Maternal stress in the early postpartum period is associated with alterations in human milk microbiome composition

Juncker, H.G.; Jakobsen, R.R.; Naninck, E.F.G.; Davids, M.; Herrema, H.; van Goudoever, J.B.; de Rooij, S.R.; Korosi, A.

DOI
10.1016/j.bbi.2024.11.020

Publication date
2025

Document Version
Final published version

Published in
Brain, behavior, and immunity

Brain, Behavior, and Immunity 124 (2025) 74–84

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Maternal stress in the early postpartum period is associated with alterations in human milk microbiome composition

Hannah G. Juncker [a,b,c,1], Rasmus R. Jakobsen [d,1], Eva F.G. Naninck [a,b], Mark Davids [e], Hilde Herrema [e], Johannes B. van Goudoever [b,c], Susanne R. de Rooij [c,f,g], Aniko Korosi [a,*]

[a] Brain Plasticity Group, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, 1012 WX Amsterdam, the Netherlands
[b] Amsterdam UMC, University of Amsterdam, Vrije Universiteit, Emma Children's Hospital, 1105 AZ Amsterdam, the Netherlands
[c] Amsterdam Reproduction and Development, 1105 AZ Amsterdam, the Netherlands
[d] Food Microbiology, Gut Health, and Fermentation, Department of Food Science, Faculty of Science, University of Copenhagen, DK-1958 Copenhagen, Denmark
[e] Departments of Internal and Experimental Vascular Medicine, Amsterdam University Medical Centers, Location AMC, 1105 AZ Amsterdam, the Netherlands
[f] Amsterdam UMC Location University of Amsterdam, Department of Epidemiology and Data Science, 1105 AZ Amsterdam, the Netherlands
[g] Amsterdam Public Health Research Institute, Aging and Later Life, Health Behaviors and Chronic Diseases, 1105 AZ Amsterdam, the Netherlands

ARTICLE INFO

Keywords:
Breast milk
Microbiome
Stress
Bacteria
Lactation

ABSTRACT

Background: Maternal stress is associated with negative early-life development and (mental)health outcomes. There is recent evidence that maternal stress in the postpartum period impacts the nutrient composition of human milk (HM). However, it is currently not known whether maternal stress is associated with changes in the HM microbiome during the critical early postpartum period.

Methods: In this prospective observational cohort study, lactating women were recruited into a high-stress (HS, n = 23) and control (CTL, n = 69) group. The HS group included mothers with infants hospitalized for at least two days. Maternal stress was assessed using validated questionnaires and cortisol concentrations in hair, saliva and HM. HM was collected at days 10 and 24 and its microbiome was analyzed using 16 s rRNA sequencing. HM bacterial composition was compared between study groups and their correlation with maternal stress levels, maternal characteristics and infant outcomes was determined.

Results: HM microbiome β-diversity differed significantly between study groups, with HS mothers displaying decreased abundance of *Streptococcus*, *Gemella*, and *Veillonella*, and increased levels of *Staphylococcus*, *Corynebacterium* and *Acinetobacter* compared to the control group. While the strongest correlation of β-diversity was with stress, HM microbiome β-diversity also correlated significantly with maternal education level and infant sex. No correlation between HM microbiome composition and HM cortisol concentrations was found.

Conclusions: This study demonstrates stress-associated alterations in the early HM microbiome that could potentially contribute to early gut colonization and subsequent (mental)health outcomes. Future research is needed to elucidate the physiological significance of these changes for infant development and health.

