Examining associations among caregiver stress, social support, and the infant gut microbiota

Sarah C. Vogel[1], Francesca R. Querdasi[2], Bridget L. Callaghan[2], Natalie H. Brito[3]

1. Boston University, Boston, MA
2. University of California Los Angeles, Los Angeles, CA
3. New York University, New York, NY

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Abstract

Previous research has found links between experiences of early life stress and development of the gut microbiota in humans, and there is a growing body of evidence for associations between caregiver well-being and the infant gut microbiome. Here, we examined how measures of caregiver stress and social support are associated with alpha diversity, beta diversity, and relative abundance of individual taxa of bacteria in the gut microbiota at 12 months of age in a typically-developing, community-based sample of infants (n=34). Caregiver social support was negatively associated with infant alpha diversity, and was associated with abundance of bacteria from several genera. We did not find associations between caregiver perceived stress and markers of infant gut microbiome diversity or composition. Results suggest that greater social support for new parents may be associated with infant health via changes in the diversity and composition of the infant gut microbiome.

Introduction

The trillions of microorganisms living in the human gastrointestinal tract, collectively known as the gut microbiota, play an important role in human health throughout the lifespan. This system is sensitive to environmental influences, especially in the first three to four years of life, which are thought to be a critical period in the colonization of the gut microbiota and the development of connections between the gut microbiota and the brain, known as the microbiota-gut-brain axis (Cowan et al., 2020). Social aspects of the early environment in particular have demonstrated associations with the gut microbiome in a few studies, including household composition (Brito et al., 2019; Stewart et al., 2018), daycare attendance (Amir et al., 2022; Roslund et al., 2020), caregiver stress/mental health (Dutton et al., 2023; Galley et al., 2023; Jahnke et al., 2021), pandemic-related social changes (Querdasi, Vogel, et al., 2023), caregiving practices (Flannery et al., 2020; Wiley et al., 2023), and socioeconomic status (Flannery et al., 2020; Lapidot et al., 2022). Taken together, available evidence suggests that aspects of caregiver well-being in infancy, including stress and social support, may play an important role in the developing infant gut microbiota. However, studies integrating sensitive, in-depth measures of caregiver well-being with measures of the infant gut microbiota are scarce. To address these gaps and expand our scientific understanding of early social influences on the developing gut microbiota, in this study we examine prospective associations between caregiver stress and multiple measures of social support and the diversity and composition of the infant gut microbiome.

There is rapid colonization of the gut microbiota occurring during and immediately following birth and large variations in diversity and composition of the gut microbiota occur throughout the first few years of life (Derrien et al., 2019; Koenig et al., 2011; Perez-Muñoz et

al., 2017; Stewart et al., 2018; Walker et al., 2017; Weng & Walker, 2013). Environmental influences such as nutrition, method of delivery, and breastfeeding behaviors all seem to play an important role in the developing gut microbiota (Bokulich et al., 2016; Derrien et al., 2019; Dominguez-Bello et al., 2010; Ferretti et al., 2018; Kim et al., 2019; Stewart et al., 2018). Likewise, the first few years of life are also an especially sensitive period of development for the main arms of the microbiota-gut-brain axis, namely the HPA-axis, the vagus nerve, and the immune system (Blair & Raver, 2012; Callaghan, 2020; Gunnar & Donzella, 2002; Propper & Holochwost, 2013; Slopen et al., 2015). Elevated stress in early life is thought to shape these systems to be more reactive to environmental stressors, tuning the central nervous system and physiological functioning to adaptively respond to environmental demands (Blair, 2010). These overlapping periods of sensitivity to environmental influence for both the gut microbiota and stress-response systems suggest that stress in early life may alter the development of the microbiota-gut-brain axis in ways important for physical and mental health throughout the lifespan (Callaghan, 2020).

Caregiver stress and the infant gut microbiota

The perinatal period is a time marked by many changes and challenges as parents adapt to the demands of caring for a new infant, which can lead to high levels of stress. Higher maternal stress has been associated with reduced likelihood of breastfeeding and potentially poorer nutritional quality of breast milk (Fallon et al., 2016), which several studies have found to be associated with differences in the composition and diversity of the gut microbiota (Kim et al., 2019; Levin et al., 2016; Matsuyama et al., 2019; Stewart et al., 2018). Specifically, breastfeeding is associated with lower gut microbiota diversity in infants as well as differences in the relative abundance of several strains of bifidobacteria (Levin et al., 2016). This raises the

possibility that higher maternal stress may be associated with *higher* infant gut microbiota diversity via reduced likelihood of breastfeeding, and that maternal stress may also influence the *composition* of the gut microbiota, both via breastfeeding behaviors and the composition of breastmilk.

Across both human and animal studies, prenatal maternal stress has consistently been associated with differences in offspring gut microbiota, however the directionality of effects and taxa implicated differ between studies (Aatsinki et al., 2020; Querdasi, Enders, et al., 2023; Rojas et al., 2023). Studies of postnatal caregiver stress and the infant gut microbiota are fewer, but provide some preliminary evidence of associations. Galley and colleagues (2023) found concurrent negative associations between maternal perceived stress and infant gut microbiota alpha diversity, a global within-individual metric of gut microbiota diversity. They also found maternal perceived stress at several time points throughout the first year of the infant's life were concurrently and prospectively associated with reduced abundance of several species from the *Bifidobacterium* genus, including *Bifidobacterium longum*, *Bifidobacterium dentum*, and *Bifidobacterium breve*, among others. These taxa represent some of the earliest colonizers of the infant gut microbiota and play an important role in the digestion of breastmilk and immune system development, among other key functions for infant health (Saturio et al., 2021). Similarly, Jahnke and colleagues (2021) found inverse associations between postnatal maternal stress and infant gut microbiota diversity, but not with differential abundance of specific taxa in the infant gut. Dutton and colleagues (2023) reported a positive association between maternal postnatal stress and infant gut microbiota alpha diversity, and found that maternal postnatal stress was associated with reduced abundance of *Lactobacillus gasseri* and *Bifidobacterium pseudocatenulatum* in infants. These findings suggest that maternal perceived stress throughout

the first year of the infant's life may be associated with differences in the composition and diversity of the infant gut microbiome in ways that are important for infant health, but we need more comprehensive studies of caregiver stress and the broader social environment to better understand these findings in context.

Caregiver social support and the gut microbiome

Available evidence suggests that caregiver stress is associated with the development of the infant gut microbiota, however the extent to which caregiver social support influences the developing gut microbiome is as yet unclear. Preliminary evidence for these associations comes from Jahnke and colleagues (2021), who found that infants of mothers reporting lower levels of family support postpartum had reduced abundance of *Bifidobacterium* at two months of age. This suggests that the *absence* of social support may be associated with lower abundance of bacteria thought to be beneficial to infant health, highlighting the importance of measuring both social supports and stressors in the same study to understand their individual and joint influences on infant health. These results provide some preliminary evidence to suggest that certain forms of caregiver social support may be implicated in the developing gut microbiota.

Indirectly, there is ample evidence for the benefits of caregiver social support on child development in general. Several studies have shown that higher caregiver social support buffers the effects of socioeconomic disadvantage and caregiver depression on several child outcomes, including global measures of child development and mother-infant bonding (McDonald et al., 2016; White et al., 2023). In another study, Nelson and colleagues (2020) examined caregiver stress, social support, and markers of inflammation in infancy. They found that higher caregiver social support received when infants were 12 months old was associated with lower levels of salivary C-reactive protein (CRP) when infants were 18 months. Moreover, greater caregiver

social support was associated with larger decreases in infant CRP between 12 and 18 months of age. These findings suggest that caregiver social support may be prospectively associated with markers of infant immune functions. Given strong connections between the gut microbiota and the immune system, as reviewed above, and in conjunction with findings from Jahnke and colleagues (2021), these data collectively suggest that caregiver social support may also be an important predictor of the infant gut microbiota.

One potential mechanism by which caregiver social support can influence the gut microbiota is via breastfeeding behaviors. Higher levels of perceived social support are associated with an increased likelihood of breastfeeding, plans to breastfeed for longer, and greater feelings of breastfeeding self-efficacy (Lyons et al., 2023; Mercan & Tari Selcuk, 2021). As reviewed above, breastfeeding plays a significant role in gut microbiome development in infancy (Kim et al., 2019; Matsuyama et al., 2019; Stewart et al., 2018). As such, higher caregiver social support may be associated with lower microbiota diversity via increased likelihood and longer duration of breastfeeding.

