta-binomial regression models that accounted for within-sample taxa correlation and variable sequencing depth from the R package corncob to test for differential abundance of taxa in infants at 3 and 12 months (18). We removed ASV that were singletons or did not have a

mean count at or above the 25th percentile in at least 10% of samples to avoid identifying rare ASV with our small sample population.

SCFA regression models and correlations

We used univariate and multivariate generalized linear regressions to examine the association of SCFA concentrations and plastic bottle usage frequency at 3 and 12 months of age, including separate models for butyric acid, propionic acid, acetic acid, and total SCFAs (sum of butyric acid, propionic acid, acetic acid, isobutyric acid, acetic acid, and isovaleric acid concentrations). We performed a natural log transformation on butyrate and proportionate concentrations at 3 and 12 months to normalize the distribution.

We then examined whether breastfeeding status modified the association of plastic bottle use with SCFA by repeating the above analyses after stratifying by whether the mother was breastfeeding her child at 3 months (Supplementary Table 4).

We conducted sensitivity analyses to determine the robustness of our analyses. We repeated the above analyses in infants whose mothers were not smokers; who did not consume antibiotics during pregnancy; or who had high educational status (i.e., had post-secondary education) [Supplemental Table 6]. Furthermore, we conducted further analysis for threats to validity among infants who were plastic bottle-fed formula compared to those who were plastic bottle-fed breastmilk. We further examined our associations by other categories of bottle use frequency and formula feeding status (breastmilk vs. formula) [Supplemental Table 8].

Growth trajectory analyses

We used univariate and multivariate generalized linear regressions to examine the association of growth outcomes and plastic bottle usage frequency at 3 and 12 months of age, including separate models for subscapular skinfolds, triceps skinfolds, abdominal skinfolds, subscapular + triceps skinfolds, and weight for length z-score.

We then examined whether breastfeeding status modified the association of plastic bottle use with anthropometric outcomes by repeating the above analyses after stratifying by whether the mother was breastfeeding her child at 3 months (Supplementary Table 5).

We conducted sensitivity analyses to determine the robustness of our analyses. We repeated the above analyses in infants whose mothers were not smokers; who did not consume antibiotics during pregnancy; or who had high educational status (i.e. had post-secondary education) [Supplemental Table 7]. Further analysis for threats to validity was conducted for infants who were plastic bottle-fed formula compared to those who were plastic bottle-fed breastmilk. We further examined our associations by other categories of bottle use frequency and formula feeding status (breastmilk vs. formula) [Supplemental Table 9].

We considered a p-value of 0.05 as significant for analyses of alpha diversity, beta diversity, short chain fatty acids, and anthropometrics. For differential abundances analyses, we considered an FDR-corrected threshold of 0.05. Additionally, we looked at an FCR-corrected threshold of 0.2 [Supplementary Table 10].

RESULTS

A total of 461 infants had plastic bottle frequency data at 3 months and were included in the growth trajectory analysis. A total of 70 infants provided stool at either time point, but 3 had missing data on plastic bottle usage frequency, leaving 67 infants in the analytic sample for microbiome as an outcome. A total of 64 infants had microbiota data at 3 months, and 49 at 12 months, with 46 having microbiota data at both time points. A total of 67 infants had SCFA data at 3 months, and 48 at 12 months, with 47 having microbiota data at both time points.

Participant characteristics

Of the 461 infants included in the study, 301 (65.2%) were Black or African American race, 312 (67.5%) were from lower-income (≤ $20,000) households, and 269 (64.0%) had mothers with pre-pregnancy BMI ≥25kg/m^2. At 3 months, 316 (68.4%) of the infants were plastic bottle fed at every feeding. A total of 231 (50.0%) of infants were ever breast fed at 3 months [Table1].

Gut microbiota alpha diversity

The estimated mean differences in microbial Shannon diversity by plastic bottle use are summarized in Table 2. Infants who used plastic bottles less frequently (i.e., less than every feeding) at 3 months had a significantly lower Shannon diversity at 3 months of age (mean difference= -0.53, 95% CI: -0.90, -0.17) compared to infants who used plastic bottles every feeding. Frequency of bottle use at 3 months was not significantly associated with Shannon diversity at 12 months of age (mean difference= -0.19, 95% CI: -0.68, 0.29) [Supplemental Figure2], and was not associated with change in diversity between 3 and 12 months of age (mean difference= 0.40, 95% CI: -0.12, 0.92). Additionally, across subgroups, mothers who were non-

smokers, mothers who did not take antibiotics during pregnancy, mothers with high education attainment; Shannon diversity at 3 months continued to be lower among those who used plastic bottles less frequently compared to those with every feeding plastic bottle at 3 months, [Supplemental Table 2].

Table 3 shows the estimated mean differences in microbial Shannon diversity by plastic bottle usage and breast-feeding status at 3 months (i.e., among 4 groups: never breastfed and every feeding plastic bottle usage (reference group), never breastfed and less than every feeding plastic bottle usage, ever breastfed and every feeding plastic bottle usage, and every breastfed and less than every feeding plastic bottle usage). Infants who were ever breastfed at 3 months and used plastic bottles less frequently at 3 months had a significantly lower change in Shannon diversity at 3 months of age (mean difference= -0.64, 95% CI: -1.03, -0.24) compared to infants who were never breastfed and had every feeding plastic bottle usage. There were no significant differences between the other two categories in comparison to the reference group (i.e., never breastfed and every feeding plastic bottle usage). The 4 groups were not associated with the Shannon index of diversity at 12 months or change in Shannon diversity from 3 to 12 months.

Gut microbiota beta diversity

Plastic bottle usage frequency was not significantly associated with Weighted UniFrac at 3 or 12 months of age [Supplemental Figure 1a and 1b]. Plastic bottle usage at 3 months with infant gut microbiota beta diversity at 3 months of age had PERMANOVA values of: $p = 0.24$, $R^2=0.019$; and at 12 months of age had PERMANOVA values of: $p = 0.78$, and $R^2=0.012$.

Gut microbiota composition

The mean relative abundances of major bacterial genus at 3 and 12 months by plastic bottle usage are shown in Fig.1. Less frequent plastic bottle usage was associated with differential abundance of 30 bacterial ASVs in the infant gut at 3 months and 26 bacterial ASV in the infant gut at 12 months of age, after adjustment for potential confounders [Supplemental Figure 3].

SCFA concentrations

Table 4 shows the estimated mean differences in SCFA concentrations (acetate, propionate, butyrate, and total SCFAs) at 3 months, 12 months, and change from 3 to 12 months by plastic bottle usage at 3 months. Infants with less than every feeding plastic bottle at 3 months had a significantly lower propionic acid concentration at 3 months of age (mean log difference= -0.53, 95% CI: -1.00, -0.06) compared to infants with every feeding. However, such significant differences were not observed in the 12 months analysis nor the change from 3 to 12 months. Acetic acid, butyric acid, and total SCFAs mean differences were not significant at 3 months, 12 months, or the change from 3 to 12 months.

Anthropometric growth variables

Table 5 shows the anthropometric growth variables measured at 12 months (subscapular skinfolds, triceps skinfolds, abdominal skinfolds, subscapular + triceps skinfolds, BMI z-score, infant length for age z-score, and weight for length z-score). Infants who were plastic bottle fed 1-3 times/day at 3 months had a significantly lower length for age z-score at 12 months (mean difference= -0.40, 95% CI: -0.72, -0.07) compared to infants with every feeding plastic bottle.

There were no significant mean differences in other growth outcomes and across different plastic bottle usages [Figure 2].

DISCUSSION

Our prospective birth cohort of racially diverse mother-infant dyads from North Carolina found that less frequent plastic bottle usage (i.e., using plastic bottles less than every feeding vs. every feeding) was associated with lower alpha microbiome diversity at 3 months of age. Less frequent plastic bottle use was also associated with a lower fecal propionic acid concentration at 3 months of age. Furthermore, infants who were plastic bottle fed 1-3 times per day (vs. every feeding) had a significantly lower length for age z-score at 12 months. However, other plastic bottle feeding categories were not associated with linear growth, nor were they associated with weight, suggesting that plastic bottle use may not greatly influence growth outcomes in infancy.

We are not aware of other human-based studies that have examined the effect of plastic bottle usage on gut microbiota composition, biodiversity, and SCFA in infancy. However, in an animal study, introducing polystyrene microplastics significantly altered the alpha diversity of mice gut microbiota, although the authors did not mention the direction of change (6). The investigators also found that the abundance of *Blautia, Bifidobacterium, Prevotella,* and *Parabacteroides* decreased, as replicated in our 3-month analysis [Supplementary Figure 3a].

Our finding that plastic bottle use was associated with lower fecal propionic acid concentration is of particular interest. Propionic acid is hypothesized to lower lipogenesis in tissues (19). A study in mice found that administering propionic acid was protective against diet-induced obesity,

insulin resistance, and reduce food intake (20). Furthermore, it is possible that having less SCFAs detected in the feces could indicate that more propionate is being retained in the gut and serum. This may explain why infants who were plastic bottle fed less than everyday had lower propionate detected in their serum and had lower weight-for-length z-scores tracked through the first 12 months compared to infants who were plastic bottle fed every day.

It is important to study plastics in infants because microplastic levels have been found to be higher in infants compared to adults, likely because infants' unique dietary exposures (3, 4). For example, infants have extensive use of plastic products such as bottles and sippy cups. Infant formula prepared in plastic bottles can release millions of microplastics (4), potentially exacerbating the gut microbiota. The Canadian CHILD birth cohort found that formula fed infants had a greater diversity at 3 months of age (21). This is further reflected in our results in which infants with less than every feeding plastic bottle and breast-fed had a lower Shannon diversity that those who were formula-fed [Supplementary Table 1]. Furthermore, a Norwegian birth cohort found that higher diversity at 3 months may be associated with an increased risk of overweight in adulthood (22). Thus, plastic bottle exposure may contribute to the risk of obesity, however, such associations were not identified in our cohort.

Strengths and Limitations

Our study is strengthened by its longitudinal design, the detailed infant growth measurement data collected along with detailed covariate data, the high sociodemographic and racial diversity, and the joint measurement of the fecal microbiome and metabolome. Thus, we could prospectively determine the plastic bottle usage, extract covariate information from electronic medical records,

and thereby increasing the accuracy of our data and reducing recall bias for association of plastic bottle use with microbiome, metabolite, and anthropometric outcomes. Furthermore, we were able to adjust for multiple potential confounders in our multivariable regression models.