1. Introduction

Postpartum maternal stress is associated with negative early-life development and health outcomes and an increased risk for psychiatric disorders and impaired cognitive functions (Reemst et al., 2022; Chan et al., 2018; Oyetunji and Chandra, 2020; Simons et al., 2019; Kingston et al., 2012). The current understanding of the biological mechanisms underlying the transmission of maternal stress to her infant and its effects on infant development and long-term health remains incomplete. Recent studies suggest that maternal stress-induced changes in human milk (HM) composition may contribute to this transmission (Reemst et al., 2022; Di Benedetto et al., 2020; Juncker et al., 2021; de Weerth et al., 2023). There is emerging evidence that maternal stress in the postpartum period is associated with an altered nutrient (e.g. fat, protein, amino acids), hormone (e.g. cortisol) and immune (e.g. antibodies, immune factors) composition of HM (Di Benedetto et al., 2020;

* Corresponding author at: Brain Plasticity group, Swammerdam Institute for Life Sciences (SILS), University of Amsterdam, Science Park 904, 1012 WX Amsterdam, The Netherlands.
E-mail address: a.korosi@uva.nl (A. Korosi).
[1] Contributed equally.

https://doi.org/10.1016/j.bbi.2024.11.020
Received 8 May 2024; Received in revised form 19 October 2024; Accepted 17 November 2024
Available online 22 November 2024

Juncker et al., 2022; Juncker et al., 2023; Moirasgenti et al., 2019; Aparicio et al., 2020).

Next to essential nutrients, hormones, and immune components, HM also contains its own microbiome, contributing to the seeding of the infant intestinal microbiome and thereby influencing immune, metabolic and cognitive health of the breastfed infant (Pannaraj et al., 2017; Jenmalm, 2018; Macpherson et al., 2017; Rautava, 2016). These HM bacteria appear to originate predominantly from the maternal digestive tract as well as the infant's oral cavity and the mother's skin (Fernandez et al., 2013; Rodríguez, 2014). The HM microbiome is dominated by *Staphylococcus* and *Streptococcus*, followed by *Corynebacteria*, *Bifidobacteria*, and *Propionibacteria* (Fernandez et al., 2013; Boix-Amoros et al., 2016; Fitzstevens et al., 2017) and varies greatly in composition between women and changes over the duration of lactation (Fernandez et al., 2013; Boix-Amoros et al., 2016; Fitzstevens et al., 2017; Cabrera-Rubio et al., 2012). Various maternal factors (e.g. mode of delivery, dietary habits and BMI) have been shown to affect the composition of the HM microbiome, (Cabrera-Rubio et al., 2012; Demmelmair et al., 2020; Moossavi et al., 2019; Daiy et al., 2022), however, whether the HM microbiome is affected by maternal stress remains uncertain.

Recently, the role of the gut microbiome in the context of stress and psychiatric disorders has garnered increasing interest (Gur and Hsiao, 2024), particularly the maternal microbiome as key for understanding the impact of prenatal stress on offspring health (Galley et al., 2023; Kimmel et al., 2024). However, the mechanism via which the maternal microbiome might mediate (adverse) effects of maternal stress on the offspring is still unclear. We here hypothesize that maternal stress induces changes in the early HM microbiome, which might be one of the mechanisms contributing to this process. There have been only two studies to our knowledge aiming to study the impact of maternal stress on HM microbiome with so far inconclusive findings (Browne et al., 2019; Fernández-Tuñas et al., 2023), with only one study (n = 26) addressing these effects in mothers with at term delivery (Browne et al., 2019). This study observed that higher maternal psychosocial stress was related to lower bacterial diversity in milk at 3 months post-delivery. In order to gain further insights into the effects of maternal stress on HM microbiome composition, we here investigated the association between maternal psychological and biological stress and the HM microbiome in the critical first month after birth. In addition, we examined HM microbiome correlations with other maternal factors and infant sex.

2. Methods

2.1. Research design and study population

The Amsterdam Mother's milk Study was a prospective observational cohort study aimed at investigating the association between maternal stress and HM composition (Juncker et al., 2022). Recruitment of participants occurred during pregnancy or within the first ten days postpartum through social media, flyers at midwifery practices or at the maternal or neonatal ward of the Amsterdam University Medical Center, The Netherlands. Inclusion criteria were: mothers aged 18 or older with the intention to breastfeed for at least the first month after birth. Exclusion criteria were maternal (gestational) diabetes, serious maternal mental health conditions, use of psychopharmaceuticals or glucocorticoid medication, major congenital diseases in the neonate or a life expectancy of the neonate of less than one month, and for the HM microbiome analysis specifically, maternal antibiotic use.