Another potential mechanism by which caregiver social support may influence the infant gut microbiome is via reductions in caregiver psychological and physiological stress. A few studies have found that higher social support for caregivers is associated with lower perceived caregiver stress and lower rates of caregiver depression and anxiety in the postnatal period (Jahnke et al., 2021; Nelson et al., 2020), which may influence patterns of parenting behaviors and parent-child interactions (Beebe et al., 2011; Feldman et al., 2009; Granat et al., 2017; Lemus et al., 2022) with important influences on the development of biological systems implicated in the gut microbiome, including infant stress-response systems (Feldman et al., 2009; Parenteau et al., 2020). Additionally, some studies have found that maternal social support

during pregnancy serves as a buffer against the physiological impacts of psychological distress (Giesbrecht et al., 2013), suggesting that another potential mechanism by which caregiver social support influences the infant gut microbiome is by preventing caregiver stress from "getting under the skin." These findings together suggest that caregiver social support may influence the gut microbiota both directly and via interactions with caregiver stress, however given the dearth of research in this area, more evidence is needed in studies examining stress and social support together to understand the individual and combined influences of caregiver stress and social support on the infant gut microbiota.

The current study

In this longitudinal, prospective study we seek to understand the extent to which variations in caregiver stress and social support work together to influence the developing gut microbiota in infancy. Specifically, we examine how these experiences are associated with alpha and beta diversity of the gut microbiota measured at 12 months of age, and differential abundance of specific taxa in the infant gut. Based on previous literature in the area of early life stress and microbiota development, we hypothesize that higher caregiver stress will be associated with *higher* gut microbiota alpha diversity in infants, whereas higher caregiver social support will be associated with *lower* alpha diversity. We also hypothesize that stress and social support will be associated with variation in beta diversity and differential abundances of specific taxa in the infant gut microbiota, but we do not have hypotheses about specific taxa affected. Finally, given the importance of breastfeeding in the development of the infant gut microbiota, and theoretical associations between breastfeeding and caregiver stress and social support, we also examine the role of breastfeeding status in these associations. We hypothesized that caregivers

reporting greater stress would be less likely to be breastfeeding at 12 months, but that caregivers reporting more social support would be more likely to still be breastfeeding at 12 months.

Methods

Participants and Procedures

One hundred and three families were recruited for a longitudinal study from community events and flyers posted around the New York City metropolitan area. Inclusion criteria for children included being born at or after 37 weeks of gestation, and no history of neurological or developmental delays. Infants and their primary caregivers participated in a lab visit when infants were 9 months of age and then participated in a phone screening and provided a stool sample when infants were 12 months old. In-lab data collection took place between May 2018 and December 2019 and remote stool sample collection took place between December 2018 and December 2020. However, to eliminate potential confounding of environmental changes due to COVID-19, for this analysis we only included infants whose caregivers provided a stool sample before March 22, 2020, which marked the beginning of stay-at-home orders in New York State (Querdasi, Vogel, et al., 2023). Thus, the final analytic sample for this study was n=34. All procedures were approved by the Institutional Review Board at New York University. Demographic characteristics of the sample are summarized in Table 1.

Measures

Caregiver stress

Caregivers completed the Perceived Stress Scale (PSS; Cohen et al., 1983) when the infant was 9 months old. The PSS is a 14-item self-report scale that assesses how often parents have perceived situations as stressful within the last month. Respondents rated items on a 5-point Likert scale ranging from 0 (never) to 4 (very often). Total scores can range from 0 to 40, and are

calculated as the sum of all responses (after 4 items are reverse scored). Scores from 0-13 are considered low perceived stress, 14-26 are considered moderate perceived stress, and 27-40 are considered high perceived stress. Sample items include: “In the last month, how often have you been upset because of something that happened unexpectedly” and “In the last month, how often have you been angered because of things that happened that were outside of your control?”

Caregiver social support

Caregivers also reported on social support at the 9-month laboratory visit. The questionnaire asked caregivers how supported they felt in certain situations. These items were rated on a 5-point scale with options: none of the time, a little of the time, some of the time, most of the time, and all of the time. Sample items include: “Is there someone available to whom you can count on to listen to you when you need to talk,” “Is there someone to help you with your daily chores,” “Do you have as much contact as you would like with someone you feel close to, someone in whom you can trust and confide in,” and “Do you feel you are supported in your everyday life?” Caregivers were also asked to indicate their sources of social support in a question reading: “Where do you receive emotional support from? Check as many as apply” with answer options: mental health counseling, support from family, support from friends, support from community, support from religious practice, support groups (example - new parent group). We derive two metrics from this questionnaire: *perceived social support*, measured as the average response of the questions regarding how caregivers feel in certain situations, and *sources of social support,* measured by the number of options caregivers selected in the “where do you receive social support” question.

Covariates

Breastfeeding. Caregivers reported on breastfeeding practices at 12 months via a sociodemographic questionnaire, in which they also reported on characteristics such as race, ethnicity, caregiver education, household composition, method of delivery, and family income. Given previous research documenting strong associations between breastfeeding status and gut microbiome diversity in infancy, we control for whether or not the infant was still breastfeeding at 12 months in these analyses. While we hypothesized that breastfeeding may serve as a mechanism by which caregiver stress and social support influence the infant gut microbiome, due to our small sample size we examine breastfeeding as a covariate, rather than as a mediator.

Infant Gut Microbiota Diversity and Composition

Caregivers collected a stool sample from an infant diaper when the infant was 12 months of age (∓3 weeks) using the OMNIgene®•GUT at-home gut microbiome collection kit (OM-200; DNAgenotek), and completed a short questionnaire reporting on the date and time of sample collection. Samples were transferred to a tube filled with stabilizing liquid and mailed back to NYU, where they were stored at room temperature for no more than two weeks, and then frozen until processing. This method has shown to be effective at preserving bacteria concentrations in stool for up to 8 weeks (Song et al., 2016). The microbiota community composition measures of interest for this analysis include alpha diversity, measured via the Shannon and Chao 1 indices, beta diversity, measured via Unifrac distance, and differential abundances of specific bacterial genera in samples.

Alpha diversity is a within-subjects measure that assesses global diversity of species in the gastrointestinal tract, and has been used in a number of other microbiota and development studies in humans (Callaghan et al., 2020; Carlson et al., 2018; Gao et al., 2019). The Chao 1 index of alpha diversity is a measure of the *richness* of the community - how many bacterial taxa

(in this case - genera) are present in each individual's sample. The Shannon index of alpha diversity, by contrast, balances *richness* with the *evenness* with which those taxa are distributed. Samples that have both high richness and evenness will have a higher Shannon value.

Beta diversity is a between-subjects metric that quantifies pairwise similarity of the entire gastrointestinal microbiome community across samples. The Unifrac distance metric uses phylogenetic relatedness to compare the samples and can either weight or not the distance matrix by low incidence microbes (weighted and unweighted Unifrac, respectively) (Lozupone & Knight, 2005). Paired samples with more phylogenetic similarity have a low Unifrac distance (i.e., low beta diversity), and vice versa. Weighted unifrac accounts for *abundance* of microbes in its measurement of community similarity, whereas unweighted unifrac only accounts for the presence/absence of each microbe in its calculation. Unifrac distance has been used extensively in human microbiota studies, including developmental studies on stress effects (Callaghan et al., 2020; Carlson et al., 2021; Michels et al., 2019).

DNA extraction, 16S rRNA sequencing and data processing. DNA extraction of the frozen stool samples was performed using the 96-well plate ZymoBIOMICS Magbead DNA/RNA Kit (Zymo Research, CA). Briefly, the samples (< 200 mg) were homogenized with glass beads in 750 µl of DNA/RNA shield (Zymo Research, CA). After centrifugation, the DNA was extracted from the supernatant using an Eppendorf epMotion 5705 automated system, following the manufacturer's protocol. The extraction included negative controls and positive controls representing defined bacterial communities (Zymo). Extracted DNA in the elution buffer was quantified using Quant-it and stored at -80°C.

The 16S rRNA V3-V4 region was amplified using Illumina adapter-ligated primers, with 2.5 µl (5 ng) DNA template in a total reaction volume of 25 µl (12.5 µl KAPA HiFi HotStart

ReadyMix, 5 µl each of forward and reverse primers) with the following cycling protocol: 95°C for 3 min, 25 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30s, and 72°C for 5 min. The Illumina Nextera XT v2 index was used to barcode sequencing libraries. Libraries were sequenced on an Illumina MiSeq using the v3 reagent kit (600 cycles) and a loading concentration of 12 pM with 10% phiX spike-in.