There were several limitations to our study. First, we relied on 16S rRNA sequencing data which may not always allow for species level resolution. Secondly, we did not have blood measurements available to measure circulation SCFAs which differ in prevalence in the stool compared to blood. Third, plastic exposure was not measured objectively such as using a biomarker. Fourth, we used a small sample from one region in North Carolina which may limit generalizability. Lastly, even though mothers may have plastic bottle fed their infant, the substance mothers put in their plastic bottle, whether it be breastmilk or formula, may differ. Nevertheless, we addressed this limitation by conducting a subgroup analysis for joint effects in plastic bottle usage frequency and status of formula feeding (breastmilk vs. formula) [Supplementary Table 1]. Another limitation is the potential of reporting bias. Mothers may have underreported plastic bottle feeding if they believed that direct breastfeeding is a better practice. Lastly, despite covariate adjustment, we cannot rule out the possibility of unmeasured or residual confounding.

CONCLUSION

Our study showed that at 3 months, infants who were plastic bottle fed in less than every feeding had a lower alpha diversity than infants who were plastic bottle fed in every feeding. Encouraging mothers to lower their use of plastic bottles may be beneficial to their infant's gut microbiota. Nevertheless, larger longitudinal gut microbiota studies are required to confirm

whether the microbiome is impacted by plastic exposure in the first few months of life, and whether the infant microbiome is causal to developing health conditions such as greater weight gain or reduced linear growth, which we did not identify in our cohort.

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TABLES

Table 1 Characteristics of Mother-Infant Pairs in the Nurture Cohort Study by Plastic Bottle Frequency at 3 months

	Every feeding N=316	4+ /day N=43	1-3/day N=60	<1/day N=43	p-value
C-section	117 (37%)	16 (37%)	20 (33%)	6 (14%)	0.03
Pre-pregnancy BMI≥25kg/m2	215 (68%)	27 (63%)	36 (60%)	18 (43%)	0.01
Maternal age (SD)	26.59 (5/40)	28.84 (5.65)	29.81 (6.50)	39.28 (5.64)	<0.001
Male sex	161 (50.95%)	21 (48.84%)	28 (47.67%)	23 (54.76%)	0.87
Black infant race	232 (73.42%)	25 (58.14%)	33 (55.00%)	11 (26.19%)	<0.001
Ever breastfed at 3 months	95 (30.06%)	36 (87.72%)	58 (96.67%)	42 (100.00%)	<0.001
Maternal low educational achievement	175 (55.38%)	18 (41.86%)	11 (18.33%)	8 (19.05%)	<0.001
Household income < $20 000	241 (77.24%)	26 (60.47%)	31 (52.54%)	14 (33.33%)	<0.001
Maternal antibiotics during pregnancy	100 (31.65%)	15 (34.88%)	11 (18.33%)	18 (42.86%)	0.06
Current smoker	45 (14.24%)	3 (6.98%)	2 (3.33%)	0 (0.00%)	0.01
Birth weight, kg (SD)	3.20 (0.53)	3.20 (0.41)	3.37 (0.49)	3.26 (0.53)	0.11
Infant gestational age in weeks (SD)	38.59 (1.51)	38.81 (1.75)	38.93 (1.41)	38.71 (1.38)	0.37
Number of people in household (SD)	2.50 (1.41)	2.09 (1.17)	2.10 (1.28)	2.38 (1.41)	0.09

Table 2 Unadjusted and multivariable-adjusted mean difference in Shannon diversity of the infant gut microbiota, assessed from infant stool at 3 and 12 months of age, by plastic bottle usage at 3 months

	Difference in Shannon diversity at 3 months (n=64)		Difference in Shannon diversity at 12 months (n=46)		Difference in the change in Shannon diversity from 3 months to 12 months) (n=46)	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
Every feeding	0.0 (ref) n= 49	0.0 (ref) n= 49	0.0 (ref) n=34	0.0 (ref) n=34	0.0 (ref) n=34	0.0 (ref) n=34
Less than every feeding	-0.46 *** (-0.72, 0.20) n= 15	-0.53 ** (-0.90, -0.17) n= 15	-0.07 (-0.44, 0.29) n=12	-0.19 (-0.68, 0.29) n=12	0.37 (-0.01, 0.76) n=12	0.40 (-0.12, 0.92) n=12

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year. Vs. ≥ $20 000 per year)
* = p < 0.05, ** = p < 0.01, and *** = p < 0.001

Table 3 Unadjusted and multivariable-adjusted mean difference in Shannon diversity of the infant gut microbiota, assessed from infant stool at 3 and 12 months of age, by plastic bottle usage at 3 months and breastfeeding status at 3 months

	Difference in Shannon diversity at 3 months (n=64)		**Difference in Shannon diversity at 12 months (n=46)**		**Difference in the change in Shannon diversity from 3 months to 12 months) (n=46)**	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
Never breast fed at 3 months and Every feeding	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=28	0.0 (ref) n=28	0.0 (ref) n=28	0.0 (ref) n=28
Never breastfed at 3 months and Less than every feeding	-0.06 (-0.58, 0.46) n=3	-0.19 (-0.77, 0.29) n=3	0.24 (-0.89, 1.37) n=1	0.11 (-1.05, 1.27) n=1	0.19 (-1.00, 1.37) n=1	0.05 (-1.20, 1.30) n=1
Ever breastfed at 3 months and Every feeding	0.09 (-0.19, 0.36) n=13	0.18 (-0.13, 0.49) n=13	0.15 (-0.35, 0.65) n=6	0.20 (-0.34, 0.73) n=6	-0.07 (-0.59, 0.46) n=6	-0.17 (-0.75, 0.40) n=6
Ever breastfed at 3 months and Less than every feeding	-0.53 *** (-0.82, -0.25) n=12	-0.64 ** (-1.03, -0.24) n=12	-0.07 (-0.47, 0.32) n=11	-0.25 (-0.80, 0.30) n=11	0.38 (-0.04, 0.79) n=11	0.46 (-0.13, 1.05) n=11

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year. Vs. ≥ $20 000 per year)

* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$

Table 4 Unadjusted and multivariable-adjusted mean difference in short-chain fatty acid concentrations (µmol/g), from infant stool provided at 3 and 12 months of age, by plastic bottle usage at 3 months

	Acetic Acid Mean difference		**Propionic Acid± Mean difference**		**Butyric Acid± Mean difference**		**Total SCFAs Mean difference**	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
SCFAs at 3 months								
Every feeding	0.0 (ref) n=52	0.0 (ref) n=52	0.0 (ref) n=52	0.0 (ref) n=52	0.0 (ref) n=51	0.0 (ref) n=51	0.0 (ref) n=52	0.0 (ref) n=52
Less than every feeding	1.90 (-23.90, 16.45) n=15	1.90 (-26.00, 29.79) n=15	-0.36 * (-0.70, -0.03) n=15	-0.53* (-1.00, -0.06) n=15	-0.55 * (-1.06, -0.03) n=14	-0.54 (-1.25, 0.16) n=14	-15.22 (-42.50, 12.05) n=15	-13.51 (-51.18, 24.16) n=15
SCFA at 12 months								
Every feeding	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36
Less than every feeding	-17.12 (-39.68, 5.44) n=12	-26.40 (-56.60, 3.80) n=12	-0.12 (-0.48, 0.25) n=12	-0.11 (-0.58, 0.35) n=12	0.00 (-0.49, 0.49) n=12	-0.08 (-0.74, 0.57) n=12	-22.13 (-54.70, 10.45) n=12	-32.72 (-75.44, 9.99) n=12
Change from 3 to 12 months								
Every feeding	0.0 (ref) n=35	0.0 (ref) n=35	0.0 (ref) n=33	0.0 (ref) n=33	0.0 (ref) n=34	0.0 (ref) n=34	0.0 (ref) n=35	0.0 (ref) n=35
Less than every feeding	-19.33 (-51.56, 12.90) n=12	-33.54 (-75.57, 8.49) n=12	0.16 (-0.29, 0.60) n=12	0.37 (-0.16, 0.89) n=12	0.63 (-0.07, 1.34) n=11	0.52 (-0.41, 1.46) n=11	-14.02 (-58.97, 30.94) n=12	-24.28 (-81.14, 32.58) n=12

±Natural log + 1 transformation used for butyric acid and propionic acid at 3 and 12 months
Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year. vs. ≥ $20 000 per year)
* = $p < 0.05$

Table 5 Unadjusted and multivariable-adjusted mean difference in growth parameters at 12 months between plastic bottle usage at 3 months