To encompass a broad spectrum of stress levels among participants, participants were included into two study groups, one control (CTL) group, consisting of mothers that gave birth to a healthy infant at term and a high stress (HS) group, consisting of women who gave birth to an infant at term who was admitted to the hospital for a minimum of two days. Hospitalization of the infant was considered the maternal stressor.

The study was conducted between November 2017 and December 2019. Written informed consent was obtained from all participants prior to participation. This study was approved by the Ethics Committee of the Amsterdam University Medical Centre, AMC on the 2nd of May 2017 (METC 2017 025, NL59994.018.16) and conducted in accordance with the Declaration of Helsinki.

2.2. Data collection

Study timeline: Fig. 1 shows the study time-line. Recruitment took place during pregnancy or within the first ten days postpartum. At the first data collection moment (within the first 10 days postpartum), participants provided a hair sample for baseline cortisol measurement and completed questionnaires regarding their general health, pregnancy, and lifetime stress experiences. On postpartum days (P) 10 and 24 participants collected two saliva samples for cortisol measurement and three HM samples for cortisol and microbiome analysis. At the end of the study, participants completed three additional stress-related questionnaires.

2.3. Sample collection and storage

Hair sample collection and storage: A strand of hair (approximately 100 hairs, 3 mm diameter) was cut by the researcher as close to the scalp as possible at the posterior vertex position. Hair was stored in the dark at room temperature until analysis. A short questionnaire was filled out by the participants in order to correct for factors influencing hair glucocorticoid concentrations. Hair samples were analysed for cortisol and cortisone as a baseline biological stress measurement reflecting the last trimester of pregnancy (Braig et al., 2016; Braig et al., 2015).

Saliva sample collection and storage: Saliva was collected two times in the morning at every collection day to measure the cortisol awakening response. Participants were instructed to chew on a swab (Salivette, Sarstedt, Germany) for one minute. The first sample was obtained within 0–10 min after waking and the second sample was obtained 30–45 min after waking (Stalder et al., 2016). Participants were requested to write down their wake-up time and the time of saliva collection. After collection, saliva samples were immediately sent to the study site where they were centrifuged, aliquotted and stored at −20 °C until analysis.

Milk sample collection and storage: At every collection day (P10 and P24), participants collected three HM samples. Participants were instructed to collect one HM sample in the morning, one in the afternoon and one in the evening on each day that they collected milk. To make sure the HM sample would contain a mixture of both foremilk and hindmilk, participants were requested to fully empty one breast before feeding their infant, mix the milk and thereafter put 5 ml of HM in a sterile polypropylene container (Sarstedt, Germany) for analysis. Participants were free to choose which breast the milk was collected from and were asked to clean the breast with water before milk collection. Participants were requested to write down the date and time of milk collection, the pumping method used (i.e. manually or electric pump etc) and the total amount of milk that was collected. Participants stored the milk samples in their freezer (−20 °C) up until collection by the researcher. At the study site, HM samples were stored at −20 °C until analysis (Stinson et al., 2023). The three daily samples were separately assessed for cortisol to take into consideration the circadian rhythm of HM cortisol, while a mixture of the three was used to assess the microbiome to make sure that potential circadian fluctuation in HM microbiome was represented in the samples (van der Voorn et al., 2016; Pundir et al., 2017);

2.4. Questionnaires

For a detailed explanation of the questionnaires used during the study, see Juncker et al. (Juncker et al., 2022). In short, to establish the participant's baseline stress levels (previous life-time stress), the participants filled out two questionnaire at the start of the study, the Life Stressor Checklist-revised (LSC-r) and the Dutch version of the Youth