Raw sequencing reads were adapter-trimmed and demultiplexed after FASTQ conversion in BaseSpace (Illumina). Divisive amplicon denoising algorithm version 2 (DADA2 1.12.1) was used to trim, dereplicate, and filter chimeric sequences before generating amplicon sequence variant (ASV) tables(1). Based on the quality score profiles of sequencing reads, forward reads were truncated at 250 bp and reverse reads were truncated at 240 bp prior to merging, ambiguities in the overlap region were not allowed, and default parameters were otherwise applied in the R *dada2*package filterAndTrim() function [truncLen=c(250,240); trimLeft=c(5,5), maxN = 0, maxEE=c(2,2), truncQ = 2]. After dereplication and merging of reads, chimeric reads were identified by consensus across samples using the DADA2 function removeBimeraDenovo(). All samples passed the imposed minimum of 7,500 reads after quality filtering for inclusion in this analysis. The MAFFT and FastTree modules in QIIME2 were used to generate a phylogenetic tree of all ASV sequences.

The ASV table, taxonomic classification table, and phylogenetic tree were imported into R 3.6.1 to generate microbial Alpha diversity (Shannon, Chao indices) and Beta diversity (weighted and unweighted Unifrac distance) metrics, and differential abundances at the genus level of taxonomy, performed using functions from the phyloseq v1.28.0 package (2).

Statistical analyses

We first performed a series of correlations between our measures of caregiver stress, social support, and both measures of alpha diversity. We then ran regressions with our measures of stress and social support as independent variables in separate models, controlling for breastfeeding. Next, we examined associations between our measures of stress and social support and beta diversity of the gut microbiota. Using the phyloseq and vegan packages in R, we quantified between-subjects differences in bacteria present in infant stool by calculating the phylogenetic similarity or dissimilarity between samples, using weighted and unweighted Unifrac distance. We first confirmed that our data did not violate homogeneity of dispersion, a necessary assumption for permutational multivariate analysis of variance (PERMANOVA). If data violated the assumption of homogeneity of dispersion, we did not proceed to the next step. Next, we ran a PERMANOVA with 9,999 permutations using the adonis2 function from the vegan package to determine how much variance in beta diversity within our sample was explained by each of our predictors (caregiver stress, caregiver perceived social support, caregiver sources of social support), controlling for current breastfeeding status.

To examine how caregiver stress and social support are associated with differential abundance of individual taxa in the gut microbiota, we used Microbiome Multivariable Associations with Linear Models v2 (MaAsLin 2) with our measures of stress or social support and breastfeeding as predictors, and default model type, transformation, and normalization parameters (Mallick et al., 2021). Because we lacked *a priori* hypotheses regarding differential abundance of specific taxa, but our power was limited due to small sample size, we took a microbiome-wide approach to differential abundance using a filtered dataset that restricted comparisons to taxa that had non-zero abundance in at least 50% of samples (17/34 samples; min_prevalence parameter in MaAsLin2 set to 0.5). P-values were corrected for multiple

comparisons within this set of analyses with a q-value threshold for significance of .25, as has been used in prior work and recommended for biomarker discovery approaches (Kelsey et al., 2021; Querdasi, Enders, et al., 2023; Querdasi, Vogel, et al., 2023). All analyses were conducted in R.

Sensitivity analyses

We followed up statistically significant associations identified in the main analyses with sensitivity analyses controlling for additional theoretically-relevant covariates, specifically number of people living in the home and number of siblings the child has. We choose these covariates based on documented associations between family structure and the infant gut microbiome (Lane et al., 2019; Stewart et al., 2018), and to account for differences in stress and social support as a function of family structure. For regression analyses, we included the main predictor of interest (perceived stress, perceived social support, or sources of social support) and either number of people living with the child, or the number of siblings the child has, as a covariate in a regression. For MaAslin models, we included the main predictor of interest and either number of people living with the child, or the number of siblings the child has as an additional fixed effect in the model. Results of sensitivity analyses are summarized in Supplementary Tables 1 and 2.

Missing Data and Attrition

Out of 103 families recruited in the larger study, 55 infants aged into the microbiota sample collection before March 22, 2020 and were contacted to provide a stool sample before the start of the pandemic. Of the 55 families, 35 families returned a stool sample and of these 35 samples, 34 were robust to processing requirements regarding sample quality and read depth and thus included in this analysis. Missing data on other measures (surveys, etc.) were accounted for

using full information maximum likelihood estimation using the Lavaan package in R for all regressions (Rosseel, 2012).

Results

Descriptive Statistics

In general, our sample reported low to moderate levels of stress on the PSS. About half of our sample fell into the "low stress" range on the PSS, about half in the "moderate stress" range and only one participant scored in the "high stress" range (Cohen et al., 1983). Our sample also reported generally moderate to high perceptions of social support, with a slight negative skew indicating that the sample is concentrated on the higher end of the spectrum. Finally, our sample reported a range of sources of social support. The median and modal number of social support sources that parents selected was 3, but with some variation across the spectrum. Full demographic characteristics of the sample and descriptive statistics can be found in Table 1.

Table 1. Demographic and descriptive characteristics

Variable	
Child Race	Two or More/Other (n=14) White (n=8) Black/African American (n=7) Asian (n=3) Unreported (n=2)
Child Ethnicity	Not Hispanic/Latino (n=18) Hispanic/Latino (n=14) Unreported (n=2)
Child Sex	Male (n=22) Female (n=12)
Method of Delivery	Vaginal (n=24) C-Section (n=9) Unreported (n=1)
Still breastfeeding at 12 months	Yes (n=14) No (n=20)

Variable	Mean (sd)	Range (possible range)	Skew
Number of people living with child	3.55 (1.15)	1-6	0.52
Number of siblings	0.72 (0.99)	0-4	1.52
Maternal Education	15.88 (2.75)	10.5-20	-0.32
Income-to-needs ratio	5.94 (6.34)	0.09-24.06	1.43
Perceived Stress Scale	13.77 (5.10)	6-28 (0-40)	0.53
Perceived Social Support	21.06 (8.39)	0-28 (0-28)	-1.25
Sources of Social Support	2.41 (1.39)	0-5 (0-5)	-0.44
Shannon index	3.85 (0.30)	3.34 - 4.63	0.25
Chao index	114.58 (32.03)	46-188	-0.19

Correlations and Descriptive Statistics

We identified a significant positive correlation between perceptions of social support and number of sources of social support ($r=0.60$, $p<.01$). Neither measure of social support was correlated with perceived stress (ps >.28). Contrary to our hypotheses, in a series of two-sample t-tests we did not find significant differences in measures of social support or perceived stress between those who were and were not still breastfeeding at 12 months (ps >.20).

Alpha Diversity Findings

A summary of regression models and results can be found in Table 2.

To test our hypotheses regarding potential associations between stress, social support, and gut microbiota diversity, we performed a series of correlations between our two measures of alpha diversity and our measures of stress and social support.

Social Support

The number of sources of caregiver support was significantly negatively associated with the Shannon index (r=-0.38, p=.03), but not with the Chao 1 index (r=-0.25, p=.16). This significant correlation between sources of social support and Shannon diversity was robust to inclusion of breastfeeding status in a regression model (**β**=-0.35, p=.02), such that infants whose caregivers reported more unique sources of social support had lower alpha diversity measured via the Shannon index. These findings are illustrated in Figure 1. We did not find significant correlations between either measure of alpha diversity and perceived social support (ps>.11).