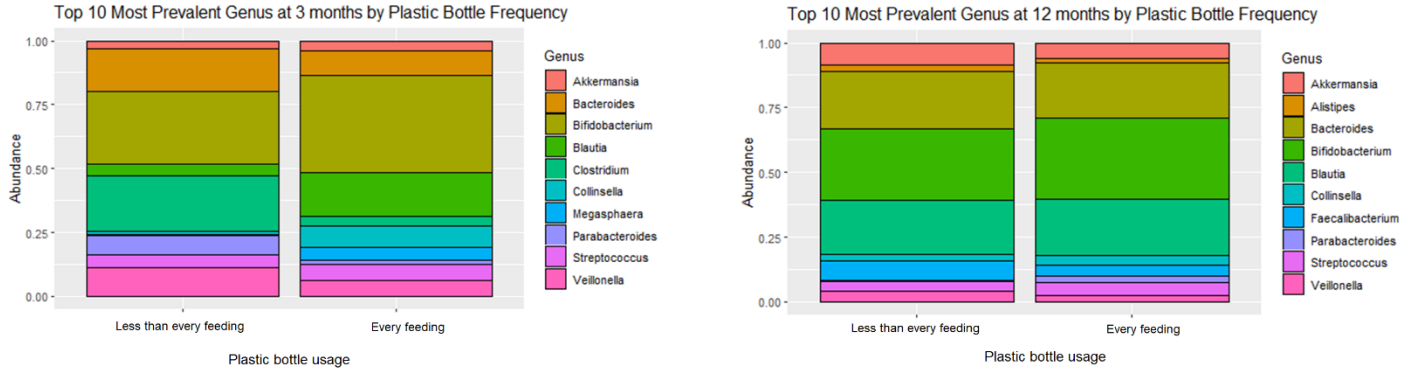
		Subscapular skinfolds	Abdominal skinfolds	Triceps skinfolds	Subscapular + Triceps skinfolds	BMI z-score	Length for age z- score	Weight for length z-score
Crude	Every feeding	n=240 reference	n=239 reference	n=239 reference	n=239 reference	n=240 reference	n=240 reference	n=250 reference
	4+/day	n=37 -0.40 (-0.89, 0.09)	n= 37 -0.12 (-0.82, 0.58)	n=37 -0.15 (-0.83, 0.54)	n=37 -0.53 (-1.58, 0.51)	n=37 -0.34 (-0.70, 0.02)	n=37 -0.08 (-0.29, 0.44)	n=37 -0.32 (-0.68, 0.03)
	1-3/day	n=55 -0.09 (-0.50, 0.33)	n=54 0.04 (-0.56, 0.64)	n=55 -0.04 (-0.63, 0.54)	n=55 -0.12 (-1.01, 0.76)	n=53 0.03 (-0.28, 0.33)	n=53 -0.38 * (-0.69, -0.06)	n=53 -0.03 (-0.33, 0.28)
	<1/day	n=39 -0.29 (-0.77, 0.19)	n=39 0.38 (-0.31, 1.06)	n=39 0.24 (-0.43, 0.92)	n=39 -0.03 (-1.05, 0.99)	n=39 -0.15 (-0.49, 0.20)	n=39 -0.19 (-0.55, 0.17)	n=39 -0.17 (-0.52, 0.18)
Adjusted	Every feeding	n=236 reference	n=235 reference	n=235 reference	n=235 reference	n=236 reference	n=236 reference	n=236 reference
	4+t/day	n=37 -0.31 (-0.81, 0.18)	n= 37 -0.01 (-0.71, 0.69)	n=37 -0.17 (-0.87. 0.53)	n=37 -0.48 (-1.54, 0.57)	n=37 -0.31 (-0.66, 0.04)	n=37 0.14 (-0.23, 0.50)	n=37 -0.28 (-0.62, 0.07)
	1-3/day	n=54 -0.07 (-0.51, 0.36)	n=53 0.08 (-0.54, 0.70)	n=54 -0.13 (-0.74, 0.47)	n=54 -0.20 (-1.13, 0.73)	n=53 -0.04 (-0.35, 0.27)	n=53 -0.40* (-0.72, -0.07)	n=53 -0.10 (-0.40, 0.21) p=0.50
	<1/day	n=39 -0.19 (-0.69, 0.31)	n=39 0.47 (-0.24, 1.19)	n=39 0.12 (-0.59, 0.83)	n=39 -0.06 (-1.13, 1.01)	n=39 -0.13 (-0.49, 0.22)	n=39 -0.16 (-0.09, 0.08)	n=39 -0.14 (-0.50, 0.21)

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year). Additionally, for weight for length z-score we controlled for birth weight for gestational age z-score instead of weight-for-length z-score at birth because this measurement was only collected at 3,6,9, and 12 months.

* = $p < 0.05$

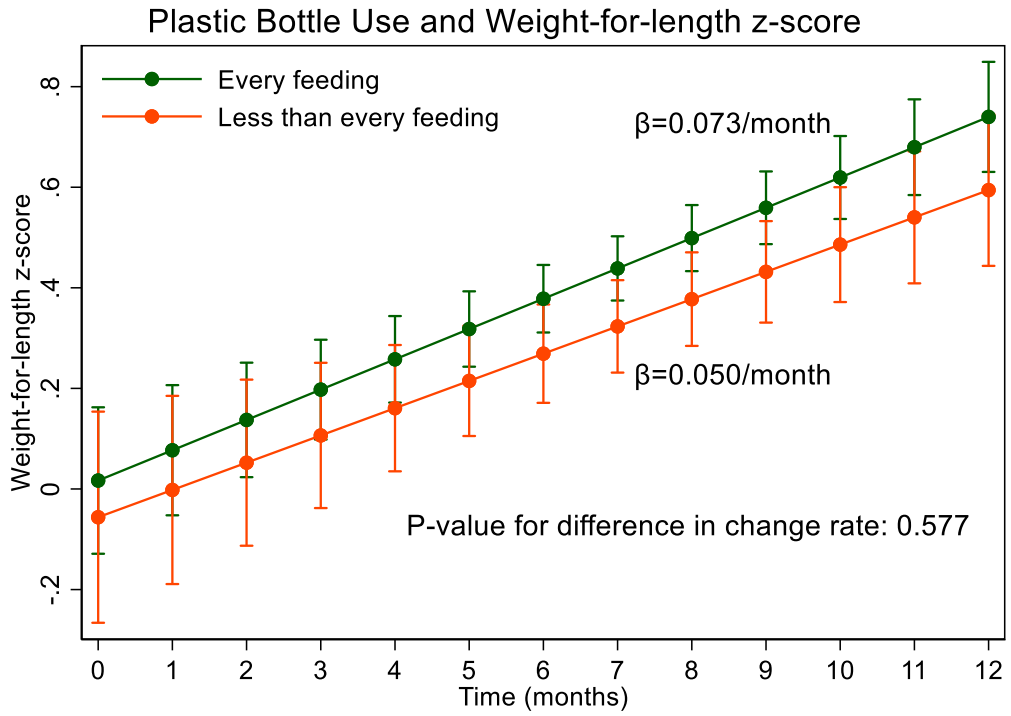
FIGURES

Fig.1



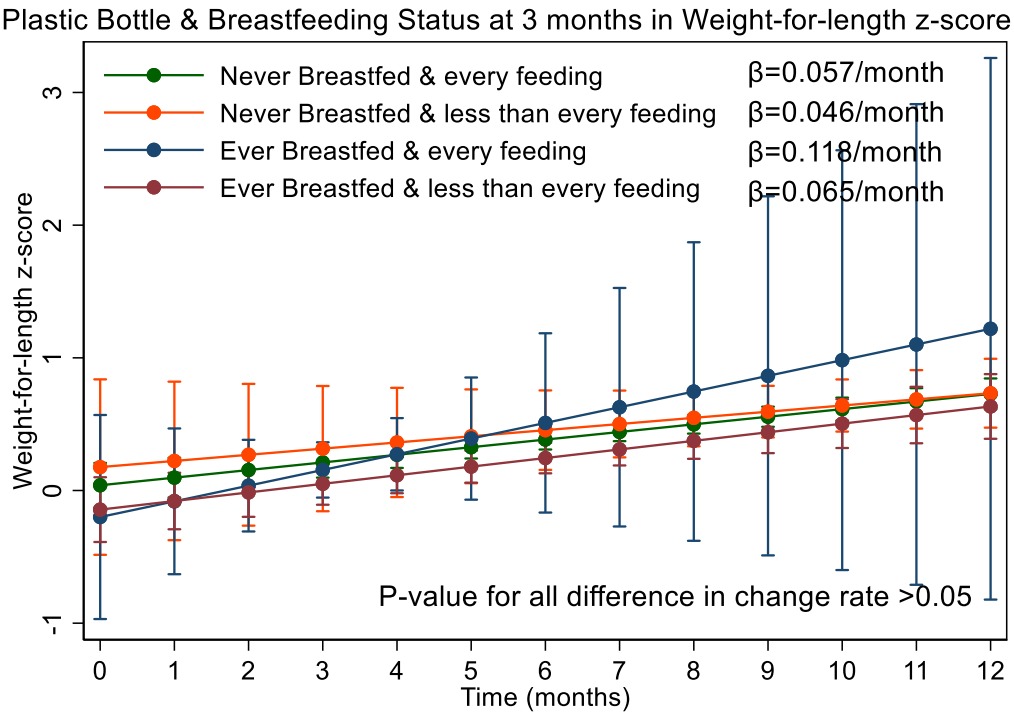
Unadjusted mean percent of a major bacterial genus at 3 and 12 months of age, by plastic bottle usage.

Fig. 2



Weight-for-length z-score trajectory in infants at 3 months who were plastic bottle fed every feeding and less than every feeding. Controlled for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year). Additionally, we controlled for birth weight for gestational age z-score instead of weight-for-length z-score at birth because this measurement was only collected at 3,6,9, and 12 months.

Fig. 3



Weight-for-length z-score trajectory in infants at 3 months who were plastic bottle fed in everyday feeding and less than everyday feeding and at 3 months who were ever or never breastfed. Controlled for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year). Additionally, we controlled for birth weight for gestational age z-score instead of weight-for-length z-score at birth because this measurement was only collected at 3,6,9, and 12 months