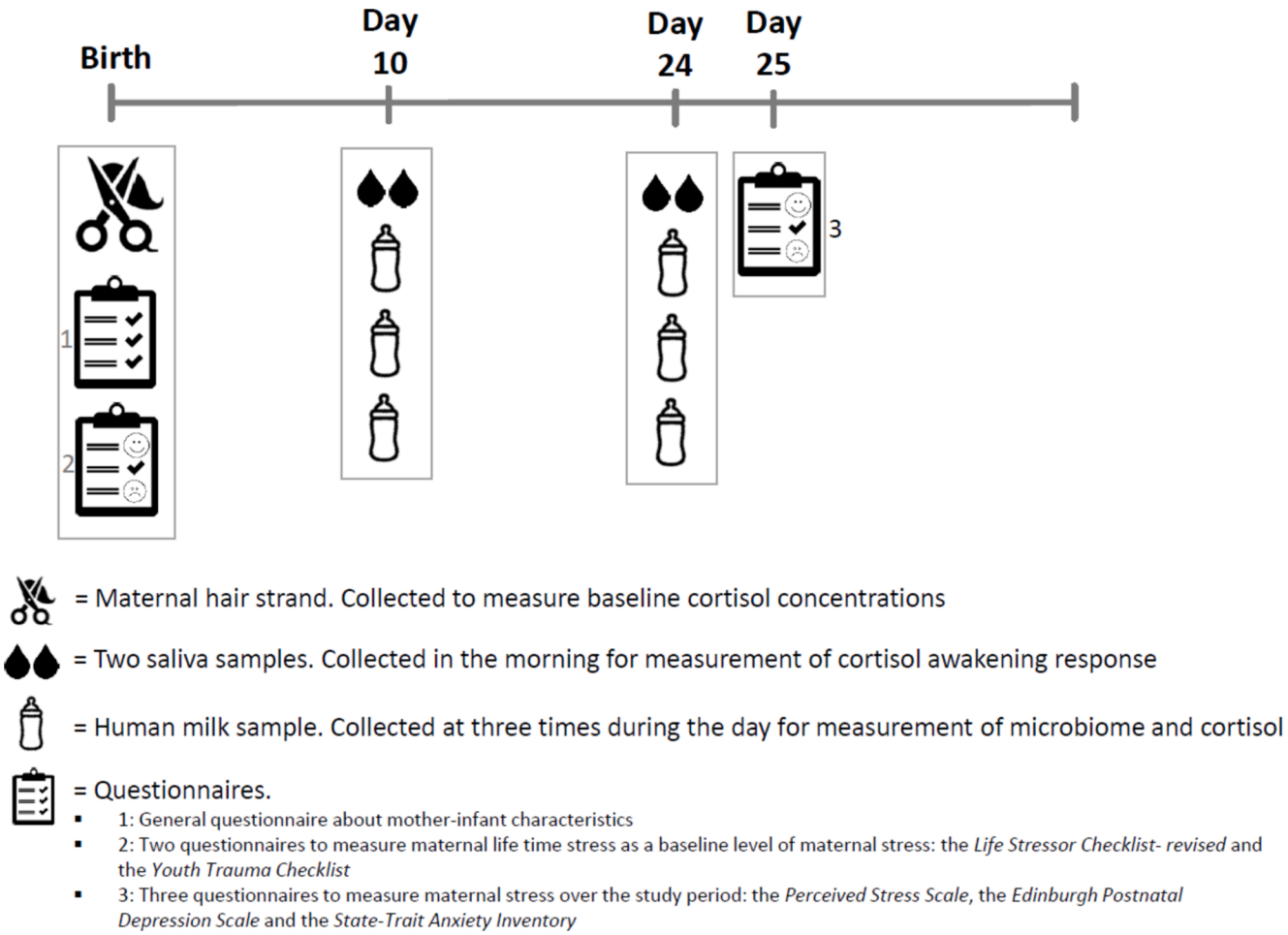

Fig. 1. Study time line.