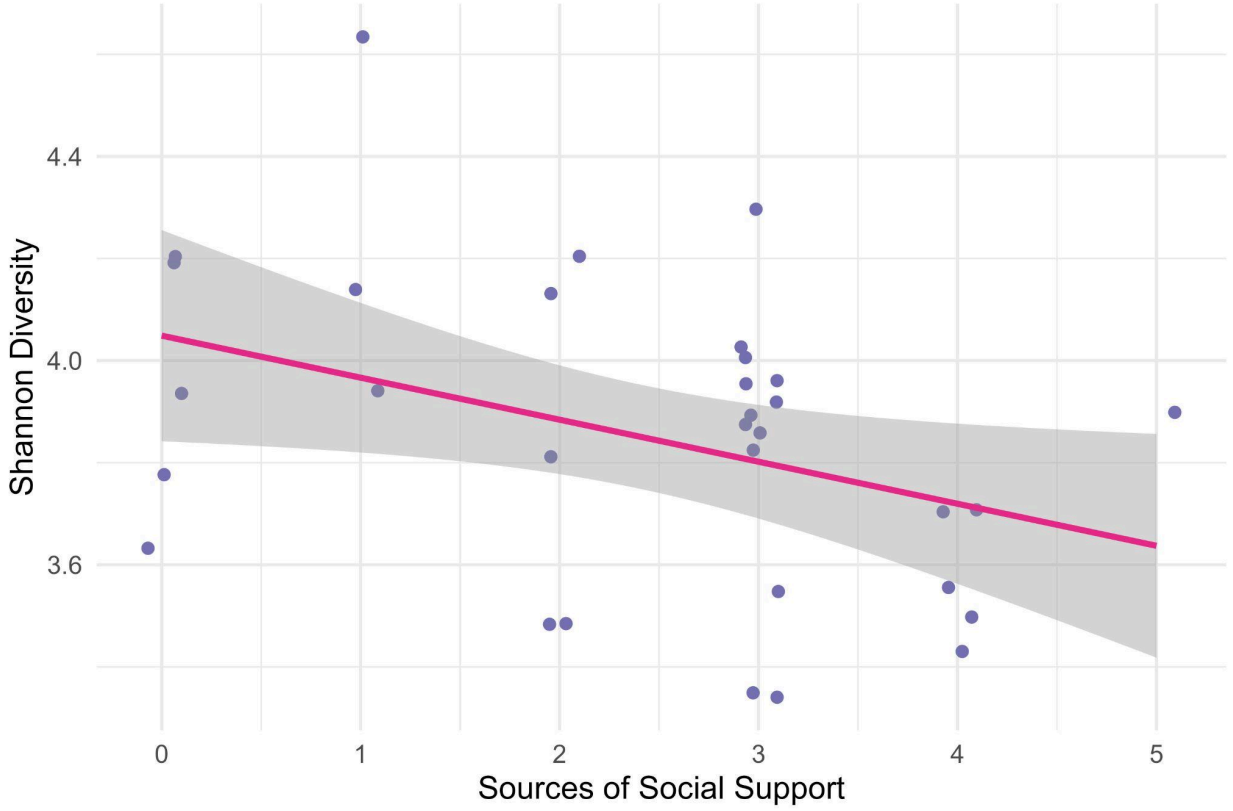


Figure 1. Plot of association between sources of social support and infant gut microbiome alpha diversity measured via the Shannon Index. Grey shading represents a 95% confidence interval, and points are jittered along the X axis for ease of visibility.

Perceived stress

Caregiver perceived stress was not associated with either measure of infant gut microbiome alpha diversity in either correlations (ps >.67) or regressions (ps > .86).

Table 2. Summary of regression models

	β	p-value	R^2
Model 1 - Shannon			0.29
Sources of support	-0.35	.02*	
Breastfeeding	-0.39	.01**	
Model 2 - Chao 1			0.22
Sources of support	-0.22	.14	
Breastfeeding	-0.39	.01**	
Model 3 - Shannon			0.21
Perceived social support	-0.20	.25	
Breastfeeding	-0.37	.02*	
Model 4 - Chao 1			0.21
Perceived social support	-0.20	.18	
Breastfeeding	-0.37	.02*	
Model 5 - Shannon			0.18
Perceived stress	0.02	.90	
Breastfeeding	-0.42	.01*	
Model 6 - Chao 1			0.16
Perceived stress	0.03	.86	
Breastfeeding	-0.40	.02*	

*p<.05, **p<.01, ***p<.001

Beta Diversity Findings

Social support

Controlling for current breastfeeding status, neither sources of social support (F=0.63, p= .47) nor perceived social support (F=0.42,p = .61) explained significant variance in *weighted* Unifrac distance. The assumption of homogeneity of dispersion was violated for *unweighted* Unifrac distance, so we did not proceed with a PERMANOVA testing variation in unweighted Unifrac distance.

Perceived stress

Controlling for current breastfeeding status, perceived stress did not explain significant variance in *weighted* Unifrac distance (F=1.98, p= .15). The assumption of homogeneity of dispersion was violated for *unweighted* Unifrac, so we did not proceed with a PERMANOVA testing variation in unweighted Unifrac distance.

Differential Abundance Findings

Social support

Sources of social support. Controlling for current breastfeeding status, the number of reported sources of caregiver social support was positively associated with abundance of the following genera: *Veillonella (***β**=1.34, p<.001, q=.02*), Clostridium sensu stricto (***β**=1.40, p=.003, q=.05*), Ruminococcus (gnavus group;* **β**=0.91, p=.006, q=.06*), Intestinibacter (***β**=1.01, p=.006, q=.06), and an unspecified genus from the Lachnospiraceae family (**β**=0.80, p=.03, q=.18).

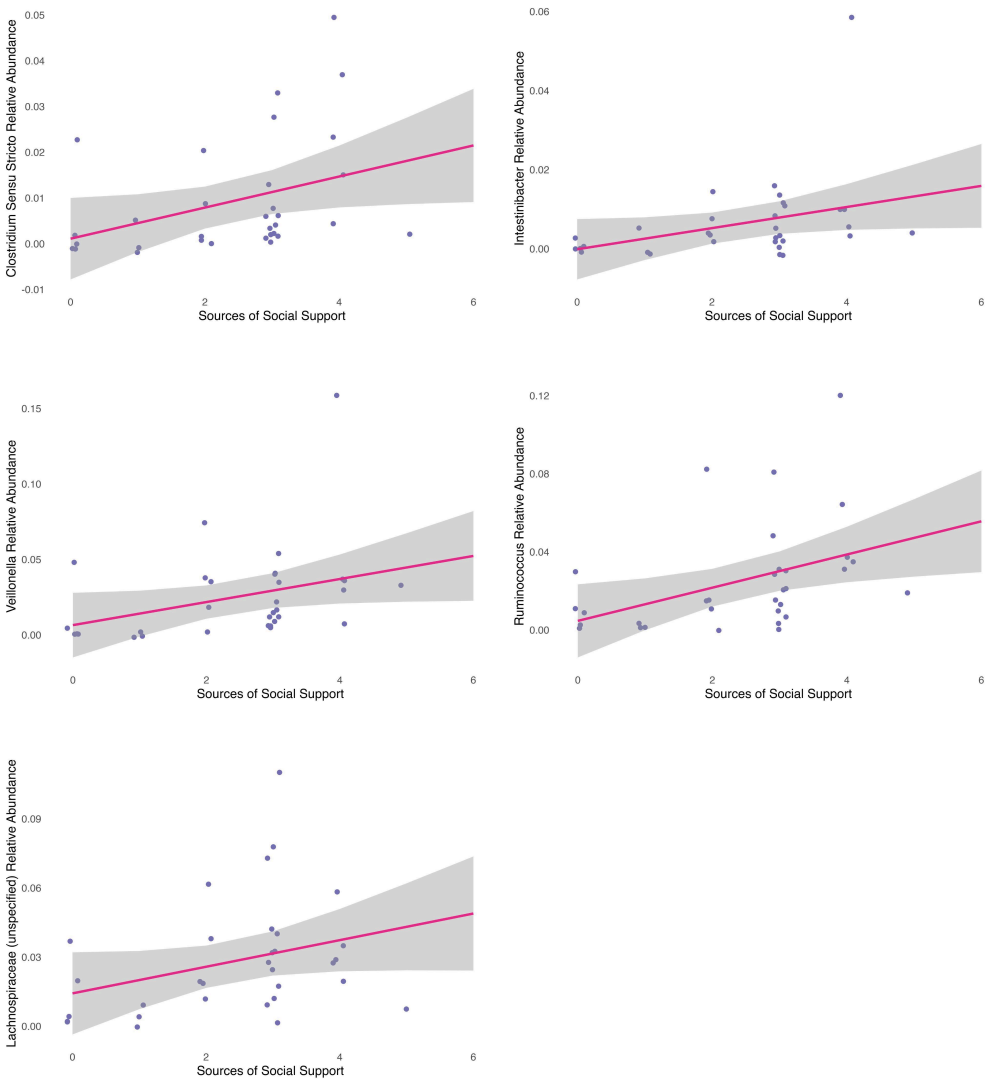
Perceived social support. Controlling for current breastfeeding status, caregiver perceived social support was positively associated with abundance of the genus *Intestinibacter* (**β**=1.11, p=.003, q=.07) and negatively associated with relative abundance of *Blautia (***β**=-0.78, p=.01, q=.22).