SUPPLEMENTARY

Figure 1A

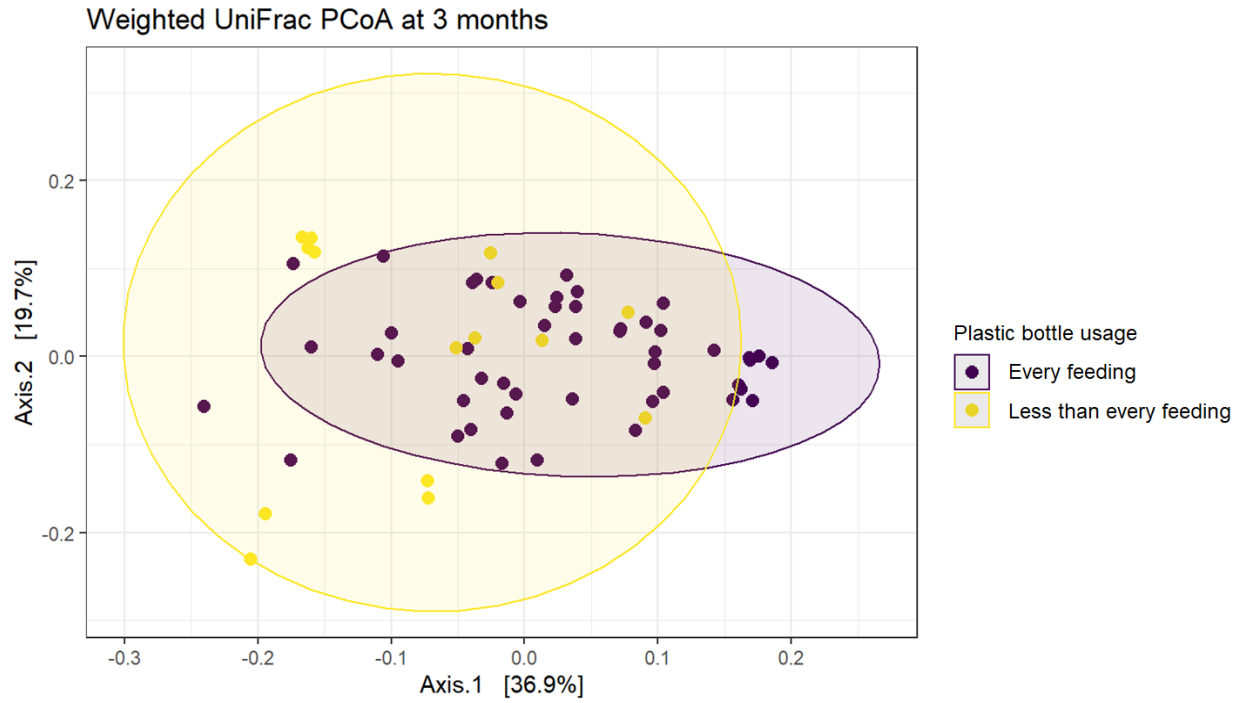
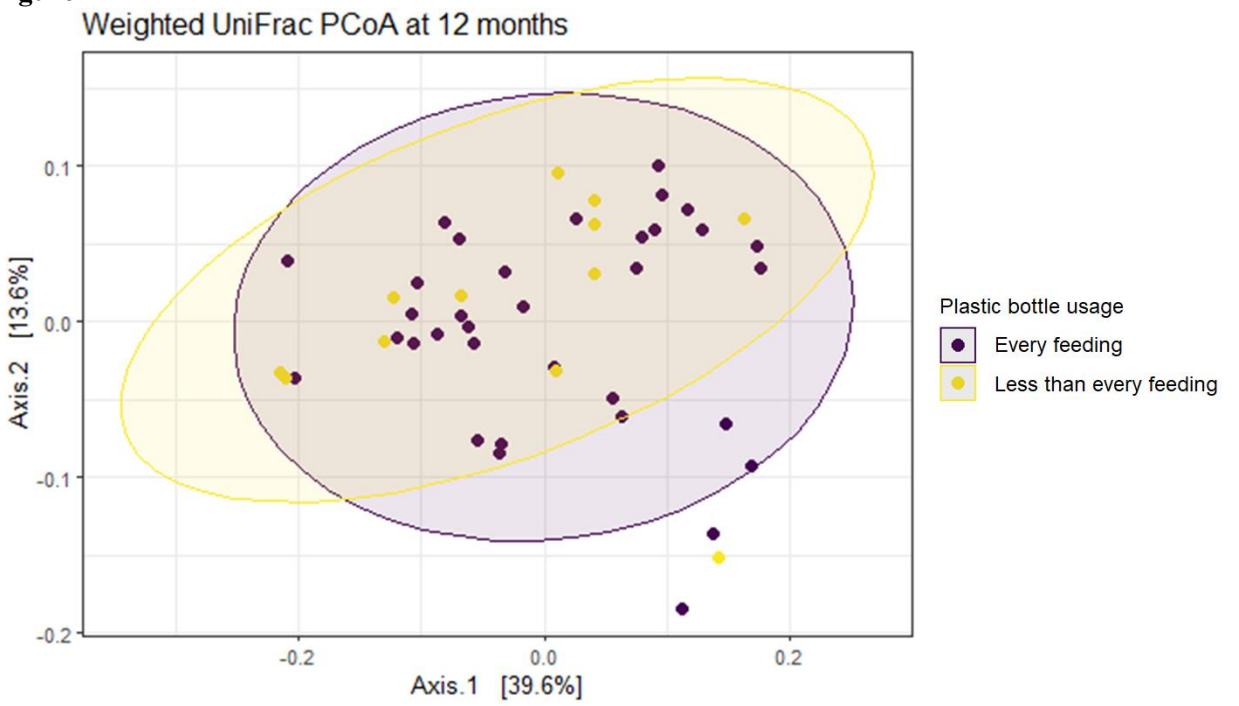
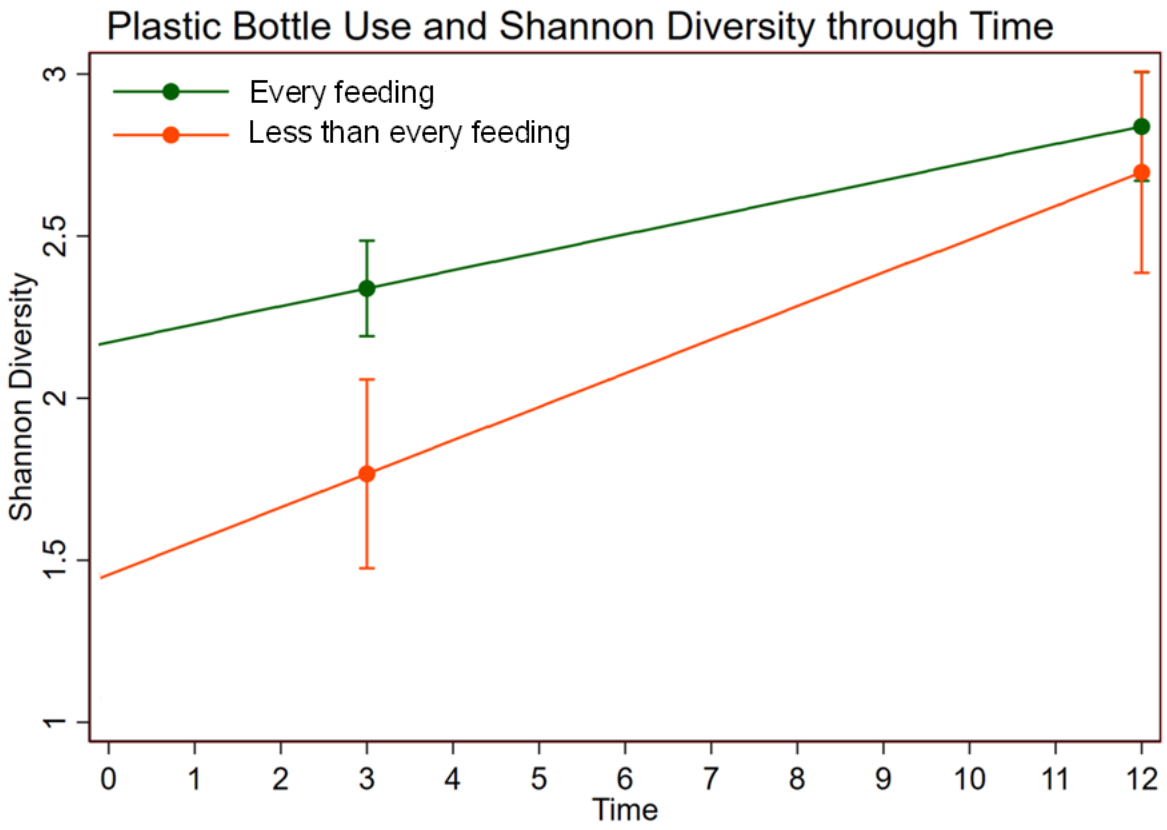


Figure 1B



Weighted UniFrac PCoA plots showing the association of plastic bottle usage at 3 months with infant gut microbiota beta diversity at (A) 3 months of age (PERMANOVA beta = 0.04, p = 0.24,R^2=0.019) and (B) 12 months of age (PERMANOVA beta = 0.02, p = 0.78, R^2=0.012). Points are colored by the plastic bottle usage.

Figure 2



Shannon diversity tracked through time with available data at 3 month and 12 months

Figure 3a

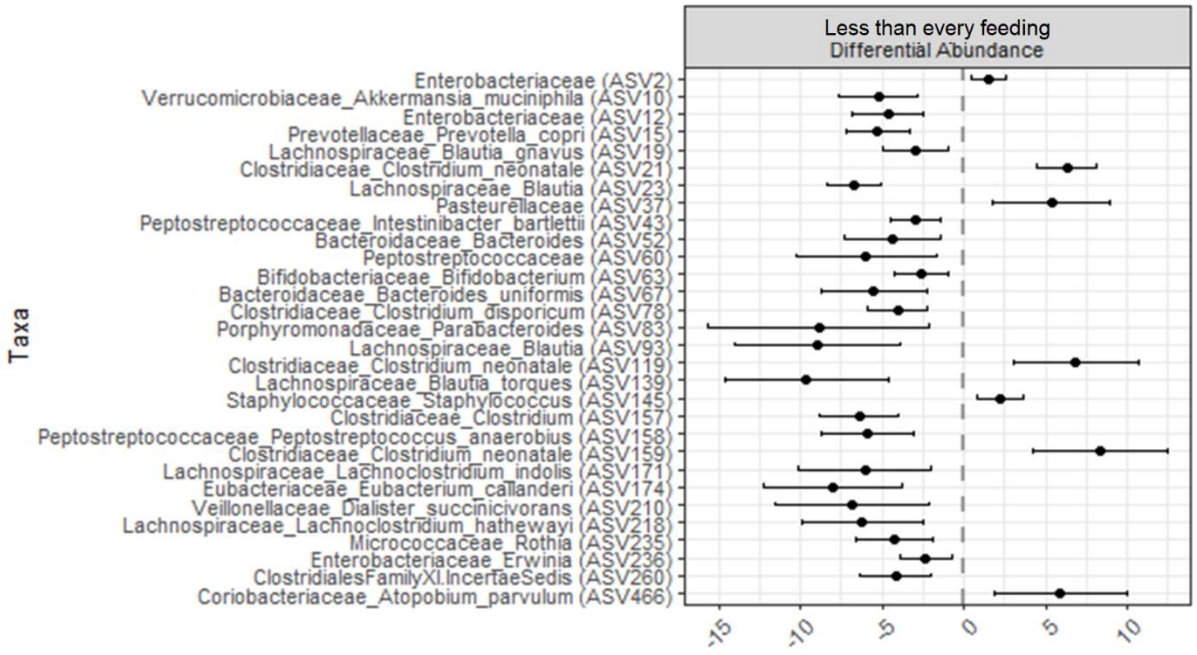
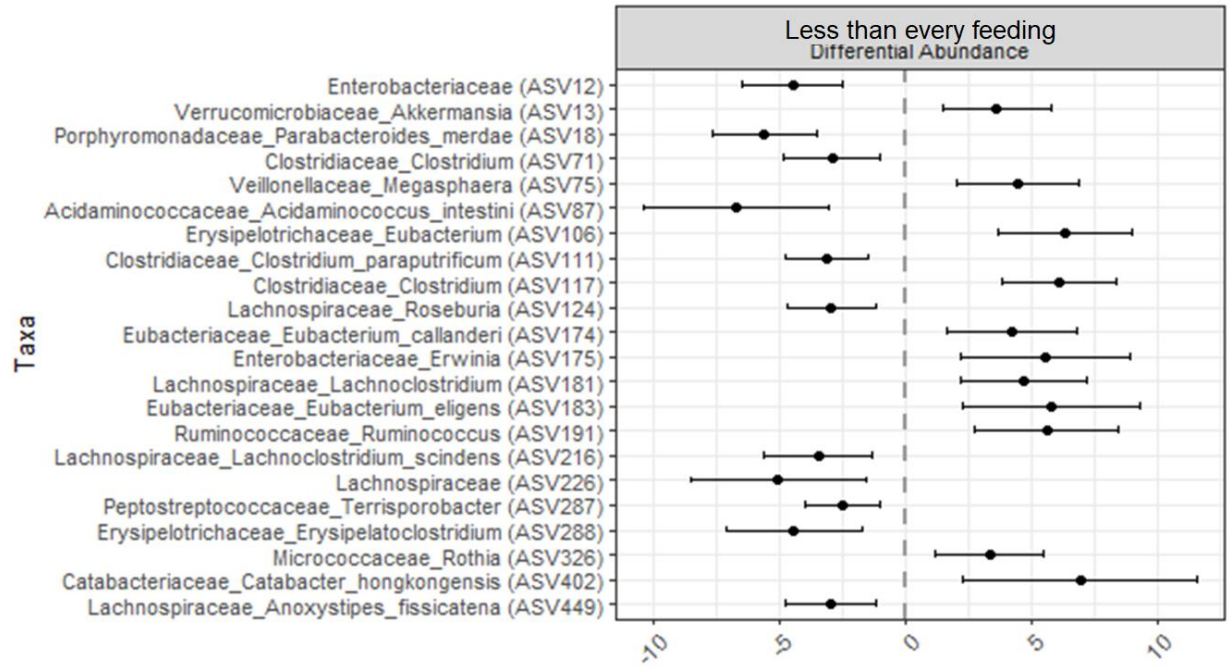