Trauma Checklist (JTV) (Choi et al., 2017; Bernstein et al., 2003). A measure of psychological stress levels *during* the study period was obtained by three questionnaires that the participants filled out at the end of the study, concerning levels of stress they experienced during the past month: the Perceived Stress Scale (PSS) (Cohen et al., 1983; Yokokura et al., 2017), Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987), and the State-Trait Anxiety Inventory (STAI) (Guillen-Riquelme and Buela-Casal, 2014). To assess maternal dietary intake during the study period, a Food Frequency Questionnaire (FFQ) was used, developed by the department of Human Nutrition of Wageningen University (the Netherlands) to assess the average intake of nutrients in the past month. The FFQ is expected to include foods that cover the daily intake of each nutrient of food of interest for at least 90 %. Infant (neuro) development (temperament) outcomes were obtained using the Infant Behaviour Questionnaire revised (IBQ-r) at three months of age. The IBQ-r, is a reliable and validated parent-report measure of infant temperament (Casalin et al., 2012; Enlow et al., 2016; Gartstein et al., 2005). Information about infant weight was self-reported by parents at week 2, 4, 8 and 12 postpartum.

2.5. Laboratory analysis

Hair cortisol/cortisone: For analyses, the proximal 3-cm hair segment was used. Wash and steroid extraction procedures were performed as described by Stalder et al, with some changes being made to allow analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Stalder et al., 2013). The lower limits of quantification were below 0.1 pg/mg for cortisol and cortisone. The inter- and intra-assay coefficients of variance were between 3.7 % and 8.8 %.

Saliva cortisol/cortisone: Cortisol and cortisone in saliva were determined using Supported Liquid Extraction (SLE+) followed by LC-MS/MS detection. Quantification was performed using an isotope dilution, with the limit of quantification being 0.3 nmol/L. The mean intra-assay variation was 6 % and 7 %, for cortisol and cortisone respectively.

HM cortisol/cortisone: 0.5 ml of HM was used to determine cortisol and cortisone concentrations. This was done using Liquid Liquid extraction followed by SLE + and LC-MS/MS detective as described earlier by Van der Voorn et al (van der Voorn et al., 2015). Quantification was done using an isotope dilution.

HM microbiome: For determination of the HM microbiome, the three milk samples of one collection day were mixed to have a good representation of the microbiome during the entire day. From a 0.5 ml HM sample, DNA was extracted from milk bacterial pellets material using a repeated bead beating protocol (method 5) (Costea et al., 2017;35 (11):1069-+.). DNA was purified using Maxwell RSC Whole Blood DNA Kit. 16S rRNA gene amplicons were generated using a single step polymerase chain reaction (PCR) protocol, using universal primers B341F and B806R, targeting the V3-V4 region (Kozich et al., 2013). PCR products were purified using Ampure XP beads and purified products were equimolar pooled. The libraries were sequenced using a MiSeq platform using V3 chemistry with 2x251 cycles.

2.6. Bioinformatics processing of raw data

16S V3V4 rRNA gene amplicon sequences were parsed using a vsearch (v2.15.2) based pipeline (Rognes et al., 2016). Briefly, paired

end reads were merged, with max differences set to 100 and allowing for staggered overlap. Contaminating sequences that were identified previously were removed.

Amplicon sequence variants (ASVs) were inferred from reads with lower than 1.5 expected error rate using the cluster unoise with centroids algorithm with a minsize of 4, after which chimeras were removed using the uchime3 denovo method. For each sample ASV abundances were determined by mapping the merged reads against the ASV sequences with identical matches defined using the usearch_global algorithm with a 0.97 distance cut off. Taxonomy was assigned using R (V4.0.5) and the dada2 (Callahan et al., 2019) assign taxonomy function using the silva (v132) (Yilmaz et al., 2014) reference database.