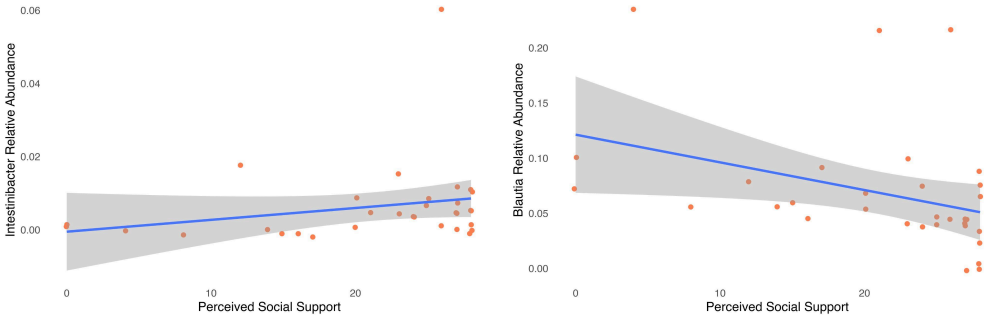
Perceived stress

Controlling for current breastfeeding status, no genera were significantly differentially abundant as a function of caregiver perceived stress.

Differential abundance findings are presented in Figure 2.

A.





B.

Figure 2. A) Associations between sources of social support and relative abundance of *Clostridium Sensu Stricto, Intestinibacter, Veillonella, Ruminococcus (gnavus group)*, and an unspecified genus from Lachnospiraceae. B) Associations between perceived social support and abundance of *Intestinibacter* and *Blautia*. Points are jittered along the X axis for ease of visibility.

Discussion

The results of this study indicate preliminary associations between caregiver social support, and the infant gut microbiota. Specifically, we found that caregivers reporting more unique sources of social support had infants with lower gut microbiota alpha diversity. Additionally, we identified a few taxa of bacteria associated with our measures of caregiver social support. We did not find evidence that caregiver perceived stress was associated with either the diversity or the composition of the gut microbiota in infancy. This is one of the first studies to examine associations between caregiver social support and the infant gut microbiota, and adds to a growing body of literature highlighting the importance of caregiver social support in promoting infant health and development. Moreover, these findings emphasize the need for sensitive, robust measurement of the social environment in infant gut microbiome research.

Stress is an incredibly common part of caregivers' lives, especially in infancy, when caregivers are dealing with the physical and mental demands of caring for a new infant. Most previous studies of caregiver stress and the infant gut microbiome have focused on *prenatal* stress and identified several metrics of the infant gut microbiota associated with experiences of caregiver stress during pregnancy (Aatsinki et al., 2020; Naudé et al., 2020; Querdasi, Enders, et

al., 2023; Rojas et al., 2023). However this study adds to a growing body of literature examining *postnatal* experiences as they are associated with the infant gut microbiota. Unlike previous studies of postnatal caregiver perceived stress on infant gut microbiota development (Dutton et al., 2023; Galley et al., 2023; Jahnke et al., 2021), we did not find evidence that variation in caregiver perceived stress was associated with measures of diversity or composition of the infant gut microbiota in our sample.

Our finding that caregiver perceived stress was not associated with any markers of the infant gut microbiota is inconsistent with previous studies of postnatal maternal stress and infant gut microbiota development. Our reduced variability in caregiver perceived stress may be one potential explanation for the discrepancies in findings between our study and others. Our sample reported overall low and moderate levels of stress, whereas the samples from both Dutton and colleagues (2023) and Galley (2023) reported higher stress levels (Dutton: range of 19-37 on the PSS, compared to 6-28 in this sample). This would suggest that perhaps caregiver stress exerts a more meaningful influence on infant gut microbiome at higher levels of stress that we did not detect in this community-based sample.

Another potential explanation for these discrepancies is differences in the measurement and timing of maternal stress and infant gut microbiota sampling. Here, we measured maternal stress at infant age nine months using the PSS (Cohen et al., 1983) and the infant gut microbiota at 12 months. Jahnke and colleagues (2021) measured perceived stress and the microbiome concurrently at infant age two months, observing positive associations between perceived stress and alpha diversity but *not* with abundance of individual taxa. Dutton and colleagues (2023) used a composite measure of maternal stress, consisting of the perceived stress scale and measures of trauma exposure, anxiety, and depression, and found this maternal stress composite measured at

birth to be associated with reduced abundance of several taxa at 2 weeks, but not six months of age, and higher alpha diversity at six months. These findings suggest that influences of maternal stress on the infant gut microbiota may be more prevalent neonatally than later in infancy. Finally, most similar to the timing and measures from our study, Galley and colleagues (2023) used the perceived stress scale to measure maternal stress and found maternal perceived stress to be associated with multiple markers of the infant gut microbiome throughout infancy. Their sample reported higher levels of stress overall than our sample, in which only two of our participants scored in the "high" range on the perceived stress scale (defined as 27 or above). Likewise, the gut microbiota goes through rapid and significant changes across the first year of life, making comparisons between studies challenging when the gut microbiota is sampled at different times. Finally, two of these studies drew from populations outside of the United States (Jahnke: Ecuador, Dutton: Democratic Republic of the Congo). Cultural differences may influence how caregivers experience and express stress, care for infants, and make choices about breastfeeding and nutrition practices, all of which may influence associations between caregiver perceived stress and the infant gut microbiota. These discrepancies highlight the need for more research in this area, particularly with larger and more diverse samples with greater variability in caregiver stress and repeated measurement of the gut microbiota across infancy.

We did find evidence for associations between both of our measures of caregiver social support and different markers of the infant gut microbiota. Specifically, infants of caregivers that reported more unique sources of social support had lower alpha diversity, measured via the Shannon index. These results held when controlling for breastfeeding. While higher alpha diversity in adults is thought to promote health, two previous studies in infants have found that higher alpha diversity is negatively associated with measures of cognition and brain development

(Carlson et al., 2018; Gao et al., 2019). These findings highlight how diverse sources of support in the lives of caregivers can both directly and indirectly promote healthy infant development via the gut microbiota.

We also found that both sources of caregiver social support and caregivers' perceptions of social support were associated with the abundances of several genera of bacteria, including *Veillonella, Clostridium sensu stricto, Ruminococcus, Intestinibacter,* and *Blautia* as well as an unspecified genus from the Lachnospiraceae family. Some of the taxa we identified, such as *Veillonella* and *Clostridium sensu stricto*, have been previously identified in studies of infant and child gut microbiota and brain and behavioral development (Acuña et al., 2021; Chen et al., 2023; Flannery et al., 2020), although the directionality of associations between taxa and child outcomes has differed between studies. Warner and colleagues (2023) identified a few species of bacteria from *Veillonella* that were differentially abundant in infants as a function of *prenatal* caregiver social wellbeing. Specifically, they found that infants of caregivers experiencing more social disadvantage had lower abundances of species from *Veillonella*, in line with our findings that caregiver social support is associated with greater *Veillonella* abundance. Similarly, Querdasi and colleagues (2023) found that children's abundance of *Clostridium sensu stricto* was positively associated with maternal experiences of childhood adversity. This is in contrast to our findings, where we found abundance of *Clostridium sensu stricto* to be positively associated with maternal social support, though the timing of stress measurement and the mechanism of effects are likely different between these findings and those presented here. Differing findings between studies in this area point towards the need for more longitudinal gut microbiome research using advanced sequencing technologies that allow for better resolution in taxa down to the level of species and more confidence in taxonomic classifications.

Of note are the differing results between our two measures of caregiver social support. The number of s*ources of social support* was negatively associated with infant alpha diversity and with the relative abundances of several genera, while we did not find associations between *perceived social support* and alpha diversity, and our differential abundance findings for perceived social support identified different genera of bacteria. There are a number of potential explanations for these differences in findings. Previous research has found that different sources of social support have differential influences on mental health in adults (Li et al., 2021). This could mean that receiving support from a *variety* of sources may promote overall well-being for both mom and baby more than receiving support from one or two sources. Relatedly, a study from Wiley and colleagues (2023) examined alloparenting behaviors, including the number of individual caregivers in contact with the infant and the number of different caregivers who hold the baby, as they were associated with the infant gut microbiome. They found that alloparenting behaviors were associated with *increased* alpha diversity of the infant gut and differential abundance of several taxa, including *Veillonella*, *Bifidobacterium*, *Intestinibacter*, *Bacteroides*, an unspecified genus from Lachnospiraceae, and *Flavonifractor*, among others. These findings would suggest that having more individual caregivers is significantly associated with the diversity and composition of the infant gut microbiome in early life. Here, we identified *Intestinibacter*, *Veillonella*, and two genera from Lachnospiraceae (*Blautia* and an unspecified genus), as being associated with our measures of social support in the same direction as the alloparenting behaviors measured by Wiley and colleagues. Taking these findings together, it suggests that our measure of *sources* of social support may be capturing heterogeneity in caregiving arrangements and mother-infant social relationships that considering maternal *perceptions* of social support does not capture alone.