Figure 3b



Differential abundance of AVS at (a) 3 months and (b) 12 months comparing less than everyday plastic bottle usage vs. everyday plastic bottle usage at 3 months, adjusting for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year).

Figure 4a

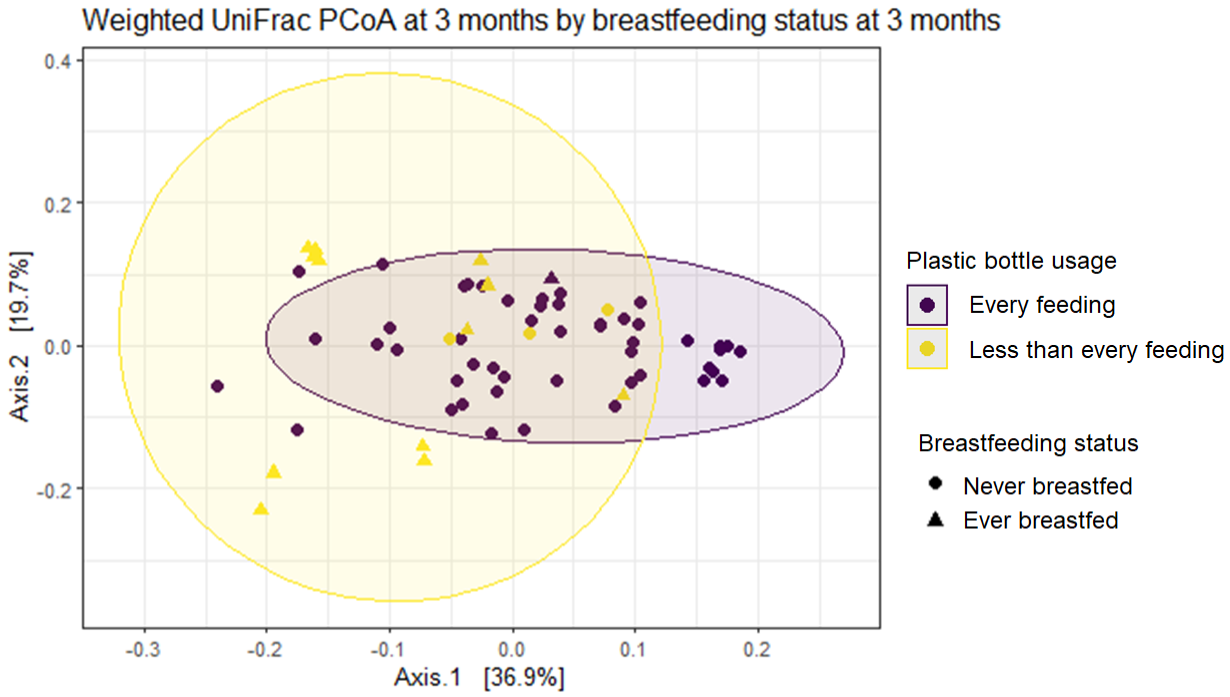
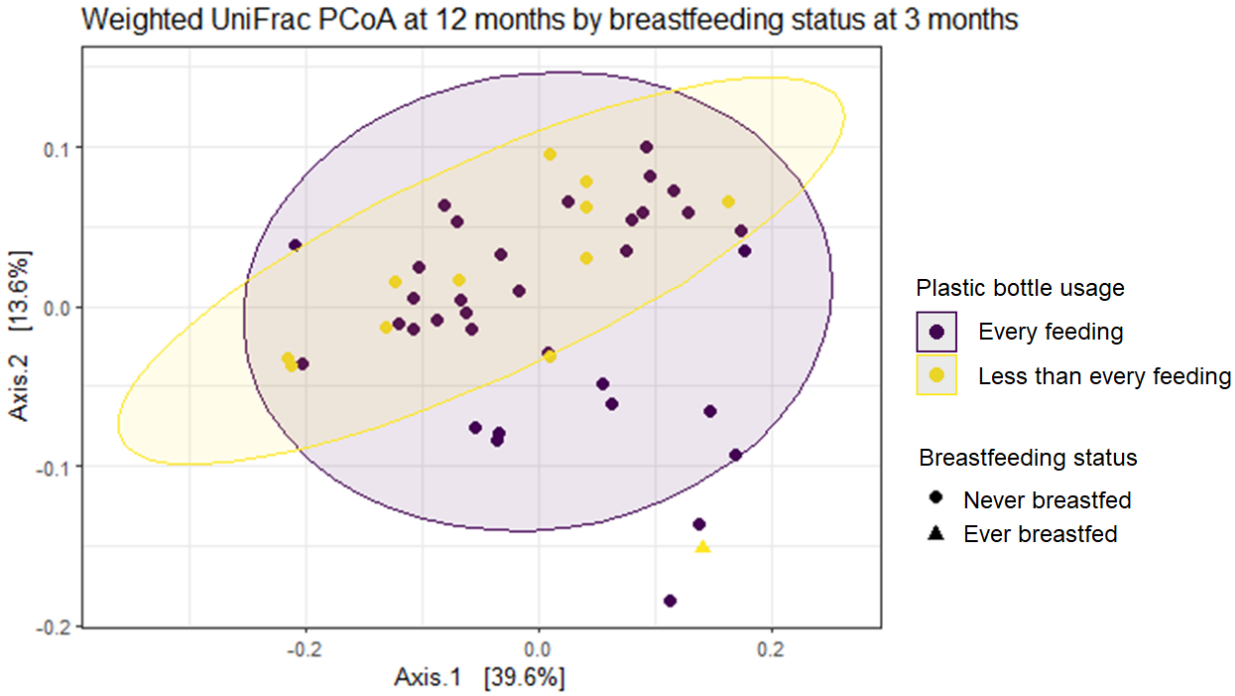


Figure 4b



Weighted UniFrac PCoA plots showing the association of plastic bottle usage at 3 months by breast feeding status at 3 months with infant gut microbiota beta diversity at (A) 3 months of age (PERMANOVA beta = 0.01, p = 0.85, R^2=0.006) and (B) 12 months of age (PERMANOVA beta = 0.02, p = 0.76, R^2=0.013). Points are colored by the plastic bottle usage. Shapes are categorized by breast feeding status.

Figure 5a

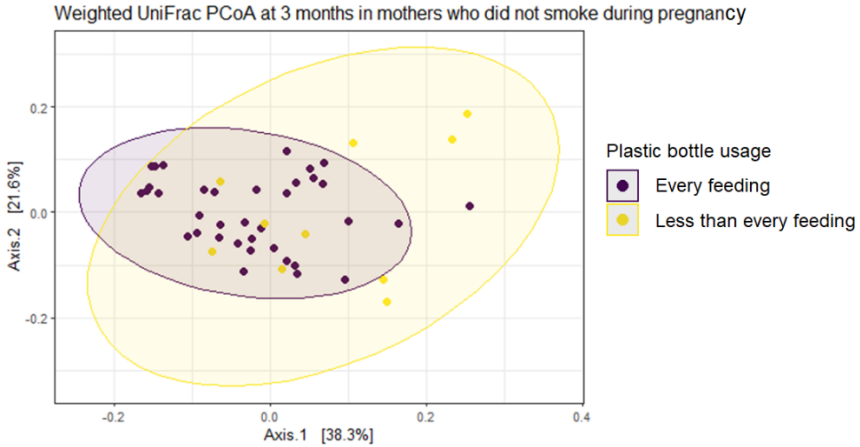


Figure 5b

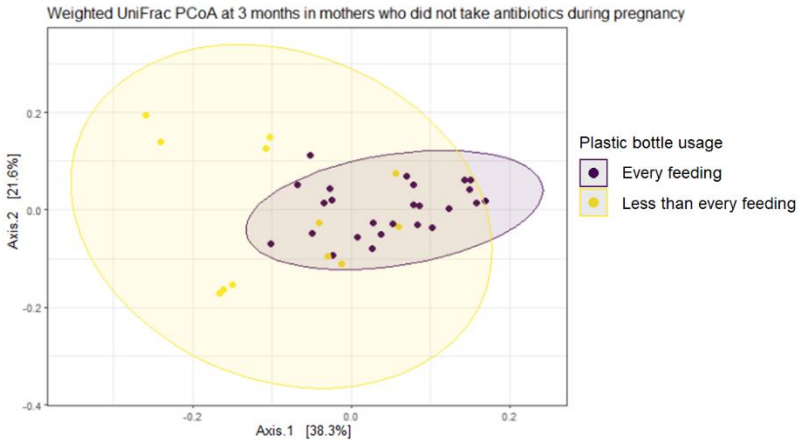
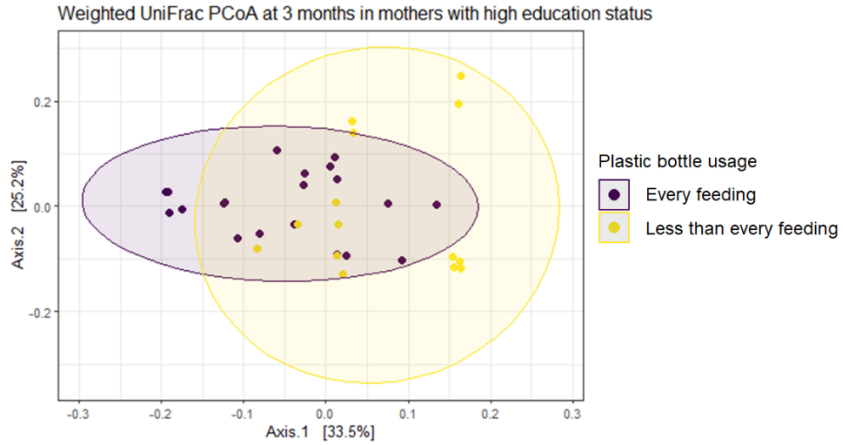