There are a number of potential mechanisms by which caregiver social support might influence the infant gut microbiota. One hypothesized mechanism is that having more social support reduces caregiver stress, however we did not find correlations between caregiver stress and social support in our sample. Previous studies of caregiver stress and social support in the perinatal period have yielded mixed results, with some reporting significant negative correlations between stress and social support (Nelson et al., 2020), and others only observing these correlations for certain forms of social support, or finding associations inconsistently across the pre- and postnatal periods (Jahnke et al., 2021). Likewise, another hypothesized mechanism by which caregiver social support influences the infant gut microbiome is via breastfeeding. Previous research has found that caregiver social support is associated with increased likelihood of breastfeeding and breastfeeding for longer (Lyons et al., 2023; Mercan & Tari Selcuk, 2021), however again we did not find that infants of caregivers reporting more perceived social support or sources of social support were more likely to still be breastfeeding at 12 months of age. There is some evidence to suggest that maternal psychosocial stress is associated with differences in energy density, fat content, and immune markers in breast milk (Groer et al., 2004; Zagoory-Sharon et al., 2024; Ziomkiewicz et al., 2021), and one study found that maternal social support was negatively associated with inflammatory markers in breast milk (Kim et al., 2014). These findings suggest that the mechanism by which maternal social support may influence the infant gut microbiome could be via changes in the nutritional and immunological composition of breastmilk, however no studies to our knowledge have directly tested this, and we did not have the data to test this hypothesis in the current study.

This study, by focusing on measures of both caregiver stress and social support, adds nuance to a growing body of literature revealing the role of the early social environment in the

development of the infant gut microbiome. A notable strength of this study is the diverse sample represented. Many studies of gut microbiome development have been done in more racially and ethnically homogenous samples, which has allowed for a foundation of research to draw from but limits generalization. Experiences of stress and social support are experienced differently and may have different impacts on physical and mental health based on culture and demographic characteristics (Abdou et al., 2010; Shavitt et al., 2016) and, as such, the role of these experiences in infant development may differ based on social and structural conditions. An additional strength of our study comes from the longitudinal nature of our study design, which allowed us to capture *prospective* associations between caregiver social support and the infant gut microbiome, rather than cross-sectional associations.

Despite these strengths, there are a number of limitations worth addressing. We relied on a small sample (n=34) for this study, which limited the kinds of analyses we could run and conclusions we could draw from these data. We were not statistically powered to control for multiple potential confounders like diet, number of people in the home, or exposure to pets, all of which previous studies have identified as contributing to the gut microbiome in infancy and childhood (Bokulich et al., 2016; Dominguez-Bello et al., 2010; Stewart et al., 2018). Our supplementary analyses suggested that some of the associations we observed between sources of social support and markers of the gut microbiome may be partially explained by measures of household composition, highlighting the importance of studies with sensitive measurement of the early social environment. We also only included one measurement of the gut microbiome in our study, but the infant gut microbiome undergoes substantial change across the first three to four years of life. Additionally, we observed some evidence of ceiling effects in our measure of perceived social support, with most participants reporting feeling highly supported. While our

skewness (-1.25) and kurtosis values (0.43) were within a normal range for this measure, some skew in a small sample such as this may limit the statistical variability we are able to detect. Future studies with larger samples, multiple measurements of the infant gut microbiome, and measurements of stress and caregiver support beginning prenatally and continuing throughout early life may help address these limitations and better characterize associations between the social environment and the development of the infant gut microbiome.

In this study, we have presented evidence that caregiver social support is associated with multiple markers of gut microbiota development in infancy. These results highlight the importance of promoting caregiver well being as a means of supporting healthy infant development. Accessible and affordable mental health care, new parent support groups, paid parental leave, available and affordable childcare, and other services that promote social bonds and new parent emotional and social wellbeing may all play important roles in setting infants up for long-term health. This study is one of the first to examine how caregiver stress and social support in infancy shape the infant gut microbiota, and sets the stage for more nuanced understanding of the various social influences on the gut microbiota in early life.

References

Aatsinki, A.-K., Keskitalo, A., Laitinen, V., Munukka, E., Uusitupa, H.-M., Lahti, L., Kortesluoma, S., Mustonen, P., Rodrigues, A. J., Coimbra, B., Huovinen, P., Karlsson, H., & Karlsson, L. (2020). Maternal prenatal psychological distress and hair cortisol levels associate with infant fecal microbiota composition at 2.5 months of age. *Psychoneuroendocrinology*, *119*, 104754.

Abdou, C. M., Dunkel Schetter, C., Campos, B., Hilmert, C. J., Dominguez, T. P., Hobel, C. J., Glynn, L. M., & Sandman, C. (2010). Communalism predicts prenatal affect, stress, and physiology better than ethnicity and socioeconomic status. *Cultural Diversity & Ethnic Minority Psychology*, *16*(3), 395–403.

Acuña, I., Cerdó, T., Ruiz, A., Torres-Espínola, F. J., López-Moreno, A., Aguilera, M., Suárez, A., & Campoy, C. (2021). Infant Gut Microbiota Associated with Fine Motor Skills. *Nutrients*, *13*(5). https://doi.org/10.3390/nu13051673

Amir, A., Erez-Granat, O., Braun, T., Sosnovski, K., Hadar, R., BenShoshan, M., Heiman, S., Abbas-Egbariya, H., Glick Saar, E., Efroni, G., & Haberman, Y. (2022). Gut microbiome development in early childhood is affected by day care attendance. *NPJ Biofilms and Microbiomes*, *8*(1), 2.

Beebe, B., Steele, M., Jaffe, J., Buck, K. A., Chen, H., Cohen, P., Kaitz, M., Markese, S., Andrews, H., Margolis, A., & Feldstein, S. (2011). MATERNAL ANXIETY SYMPTOMS AND MOTHER-INFANT SELF- AND INTERACTIVE CONTINGENCY. *Infant Mental Health Journal*, *32*(2), 174–206.

Blair, C. (2010). Stress and the Development of Self-Regulation in Context. *Child Development Perspectives*, *4*(3), 181–188.

Blair, C., & Raver, C. C. (2012). Child development in the context of adversity: experiential canalization of brain and behavior. *The American Psychologist*, *67*(4), 309–318.

Bokulich, N. A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H., D Lieber, A., Wu, F., Perez-Perez, G. I., Chen, Y., Schweizer, W., Zheng, X., Contreras, M., Dominguez-Bello, M. G., & Blaser, M. J. (2016). Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Science Translational Medicine*, *8*(343), 343ra82.

Brito, I. L., Gurry, T., Zhao, S., Huang, K., Young, S. K., Shea, T. P., Naisilisili, W., Jenkins, A. P., Jupiter, S. D., Gevers, D., & Alm, E. J. (2019). Transmission of human-associated microbiota along family and social networks. *Nature Microbiology*, *4*(6), 964–971.

Callaghan, B. (2020). Nested sensitive periods: how plasticity across the microbiota-gut-brain axis interacts to affect the development of learning and memory. *Current Opinion in Behavioral Sciences*, *36*, 55–62.

Callaghan, B. L., Fields, A., Gee, D. G., Gabard-Durnam, L., Caldera, C., Humphreys, K. L., Goff, B., Flannery, J., Telzer, E. H., Shapiro, M., & Tottenham, N. (2020). Mind and gut: Associations between mood and gastrointestinal distress in children exposed to adversity. *Development and Psychopathology*, *32*(1), 309–328.

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Goldman, B. D., Ahn, M., Styner, M. A., Thompson, A. L., Geng, X., Gilmore, J. H., & Knickmeyer, R. C. (2018). Infant Gut Microbiome Associated With Cognitive Development. *Biological Psychiatry*, *83*(2), 148–159.

Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Rosin, S. P., Fine, J. P., Mu, W., Zopp, J. B., Kimmel, M. C., Styner, M. A., Thompson, A. L., Propper, C. B., & Knickmeyer, R. C. (2021). Infant gut microbiome composition is associated with non-social fear behavior in a

pilot study. *Nature Communications*, *12*(1), 3294.

Chen, J., Li, H., Zhao, T., Chen, K., Chen, M.-H., Sun, Z., Xu, W., Maas, K., Lester, B. M., & Cong, X. S. (2023). The Impact of Early Life Experiences and Gut Microbiota on Neurobehavioral Development in Preterm Infants: A Longitudinal Cohort Study. *Microorganisms*, *11*(3). https://doi.org/10.3390/microorganisms11030814

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). Perceived stress scale (PSS). *Journal of Health and Social Behavior*, *24*, 285.

Cowan, C. S. M., Dinan, T. G., & Cryan, J. F. (2020). Annual Research Review: Critical windows–the microbiota–gut–brain axis in neurocognitive development. *Journal of Child*. https://onlinelibrary.wiley.com/doi/abs/10.1111/jcpp.13156

Derrien, M., Alvarez, A.-S., & de Vos, W. M. (2019). The Gut Microbiota in the First Decade of Life. *Trends in Microbiology*, *27*(12), 997–1010.

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(26), 11971–11975.

Dutton, C. L., Maisha, F. M., Quinn, E. B., Morales, K. L., Moore, J. M., & Mulligan, C. J. (2023). Maternal Psychosocial Stress Is Associated with Reduced Diversity in the Early Infant Gut Microbiome. *Microorganisms*, *11*(4). https://doi.org/10.3390/microorganisms11040975

Fallon, V., Groves, R., Halford, J. C. G., Bennett, K. M., & Harrold, J. A. (2016). Postpartum Anxiety and Infant-Feeding Outcomes. *Journal of Human Lactation: Official Journal of International Lactation Consultant Association*, *32*(4), 740–758.

Feldman, R., Granat, A., Pariente, C., Kanety, H., Kuint, J., & Gilboa-Schechtman, E. (2009). Maternal depression and anxiety across the postpartum year and infant social engagement, fear regulation, and stress reactivity. *Journal of the American Academy of Child and Adolescent Psychiatry*, *48*(9), 919–927.

Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., Armanini, F., Truong, D. T., Manara, S., Zolfo, M., Beghini, F., Bertorelli, R., De Sanctis, V., Bariletti, I., Canto, R., Clementi, R., Cologna, M., Crifò, T., Cusumano, G., … Segata, N. (2018). Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. *Cell Host & Microbe*, *24*(1), 133–145.e5.

Flannery, J. E., Stagaman, K., Burns, A. R., Hickey, R. J., Roos, L. E., Giuliano, R. J., Fisher, P. A., & Sharpton, T. J. (2020). Gut Feelings Begin in Childhood: the Gut Metagenome Correlates with Early Environment, Caregiving, and Behavior. *mBio*, *11*(1). https://doi.org/10.1128/mBio.02780-19

Galley, J. D., Mashburn-Warren, L., Blalock, L. C., Lauber, C. L., Carroll, J. E., Ross, K. M., Hobel, C., Coussons-Read, M., Dunkel Schetter, C., & Gur, T. L. (2023). Maternal anxiety, depression and stress affects offspring gut microbiome diversity and bifidobacterial abundances. *Brain, Behavior, and Immunity*, *107*, 253–264.

Gao, W., Salzwedel, A. P., Carlson, A. L., Xia, K., Azcarate-Peril, M. A., Styner, M. A., Thompson, A. L., Geng, X., Goldman, B. D., Gilmore, J. H., & Knickmeyer, R. C. (2019). Gut microbiome and brain functional connectivity in infants-a preliminary study focusing on the amygdala. *Psychopharmacology*, *236*(5), 1641–1651.

Giesbrecht, G. F., Poole, J. C., Letourneau, N., Campbell, T., Kaplan, B. J., & APrON Study Team. (2013). The buffering effect of social support on hypothalamic-pituitary-adrenal axis

function during pregnancy. *Psychosomatic Medicine*, *75*(9), 856–862.

Granat, A., Gadassi, R., Gilboa-Schechtman, E., & Feldman, R. (2017). Maternal depression and anxiety, social synchrony, and infant regulation of negative and positive emotions. *Emotion* , *17*(1), 11–27.

Groer, M., Davis, M., & Steele, K. (2004). Associations between human milk SIgA and maternal immune, infectious, endocrine, and stress variables. *Journal of Human Lactation: Official Journal of International Lactation Consultant Association*, *20*(2), 153–158; quiz 159–163.

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*, *27*(1-2), 199–220.

Jahnke, J. R., Roach, J., Azcarate-Peril, M. A., & Thompson, A. L. (2021). Maternal precarity and HPA axis functioning shape infant gut microbiota and HPA axis development in humans. *PloS One*, *16*(5), e0251782.

Kelsey, C. M., Prescott, S., McCulloch, J. A., Trinchieri, G., Valladares, T. L., Dreisbach, C., Alhusen, J., & Grossmann, T. (2021). Gut microbiota composition is associated with newborn functional brain connectivity and behavioral temperament. *Brain, Behavior, and Immunity*, *91*, 472–486.

Kim, E. S., Jeong, M. J., Kim, S., Shin, H. A., Lee, H. K., Shin, K., & Han, J. H. (2014). Maternal Psychosocial Factors that Affect Breastfeeding Adaptation and Immune Substances in Human Milk. *Korean Journal of Women Health Nursing*, *20*(1), 14–28.

Kim, H., Sitarik, A. R., Woodcroft, K., Johnson, C. C., & Zoratti, E. (2019). Birth Mode, Breastfeeding, Pet Exposure, and Antibiotic Use: Associations With the Gut Microbiome and Sensitization in Children. *Current Allergy and Asthma Reports*, *19*(4), 22.

Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., Angenent, L. T.,

& Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *108 Suppl 1*, 4578–4585.

Lane, A. A., McGuire, M. K., McGuire, M. A., Williams, J. E., Lackey, K. A., Hagen, E. H., Kaul, A., Gindola, D., Gebeyehu, D., Flores, K. E., Foster, J. A., Sellen, D. W., Kamau-Mbuthia, E. W., Kamundia, E. W., Mbugua, S., Moore, S. E., Prentice, A. M., Kvist, L. J., Otoo, G. E., … Meehan, C. L. (2019). Household composition and the infant fecal microbiome: The INSPIRE study. *American Journal of Physical Anthropology*, *169*(3), 526–539.

Lapidot, Y., Reshef, L., Maya, M., Cohen, D., Gophna, U., & Muhsen, K. (2022). Socioeconomic disparities and household crowding in association with the fecal microbiome of school-age children. *NPJ Biofilms and Microbiomes*, *8*(1), 10.

Lemus, A., Vogel, S. C., Greaves, A. N., & Brito, N. H. (2022). Maternal anxiety symptoms associated with increased behavioral synchrony in the early postnatal period. *Infancy: The Official Journal of the International Society on Infant Studies*. https://doi.org/10.1111/infa.12473

Levin, A. M., Sitarik, A. R., Havstad, S. L., Fujimura, K. E., Wegienka, G., Cassidy-Bushrow, A. E., Kim, H., Zoratti, E. M., Lukacs, N. W., Boushey, H. A., Ownby, D. R., Lynch, S. V., & Johnson, C. C. (2016). Joint effects of pregnancy, sociocultural, and environmental factors on early life gut microbiome structure and diversity. *Scientific Reports*, *6*, 31775.

Li, F., Luo, S., Mu, W., Li, Y., Ye, L., Zheng, X., Xu, B., Ding, Y., Ling, P., Zhou, M., & Chen, X. (2021). Effects of sources of social support and resilience on the mental health of different age groups during the COVID-19 pandemic. *BMC Psychiatry*, *21*(1), 16.

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228–8235.

Lyons, G. C., Kay, M. C., Duke, N. N., Bian, A., Schildcrout, J. S., Perrin, E. M., Rothman, R. L., Yin, H. S., Sanders, L. M., Flower, K. B., Delamater, A. M., & Heerman, W. J. (2023). Social Support and Breastfeeding Outcomes Among a Racially and Ethnically Diverse Population. *American Journal of Preventive Medicine*, *64*(3), 352–360.

Mallick, H., Rahnavard, A., McIver, L. J., Ma, S., Zhang, Y., Nguyen, L. H., Tickle, T. L., Weingart, G., Ren, B., Schwager, E. H., Chatterjee, S., Thompson, K. N., Wilkinson, J. E., Subramanian, A., Lu, Y., Waldron, L., Paulson, J. N., Franzosa, E. A., Bravo, H. C., & Huttenhower, C. (2021). Multivariable association discovery in population-scale meta-omics studies. *PLoS Computational Biology*, *17*(11), e1009442.