Figure 5c



Weighted UniFrac PCoA plots showing the association of plastic bottle usage at 3 months with infant gut microbiota beta diversity at 3 months among (A) mothers who did not smoke during pregnancy (PERMANOVA beta = 0.03, p = 0.45, R^2=0.018), (B) mothers who did not take antibiotics during pregnancy (PERMANOVA beta = 0.03, p = 0.30, R^2=0.027), and (C) mothers with high education status (PERMANOVA beta = 0.05, p = 0.13, R^2=0.046). Points are colored by plastic bottle usage.

Table 1 Unadjusted and multivariable-adjusted mean difference in Shannon diversity of the infant gut microbiota, assessed from infant stool at 3 and 12 months of age, by plastic bottle usage at 3 months and formula feeding status (breastmilk vs. formula) at 3 months

	Difference in Shannon diversity at 3 months (n=64)		**Difference in Shannon diversity at 12 months (n=46)**		**Difference in the change in Shannon diversity from 3 months to 12 months) (n=46)**	
	Crude	**Adjusted**	**Crude**	**Adjusted**	**Crude**	**Adjusted**
Every feeding and formula feeding	1.0 (ref) n=49	0.0 (ref) n=49	1.0 (ref) n=34	0.0 (ref) n=34	0.0 (ref) n=34	0.0 (ref) n=34
Less than every feeding and formula feeding	-0.30 (-0.63, 0.03) n=8	-0.39 (-0.80, 0.02) n=8	-0.08 (-0.56, 0.42) n=6	-0.18 (-0.75, 0.40) n=6	0.21 (-0.29, 0.72) n=6	0.26 (-0.35, 0.88) n=6
Less than every feeding and breastmilk	-0.64 (-0.99, -0.30) n=7	-0.72 ** (-1.16, -0.28) n=7	-0.07 (-0.56, 0.42) n=6	-0.21 (-0.80, 0.38) n=6	0.53 * (-0.02, 1.04) n=6	0.54 (-0.09, 1.17) n=6

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year)
* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$

Table 2 Sensitivity Analysis in multivariable-adjusted mean difference in Shannon diversity of the infant gut microbiota, assessed from infant stool at 3 months of age, by plastic bottle usage at 3 months

	Total cohort	**Mothers who were non-smokers**	**Mothers who did not take anti-biotics during pregnancy**	**Mothers with high education status**
Every feeding	0.0 (ref) n=49	0.0 (ref) n=38	0.0 (ref) n=25	0.0 (ref) n=20
Less than every feeding	-0.53 (-0.90, -0.17) n=15	-0.49 * (-0.88, -0.10) n=15	-0.49 * (-0.96, -0.03) n=12	-0.66 * (-1.17, -0.14) n=14

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year)
* = $p < 0.05$

Table 3 Multivariable-adjusted mean difference in growth outcomes at 12 months of age, by plastic bottle usage at 3 months and breast-feeding status at 3 months

	Subscapular N=299	Abdominal N=297	Triceps N=298	Subscapular + Triceps N=298	Weight for length z-score N=298	BMI z-score N=298	Length-for-weight z-score N=298
Not breastfed & Every feeding	0.0 (ref)	0.0 (ref)	0.0 (ref)	0.0 (ref)	0.0 (ref)	0.0 (ref)	0.0 (ref)
Not breastfed & 4+ plastic use/day	0.04 (-0.56, 0.66)	0.36 (-0.52, 1.25)	0.43 (-0.44, 1.31)	0.48 (-0.84, 1.81)	-0.08 (-0.52, 0.35)	-0.12 (-0.56, 0.31)	0.27 (-0.18, 0.71)
Not breastfed & 1-3 plastic use/day	0.31 (-0.33, 0.95)	0.14 (-0.79, 1.07)	0.23 (-0.69, 1.15)	0.55 (-0.84, 1.93)	0.17 (-0.29, 0.62)	0.22 (-0.24, 0.67)	-0.28 (-0.75, 0.19)
Not breastfed & <1 plastic use/day	0.06 (-1.09, 1.21)	-0.11 (-0.77, 1.56)	0.20 (-1.44, 1.85)	0.28 (-2.21, 2.77)	0.01 (-0.81, 0.82)	-0.02 (-0.85, 0.80)	0.21 (-0.63, 1.05)
Breastfed & Every feeding	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Breastfed & 4+ plastic use/day	-0.53 (-1.48, 0.42)	-0.37 (-1.75, 1.00)	-1.38 * (-2.74, -0.02)	-1.91 (-3.96, 0.15)	-0.33 (-1.01, 0.34)	-0.28 (-0.95, 0.40)	-0.49 (-1.18, 0.20)
Breastfed & 1-3 plastic use/day	-0.11 (-0.79, 0.56)	0.58 (-0.43, 1.59)	-0.19 (-1.16, 0.78)	-0.30 (-1.77, 1.16)	0.03 (-0.46, 0.53)	0.10 (-0.39, 0.60)	-0.51 * (-1.01, -0.01)
Breastfed & <1 plastic use/day	-0.59 (-1.46, 0.29)	1.18 (-0.10, 2.45)	-0.03 (-1.29, 1.23)	-0.62 (-2.53, 1.28)	0.16 (-0.46, 0.78)	0.21 (-0.42, 0.84)	-0.31 (-0.95, 0.33)

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year)

* = $p < 0.05$

n/a: not enough or 0 people in the category breastfed & everyday plastic

Table 4 Multivariable-adjusted mean difference in short-chain fatty acid concentrations (µmol/g), from infant stool provided at 3 and 12 months of age, according to plastic bottle usage at 3 months and breastfeeding at 3 months

	Acetic Acid Mean difference	**Propionic Acid± Mean difference**	**Butyric Acid± Mean difference**	**Total SCFAs Mean difference**
SCFAs at 3 months				
Never breastfeeding & Every feeding	0.0 (ref) N=37	0.0 (ref) N=38	0.0 (ref) N=37	0.0 (ref) N=37
Ever breastfeeding & Every feeding	8.72 (-14.11, 31.57) N=15	0.05 (-0.34, 0.44) N=14	-0.31 (-0.88, 0.27) N=14	2.69 (-28,25, 33.63) N=15
Never breastfeeding & Less than every feeding	-5.50 (-52.12, 41.11) N=3	-0.69 (-1.48, 0.08) N=3	-0.56 (-1.71, 0.59) N=3	-28.98 (-92.13, 34.17) N=3
Ever breastfeeding & Less than every feeding	6.44 (24.68, 37.56) N=12	-0.46 (-0.98, 0.07) N=12	-0.62 (-1.41, 0.17) N=11	-7.67 (-49.83, 34.49) N=12
SCFA at 12 months				
Never breastfeeding & Every feeding	0.0 (ref) N=30	0.0 (ref) N=30	0.0 (ref) N=30	0.0 (ref) N=30
Ever breastfeeding & Every feeding	6.13 (-27.17, 39.47) N=6	-0.04 (-0.56, 0.48) N=6	0.39 (-0.33, 1.11) N=6	18.57 (-28.32, 65.46) N=6
Never breastfeeding & Less than every feeding	-54.89 (-127.86, 18.06) N=1	-0.11 (-1.24, 1.02) N=1	-0.47 (-2.04, 1.11) N=1	-68.84 (-171.52, 33.84) N=1
Ever breastfeeding & Less than every feeding	-19.66 (-53.66, 14.34) N=11	-0.12 (-0.65, 0.41) N=11	0.03 (-0.71, 0.76) N=11	-23.51 (-71.36, 24.34) N=11
Change from 3 to 12 months				
Never breastfeeding & Every feeding	0.0 (ref) N=29	0.0 (ref) N=28	0.0 (ref) N=29	0.0 (ref) N=29
Ever breastfeeding & Every feeding	16.72 (-29.24, 62.67) N=6	-0.04 (-0.66, 0.57) N=5	0.63 (-0.43, 1.69) N=5	42.42 (-18.80, 103.65) N=6
Never breastfeeding & Less than every feeding	-61.76 (-162.21, 38.69) N=1	0.16 (-1.09, 1.41) N=1	0.85 (-1.32, 3.03) N=1	50.10 (-183.92, 83.72) N=1
Ever breastfeeding & Less than every feeding	-25.75 (-73.31, 21.80) N=11	0.41 (-0.20, 1.02) N=11	0.52 (-0.54, 1.59) N=10	-15.12 (-78.48, 48.23) N=11

±Natural log + 1 transformation used for butyric acid and propionic acid at 3 and 12 months

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year. Vs. ≥ $20 000 per year)

* = $p < 0.05$

Table 5 Unadjusted and multivariable-adjusted mean difference in growth parameters at 12 months between binary plastic bottle usage at 3 months

		Subscapular skinfolds	Abdominal skinfolds	Triceps skinfolds	Subscapular + Triceps skinfolds	BMI z-score	Length for age z- score	Weight for length z-score
Crude	Every feeding	n=240 reference	n=239 reference	n=239 reference	n=239 reference	n=240 reference	n=240 reference	n=250 reference
	Less than every feeding	n=131 -0.23 (-0.54, 0.07)	n=130 0.10 (-0.33, 0.53)	n=131 0.01 (-0.41, 0.44)	n=131 -0.21 (-0.85, 0.43)	n=129 -0.13 (-0.35, 0.09)	n=129 -0.19 (-0.42, 0.04)	n=129 -0.15 (-0.37, 0.06)
Adjusted	Every feeding	n=236 reference	n=235 reference	n=235 reference	n=235 reference	n=236 reference	n=236 reference	n=236 reference
	Less than every feeding	n=130 -0.18 (-0.50, 0.15)	n=129 0.16 (-0.30, 0.62)	n=130 -0.07 (-0.53, 0.38)	n=130 -0.25 (-0.94, 0.44)	n=129 -0.15 (-0.38, 0.08)	n=129 -0.16 (-0.41, 0.08)	n=129 -0.17 (-0.39, 0.06)

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year)

* = $p < 0.05$

Table 6 Sensitivity Analysis in multivariable-adjusted mean difference in short-chain fatty acid concentrations (µmol/g), from infant stool provided at 3 and 12 months of age, by plastic bottle usage at 3 months

	Acetic Acid Mean difference			**Propionic Acid± Mean difference**			**Butyric Acid± Mean difference**			**Total SCFAs Mean difference**		
	Mothers who were non-smokers	**Mothers who did not take antibiotics during pregnancy**	**Mothers with high education status**	**Mothers who were non-smokers**	**Mothers who did not take antibiotics during pregnancy**	**Mothers with high education status**	**Mothers who were non-smokers**	**Mothers who did not take antibiotics during pregnancy**	**Mothers with high education status**	**Mothers who were non-smokers**	**Mothers who did not take antibiotics during pregnancy**	**Mothers with high education status**
SCFAs at 3 months												
Every feeding	0.0 (ref) n=40	0.0 (ref) n=28	0.0 (ref) n=22	0.0 (ref) n=38	0.0 (ref) n=27	0.0 (ref) n=22	0.0 (ref) n=39	0.0 (ref) n=28	0.0 (ref) n=22	0.0 (ref) n=40	0.0 (ref) n=28	0.0 (ref) n=22
Less than every feeding	3.26 (-26.19, 32.71) n=15	7.03 (-28.95, 43.00) n=12	-2.48 (-36.12, 31.17) n=14	-0.55* (-1.08, -0.02) n=15	-0.39 (-0.98, 0.21) n=12	-0.81** (-1.39, -0.23) n=14	-0.24 (-1.02, 0.55) n=14	-0.63 (-1.40, 0.14) n=11	-0.63 (-1.56, 0.19) n=13	-8.15, (-48.81, 32.50) n=15	-5.92 (-54.96, 43.12) n=12	-19.74 (-63.80, 24.31) n=14
SCFA at 12 months												
Every feeding	0.0 (ref) n=26	0.0 (ref) n=18	0.0 (ref) n=14	0.0 (ref) n=26	0.0 (ref) n=18	0.0 (ref) n=14	0.0 (ref) n=26	0.0 (ref) n=18	0.0 (ref) n=14	0.0 (ref) n=26	0.0 (ref) n=18	0.0 (ref) n=14
Less than every feeding	-19.88 (-54.00, 14.23) n=12	-42.88 * (-79.71, -6.07) n=11	-25.44 (-58.35, 7.46) n=11	0.06 (-0.46, 0.59) n=12	-0.18 (-0.84, 0.48) n=11	-0.21 (-0.82, 0.40) n=11	0.14 (-0.62, 0.91) n=12	-0.40 (-1.21, 0.41) n=11	-0.28 (-1.14, 0.58) n=11	-17.16 (-65.31, 30.99) n=12	-61.29 (-115.04, -7.54) n=11	-37.54 (-89.74, 14.67) n=11
Change from 3 to 12 months												
Every feeding	0.0 (ref) n=26	0.0 (ref) n=17	0.0 (ref) n=13	0.0 (ref) n=24	0.0 (ref) n=16	0.0 (ref) n=13	0.0 (ref) n=25	0.0 (ref) n=17	0.0 (ref) n=13	0.0 (ref) n=26	0.0 (ref) n=17	0.0 (ref) n=13
Less than every feeding	-30.35 (-81.84, 21.15) n=12	-50.41 (-101.82, 1.01) n=11	-31.67 (-84.33, 20.99) n=11	0.51 (-0.06, 1.08) n=12	0.14 (-0.60, 0.88) n=11	0.75* (0.04, 1.45) n=11	0.50 (-0.49, 1.49) n=11	0.46 (-0.63, 1.55) n=10	0.32 (-0.76, 1.41) n=10	-16.76 (-84.08, 50.56) n=12	-54.62 (-119.50, 10.26) n=11	-25.10 (-92.59, 42.39) n=11

±Natural log + 1 transformation used for butyric acid and propionic acid at 3 and 12 months

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year. Vs. ≥ $20 000 per year)

* = $p < 0.05$, ** = $p < 0.01$

Table 7 Sensitivity Analysis in multivariable-adjusted mean difference in growth parameters at 12 months, by plastic bottle usage at 3 months

		Subscapular skinfolds	Abdominal skinfolds	Triceps skinfolds	Subscapular + Triceps skinfolds	BMI z-score	Length for age z- score	Weight for length z-score
Mothers who were non-smokers	Every feeding	0.0 (ref) n=176	0.0 (ref) n=175	0.0 (ref) n=175	0.0 (ref) n=175	0.0 (ref) n=176	0.0 (ref) n=176	0.0 (ref) n=176
	4+times/day	-0.32 (-0.83, 0.19) n=31	0.02 (-0.69, 0.74) n=31	-0.08 (-0.81, 0.65) n=31	-0.40 (-1.49, 0.70) n=31	-0.26 (-0.62, 0.09) n=31	0.13 (-0.24, 0.50) n=31	-0.23 (-0.59, 0.12) n=31
	1-3/day	-0.09 (-0.54, 0.36) n=39	0.08 (-0.55, 0.72) n=38	-0.14 (-0.78, 0.50) n=39	-0.23 (-1.19, 0.73) n=39	-0.07 (-0.39, 0.24) n=38	-0.38 * (-0.71, -0.05) n=38	-0.13 (-0.44, 0.19) n=38
	<1/day	-0.19 (-0.70, 0.32) n=17	0.49 (-0.22, 1.20) n=17	0.15 (-0.56, 0.87) n=17	-0.03 (-1.11, 1.05) n=17	-0.11 (-0.47, 0.24) n=17	-0.12 (-0.49, 0.25) n=17	-0.13 (-0.48, 0.22) n=17
Mothers who did not take anti-biotics during pregnancy	Every feeding	0.0 (ref) n=140	0.0 (ref) n=140	0.0 (ref) n=140	0.0 (ref) n=140	0.0 (ref) n=140	0.0 (ref) n=140	0.0 (ref) n=140
	4+times/day	-0.26 (-0.82, 0.30) n=24	-0.17 (-0.98, 0.65) n=24	-0.24 (-1.09, 0.62) n=24	-0.50 (-1.74, 0.76) n=24	-0.02 (-0.43, 0.38) n=24	-0.14 (-0.58, 0.31) n=24	-0.05 (-0.45, 0.35) n=24
	1-3/day	0.06 (-0.41, 0.53) n=32	0.20 (-0.48, 0.89) n=32	-0.11 (-0.82, 0.61) n=32	-0.05 (-1.09, 1.00) n=32	0.17 (-0.17, 0.51) n=32	-0.30 (-0.67, 0.07) n=32	0.12 (-0.21, 0.46) n=32
	<1/day	-0.24 (-0.87, 0.38) n=11	0.61 (-0.31, 1.52) n=11	0.32 (-0.65, 1.28) n=11	0.07 (-1.33, 1.48) n=11	0.20 (-0.25, 0.66) n=11	-0.11 (-0.61, 0.39) n=11	0.19 (-0.26, 0.64) n=11
Mothers with high education status	Every feeding	0.0 (ref) n=97	0.0 (ref) n=96	0.0 (ref) n=97	0.0 (ref) n=97	0.0 (ref) n=97	0.0 (ref) n=97	0.0 (ref) n=97
	4+times/day	-0.04 (-0.69, 0.92) n=22	0.40 (-0.54, 1.33) n=22	-0.03 (-0.98, 0.93) n=22	-0.06 (-1.48, 1.35) n=22	-0.13 (-0.59, 0.32) n=22	-0.13 (-0.59, 0.33) n=22	-0.15 (-0.59, 0.29) n=22
	1-3/day	0.02 (-0.51, 0.55) n=34	0.24 (-0.53, 1.01) n=33	-0.08 (-0.86, 0.70) n=34	-0.06 (-1.21, 1.10) n=34	-0.01 (-0.38, 0.37) n=33	-0.39 (-0.77, -0.01) n=33	-0.06 (-0.42, 0.30) n=33
	<1/day	-0.06 (-0.67, 0.55) n=15	0.68 (-0.20, 1.55) n=15	0.22 (-0.68, 1.11) n=15	0.16 (-1.17, 1.48) n=15	0.03 (-0.40, 0.46) n=15	-0.29 (-0.72, 0.14) n=15	-0.02 (-0.43, 0.40) n=15

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year)

* = $p < 0.05$

Table 8 Multivariable-adjusted mean difference in short-chain fatty acid concentrations (µmol/g), from infant stool provided at 3 and 12 months of age, by plastic bottle usage at 3 months and formula feeding status (breastmilk vs. formula) at 3 months

	Acetic Acid Mean difference	**Propionic Acid± Mean difference**	**Butyric Acid± Mean difference**	**Total SCFAs Mean difference**
SCFAs at 3 months				
Every feeding and formula feeding	0.0 (ref) n=58	0.0 (ref) n=50	0.0 (ref) n=51	0.0 (ref) n=52
Less than every feeding and formula feeding	12.69 (-19.33, 44.71) n=8	-0.22 (-0.74, 0.29) n=8	-0.18 (-0.97, 0.60) n=8	4.74 (-38.13, 47.60) n=8
Less than every feeding and breastmilk	-11.51 (-45.64, 22.61) n=7	-0.91 * (-1.46, 0.36) n=7	-1.05* (-1.93, -0.18) n=6	-36.17 (-81.86, 9.51) n=7
SCFA at 12 months				
Every feeding and formula feeding	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36	0.0 (ref) n=36
Less than every feeding and formula feeding	-25.55 (-61. 89, 10.79) n=6	0.05 (-0.50, 0.60) n=6	-0.00 (-0.79, 0.79) n=6	-24.88 (-76.08, 26.32) n=6
Less than every feeding and breastmilk	-27.30 (-64.35, 9.76) n=6	-0.29 (-0.85, 0.28) n=6	-0.18 (-0.98, 0.63) n=6	-41.09 (-93.30, 11.11) n=6
Change from 3 to 12 months				
Every feeding and formula feeding	0.0 (ref) n=35	0.0 (ref) n=33	0.0 (ref) n=34	0.0 (ref) n=35
Less than every feeding and formula feeding	-49.20 (-98.67, 0.27) n=6	0.05 (-0.54, 0.65) n=6	0.26 (-0.83, 1.34) n=6	-43.39 (-110.5, 23.75) n=6
Less than every feeding and breastmilk	-16.63 (-67.26, 34.01) n=6	0.71 * (0.10, 1.31) n=6	0.86 (-0.30, 2.02) n=5	-3.64 (-72.36, 65.08) n=6