Matsuyama, M., Gomez-Arango, L. F., Fukuma, N. M., Morrison, M., Davies, P. S. W., & Hill, R. J. (2019). Breastfeeding: a key modulator of gut microbiota characteristics in late infancy. *Journal of Developmental Origins of Health and Disease*, *10*(2), 206–213.

McDonald, S., Kehler, H., Bayrampour, H., Fraser-Lee, N., & Tough, S. (2016). Risk and protective factors in early child development: Results from the All Our Babies (AOB) pregnancy cohort. *Research in Developmental Disabilities*, *58*, 20–30.

Mercan, Y., & Tari Selcuk, K. (2021). Association between postpartum depression level, social support level and breastfeeding attitude and breastfeeding self-efficacy in early postpartum women. *PloS One*, *16*(4), e0249538.

Michels, N., Van de Wiele, T., Fouhy, F., O'Mahony, S., Clarke, G., & Keane, J. (2019). Gut microbiome patterns depending on children's psychosocial stress: Reports versus biomarkers. *Brain, Behavior, and Immunity*, *80*, 751–762.

Naudé, P. J. W., Claassen-Weitz, S., Gardner-Lubbe, S., Botha, G., Kaba, M., Zar, H. J., Nicol, M. P., & Stein, D. J. (2020). Association of maternal prenatal psychological stressors and distress with maternal and early infant faecal bacterial profile. *Acta Neuropsychiatrica*, *32*(1), 32–42.

Nelson, B. W., Wright, D. B., Allen, N. B., & Laurent, H. K. (2020). Maternal stress and social support prospectively predict infant inflammation. *Brain, Behavior, and Immunity*, *86*, 14–21.

Parenteau, A. M., Alen, N. V., Deer, L. K., Nissen, A. T., Luck, A. T., & Hostinar, C. E. (2020). Parenting matters: Parents can reduce or amplify children's anxiety and cortisol responses to acute stress. *Development and Psychopathology*, *32*(5), 1799–1809.

Perez-Muñoz, M. E., Arrieta, M.-C., Ramer-Tait, A. E., & Walter, J. (2017). A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. *Microbiome*, *5*(1), 48.

Propper, C. B., & Holochwost, S. J. (2013). The influence of proximal risk on the early development of the autonomic nervous system. *Developmental Review: DR*, *33*(3), 151–167.

Querdasi, F. R., Enders, C., Karnani, N., Broekman, B., Yap Seng, C., Gluckman, P. D., Mary Daniel, L., Yap, F., Eriksson, J. G., Cai, S., Chong, M. F.-F., Toh, J. Y., Godfrey, K., Meaney, M. J., & Callaghan, B. L. (2023). Multigenerational adversity impacts on human gut microbiome composition and socioemotional functioning in early childhood. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(30), e2213768120.

Querdasi, F. R., Vogel, S. C., Thomason, M. E., Callaghan, B. L., & Brito, N. H. (2023). A

comparison of the infant gut microbiome before versus after the start of the covid-19 pandemic. *Scientific Reports*, *13*(1), 13289.

Rojas, L., van de Wouw, M., Wang, Y., Vaghef-Mehrabani, E., Dewey, D., Reimer, R. A., Letourneau, N., Campbell, T., Arrieta, M.-C., & Giesbrecht, G. F. (2023). Long-term and trimester-specific effects of prenatal stress on the child gut microbiota. *Psychoneuroendocrinology*, *158*, 106380.

Roslund, M. I., Puhakka, R., Grönroos, M., Nurminen, N., Oikarinen, S., Gazali, A. M., Cinek, O., Kramná, L., Siter, N., Vari, H. K., Soininen, L., Parajuli, A., Rajaniemi, J., Kinnunen, T., Laitinen, O. H., Hyöty, H., Sinkkonen, A., & ADELE research group. (2020). Biodiversity intervention enhances immune regulation and health-associated commensal microbiota among daycare children. *Science Advances*, *6*(42). https://doi.org/10.1126/sciadv.aba2578

Rosseel, Y. (2012). lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software*, *48*, 1–36.

Saturio, S., Nogacka, A. M., Alvarado-Jasso, G. M., Salazar, N., de Los Reyes-Gavilán, C. G., Gueimonde, M., & Arboleya, S. (2021). Role of Bifidobacteria on Infant Health. *Microorganisms*, *9*(12). https://doi.org/10.3390/microorganisms9122415

Shavitt, S., Cho, Y. I., Johnson, T. P., Jiang, D., Holbrook, A., & Stavrakantonaki, M. (2016). Culture Moderates the Relation Between Perceived Stress, Social Support, and Mental and Physical Health. *Journal of Cross-Cultural Psychology*, *47*(7), 956–980.

Slopen, N., Loucks, E. B., Appleton, A. A., Kawachi, I., Kubzansky, L. D., Non, A. L., Buka, S., & Gilman, S. E. (2015). Early origins of inflammation: An examination of prenatal and childhood social adversity in a prospective cohort study. *Psychoneuroendocrinology*, *51*, 403–413.

Song, S. J., Amir, A., Metcalf, J. L., Amato, K. R., Xu, Z. Z., Humphrey, G., & Knight, R. (2016). Preservation Methods Differ in Fecal Microbiome Stability, Affecting Suitability for Field Studies. *mSystems*, *1*(3). https://doi.org/10.1128/mSystems.00021-16

Stewart, C. J., Ajami, N. J., O'Brien, J. L., Hutchinson, D. S., Smith, D. P., Wong, M. C., Ross, M. C., Lloyd, R. E., Doddapaneni, H., Metcalf, G. A., Muzny, D., Gibbs, R. A., Vatanen, T., Huttenhower, C., Xavier, R. J., Rewers, M., Hagopian, W., Toppari, J., Ziegler, A.-G., … Petrosino, J. F. (2018). Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature*, *562*(7728), 583–588.

Walker, R. W., Clemente, J. C., Peter, I., & Loos, R. J. F. (2017). The prenatal gut microbiome: are we colonized with bacteria in utero? *Pediatric Obesity*, *12 Suppl 1*, 3–17.

Warner, B. B., Rosa, B. A., Ndao, I. M., Tarr, P. I., Miller, J. P., England, S. K., Luby, J. L., Rogers, C. E., Hall-Moore, C., Bryant, R. E., Wang, J. D., Linneman, L. A., Smyser, T. A., Smyser, C. D., Barch, D. M., Miller, G. E., Chen, E., Martin, J., & Mitreva, M. (2023). Social and psychological adversity are associated with distinct mother and infant gut microbiome variations. *Nature Communications*, *14*(1), 1–19.

Weng, M., & Walker, W. A. (2013). The role of gut microbiota in programming the immune phenotype. *Journal of Developmental Origins of Health and Disease*, *4*(3), 203–214.

White, L. K., Kornfield, S. L., Himes, M. M., Forkpa, M., Waller, R., Njoroge, W. F. M., Barzilay, R., Chaiyachati, B. H., Burris, H. H., Duncan, A. F., Seidlitz, J., Parish-Morris, J., Elovitz, M. A., & Gur, R. E. (2023). The impact of postpartum social support on postpartum mental health outcomes during the COVID-19 pandemic. *Archives of Women's Mental Health*, 1–11.

Wiley, K. S., Gregg, A. M., Fox, M. M., Lagishetty, V., Sandman, C. A., Jacobs, J. P., & Glynn,

L. M. (2023). Contact with caregivers is associated with composition of the infant gastrointestinal microbiome in the first 6 months of life. *American Journal of Biological Anthropology*. https://doi.org/10.1002/ajpa.24858

Zagoory-Sharon, O., Yirmiya, K., Peleg, I., Shimon-Raz, O., Sanderlin, R., & Feldman, R. (2024). Breast milk oxytocin and s-IgA modulate infant biomarkers and social engagement; The role of maternal anxiety. *Comprehensive Psychoneuroendocrinology*, *17*(100219), 100219.

Ziomkiewicz, A., Babiszewska, M., Apanasewicz, A., Piosek, M., Wychowaniec, P., Cierniak, A., Barbarska, O., Szołtysik, M., Danel, D., & Wichary, S. (2021). Psychosocial stress and cortisol stress reactivity predict breast milk composition. *Scientific Reports*, *11*(1), 11576.