±Natural log + 1 transformation used for butyric acid and propionic acid at 3 and 12 months
Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year. Vs. ≥ $20 000 per year)
* = $p < 0.05$

Table 9 Multivariable-adjusted mean difference in growth parameters at 12 months, by binary plastic bottle usage at 3 months and formula feeding status (breastmilk vs. formula) at 3 months

	Subscapular skinfolds	Abdominal skinfolds	Triceps skinfolds	Subscapular + Triceps skinfolds	BMI z-score	Length for age z- score	Weight for length z-score
Every feeding and formula feeding	0.0 (ref) n=209	0.0 (ref) n=208	0.0 (ref) n=208	0.0 (ref) n=208	0.0 (ref) n=209	0.0 (ref) n=209	0.0 (ref) n=209
Less than every feeding and formula feeding	-0.26 (-0.61, 0.09) n=74	0.08 (-0.42, 0.58) n=73	-0.14 (-0.64, 0.35) n=74	-0.41 (-1.16, 0.34) n=74	-0.16 (-0.41, 0.09) n=73	-0.11 (-0.37, 0.16) n=73	-0.17 (-0.42, 0.07) n=73
Less than every feeding and breastmilk	0.11 (-0.41, 0.63) n=17	0.38 (-0.36, 1.12) n=17	0.17 (-0.57, 0.91) n=17	0.28 (-0.84, 1.39) n=17	-0.10 (-0.47, 0.27) n=17	-0.32 (-0.71, 0.06) n=17	-0.14 (-0.51, 0.22) n=17

Multivariable-adjusted linear models included adjustment for birth weight (kg), gestational age (weeks), maternal age (years), household income (<$20 000 per year vs. ≥ $20 000 per year)
* = $p < 0.05$

Table 10 Differential abundance for significant ASVs at FDR corrected p<0.20 at 3 and 12 months, by plastic bottle usage at 3 months

Significant ASVs at 3 moths	ASV2 ASV8 ASV10 ASV12 ASV15 ASV17 ASV19 ASV21 ASV23 ASV31 ASV37 ASV40 ASV43 ASV52 ASV54 ASV60 ASV62 ASV63 ASV65 ASV67 ASV78 ASV83 ASV85 ASV93 ASV94 ASV99 ASV115 ASV119 ASV125 ASV139 ASV145 ASV157 ASV158 ASV159 ASV171 ASV174 ASV181 ASV210 ASV217 ASV218 ASV235 ASV236 ASV256 ASV260 ASV304 ASV347 ASV376 ASV398 ASV466
Significant ASVs at 12 months	ASV8 ASV12 ASV13 ASV14 ASV18 ASV19 ASV32 ASV42 ASV50 ASV67 ASV71 ASV75 ASV80 ASV83 ASV87 ASV106 ASV111 ASV117 ASV122 ASV124 ASV134 ASV139 ASV148 ASV153 ASV161 ASV174 ASV175 ASV181 ASV183 ASV191 ASV204 ASV216 ASV226 ASV232 ASV269 ASV287 ASV288 ASV326 ASV338 ASV353 ASV390 ASV402 ASV421 ASV427 ASV449 ASV534

Heather Jianbo Zhao

Email: jzhao62@jhmi.edu or heatherzhao1@gmail.com

EDUCATION

September 2020 – May 2022	**Johns Hopkins Bloomberg School of Public Health,** Baltimore, MD Master of Science in Clinical Epidemiology (ScM) 4.0/4.0 GPA Maternal & Child Health Certificate Pharmacoepidemiology Certificate
September 2016- May 2020	**McMaster University**, Hamilton, ON, Canada Honours Bachelor of Health Science (BHSc) 3.9/4.0 GPA

WORK & RESEARCH EXPERIENCE

October 2020 — Present **Lead Epidemiologist & Statistician**, Baltimore MD
Mueller Lab | Johns Hopkins Bloomberg School of Public Health

- Led, coordinated, & presented scoping review to summarize findings for maternal short-chain fatty acids and its relationship with preeclampsia; presented review and expertise to stakeholders
- Conducted descriptive analysis and regression model building using prospective data from the Boston Birth Cohort with over 24 000 participants to explore the effect of breastfeeding and childhood blood pressure
- Oversaw study design for plastic exposure and the infant gut microbiome. Cleaned and merged large datasets; performed statistical, analytical, and modelling methods for diversity and differential abundance

November 2021 — Present **Research Analyst**, Baltimore MD
DASH4d Clinical Trials Team | Johns Hopkins School of Medicine

- Performed quality control checks on patient electronic data for cardiovascular and renal outcomes
- Collaborated and consulted with physicians in validating clinical data collection for clinical trial investigating sodium consumption among diabetics
- Oversaw data cleaning of over 100 participants in the study, and corrected for the inclusion criteria of ~20 individuals via data verification

May 2021 — Present **Lab Instructor**, Baltimore MD
Dept. of Biostatistics & Dept. of Epidemiology | Johns Hopkins Bloomberg School of Public Health

- Provided mentorship to over 300 students in graduate courses (Epidemiologic Methods III, Data Analysis I, and Epidemiologic Inference in Public Health I) and undergraduate course (Biostatistics in Public Health)
- Graded assignments and tests, hosted office hours to improve students' comprehension of research methods and analytical concepts; Developed infographics to resolve common misconceptions

January 2018 — August 2020 **Epidemiologist**, Hamilton ON Canada

Health Research Methods Evidence & Impact | McMaster University

- Led and facilitated research protocol for a systematic review and meta-analysis on plant-based diets and cardiovascular health in young adults; guided 2 junior researchers on abstract and full-length review. Conducted critical appraisal of included literatures using the GRADE system
- Designed a cross-sectional study and created a questionnaire investigating adherence and awareness of 2019 Canada's Food Guide among young adults

October 2017 — March 2019 **Clinical Medical Researcher**, Hamilton ON Canada
Emergency Department | Hamilton Health Sciences

- Worked with clients to refine electronic medical record data; maintained surveillance data-gathering
- Developed randomization coding for 2 multi-site blocked clinical trial

PUBLICATIONS

PEER-REVIEWED JOURNAL ARTICLES

[1] **Zhao HJ,** Kirkpatrick S, de Souza RJ. Knowledge and adherence to the 2019 Canada's Food Guide among Young Adults attending an urban university in Canada: A Cross-Sectional Survey (2022). *In print* at *Canadian Journal of Dietary Practice and Nutrition*
[2] Han Y, **Zhao HJ,** Zhang M, Liu T, Hong X, Wang X, Mueller N. Breastfeeding association with childhood blood pressure. *Manuscript in progress.*
[3] **Zhao JH**, Liu T, MacArthur K, Mueller N. Short-chain Fatty Acids and Preeclampsia. *Manuscript in progress.*
[4] **Zhao JH**, Ostbye T, Hoyo C, Benjamin-Neelon SE, Mueller N. Postnatal Plastic Exposure and Infant's Gut and Oral Microbiome. *Manuscript in progress.*

ABSTRACTS

[1] **Zhao JH**, Liu T, MacArthur K, Mueller N. Short-chain Fatty Acids are lower in Women with Preeclampsia. *AHA Epidemiology, Prevention, Lifestyle & Cardiometabolic Health 2022 Conference;* abstract accepted.
[2] Han Y, **Zhao HJ,** Zhang M, Liu T, Hong X, Wang X, Mueller N. The association of breastfeeding with lower blood pressure is stronger in children born vaginally compared to caesarean section. *AHA Epidemiology, Prevention, Lifestyle & Cardiometabolic Health 2022 Conference*; abstract accepted.

PRESENTATIONS

[1] Plant-Based Diet and the Impact on Young Adults' Cardiovascular Health: A Systematic Review and Meta Analysis. Poster presentation at the Annual Health Science Research Fair, McMaster University, Hamilton, ON, March 5, 2019
[2] Emergency Scanning for Pulmonary Embolism: Clinical Decision Making. Poster presentation at Juravinski Emergency Research Conference, Hamilton Health Sciences, Hamilton ON, October 25, 2018

HONORS, AWARDS, & SCHOLARSHIPS

2022 Charlotte Ferencz Scholarship for Maternal & Child Health: BSPH Epidemiology Dept.
2022 Conference Travel Award: BSPH Epidemiology Dept.
2021 caRING Johns Hopkins Community Service Award
2021 Anna Baetjer Society Public Health Art Award
2020 Masters Tuition Scholarship $45 000 of BSPH Epidemiology Dept.
2020 Provost Award & Senate Scholarship of McMaster University
2019 Scholarship: McMaster World's Challenge for United Nations' Sustainable Development

SELECTED LEADERSHIP EXPERIENCE & VOLUNTEER ACTIVITIES

2020—Present Perspectives Global Health Magazine Johns Hopkins: *Founder & Editor-in-Chief*
2021—Present Epidemiology Inclusion, Diversity, Equity, Anti-racism, and Science: *Outreach Member*
2020—Present Johns Hopkins Student Assembly: *Co-VP of Sociocultural Affairs & Quality of Life*
2020—2021 Health Education and Training Johns Hopkins (JH HEAT corps): *Covid-19 Instructor*
2019—2020 Hamilton Inasmuch Shelter: *Volunteer*
2017—2018 Dr. Curnew Cardiology Center: *Mandarin Translator*

TECHNICAL & LANGUAGE COMPETENCIES

- **Programming**: STATA, R, Python, SAS, SQL
- **Technological Tools:** Covidence, RevMan, EndNote, Redcap, Sovera, MEDITECH EHR, and Microsoft Word/ PowerPoint / Excel/ Outlook
- **Language Skills**: English (native speaker), Mandarin Chinese (fluent)
- **Unique competencies:** acrylic and oil painting, website design