4. Results

4.1. Study population and stress measurements

A total of 116 lactating women participated in the study, with 86 in the CTL group and 30 in the HS group. Due to maternal antibiotics use, four women in the CTL group and one in the HS group were excluded for the current analysis. Drop-out during the study resulted in 69 CTL and 23 HS participants at day 10, and 66 CTL and 22 HS participants at day 24 (Fig. 2).

Maternal and infant characteristics did not significantly differ between groups except for infant sex, where HS group had a higher percentage of male infants (74 % vs 48 %, $p = 0.03$) (Table 1). Type of human milk pumping was compared between groups for each collection moment separately (three collection moments on p10 and three on p24). Way of HM pumping did not differ between study groups (p10 p = 0.39, 0.27, 0.34; p24 p = 0.66, 0.69, 0.35; exact data not shown. Median (IQR) hospitalization duration of infants in the HS groups was 7 days. At day 10, five infants in the HS group were still hospitalized, whereas none were hospitalized at day 24. None of the mothers were hospitalized themselves during the study period, nor did they room in with their infant. Maternal dietary intake as measured by FFQ did not differ between study groups (Table S1).

Baseline stress measurements (i.e. the questionnaire based life-time stress exposure scores and hair cortisol concentrations) did not differ between study groups. Perceived stress measured over the study period was higher in the HS group compared to the CTL group, indicated by higher scores on the PSS, EPDS, STAI-s and STAI-t ($p < 0.01$). There were no differences in HM or saliva cortisol or cortisone levels between study groups (Table 2).

4.2. Human milk microbiome composition and diversity

16S amplicon sequencing showed that the HM microbiome was highly variable between individuals, with the majority dominated by the *Streptococcus* genus while a subset were dominated by *Acinetobacter* or *Staphylococcus* instead (Fig. 3**A**). Agglomerated to phylum level, most ASVs were assigned to Firmicutes, followed by Proteobacteria and a smaller proportion to Actinobacteria (**Figure S1A**). We then compared the overall bacterial composition of HS vs CTL samples at different taxonomic ranks. At the genus level, stress-HM exhibited overall lower relative abundance of *Streptococcus* and higher abundance of *Staphylococcus* (Fig. 3**B**). The overall difference in HM bacterial composition between stress groups was more pronounced at day 10 (Fig. 3C), but differential abundances trends were similar across time points when tested independently (data not shown). We then compared the bacterial diversity of the HM bacterial communities. α-diversity (within-sample diversity) did not vary between CTL and HS groups (Fig. 3**D**), but β-diversity (between-sample composition (dis)similarities) showed significant ($p < 0.05$) differences in bacterial compositions when comparing CTL and HS groups (Fig. 3**E**). When testing how HM microbiome changed across the two collection days (day 10 and day 24), the overall HM bacterial profiles showed a decrease in *Staphylococcus* day 24 vs day 10 but neither α-diversity nor β-diversity differed significantly (**Figure S1B-D**). We then tested whether the stress associated differences were time dependent, and found that the correlation of milk bacterial β-diversity and stress remained significant also when examining each time point independently ($p < 0.05$) (**Figure S1E**).

4.3. Differential abundance and microbial interactions

To quantify which HM bacteria constituted the difference in β-diversity between HS and CTL samples we performed differential abundance analysis. When summarised at genus level, we found that HS samples had significantly lower relative abundances of *Streptococcus*, *Gemella* and *Veillonella* and higher *Corynebacterium* (Fig. 3**F**). Since genus-level differences provides a general overview of the bacteria differences, we also performed an ASV-level differential abundance analysis to compare differences of each uniquely detected bacteria amplicon sequence. We found several *Klebsiella* and *Acinetobacter* and single *Enterobacter* and *Stenotrophomonas* ASVs at significantly higher levels in HS samples, while a *Streptococcus* and a *Gemella* ASV were significantly lower (Fig. 3**G**).

Next, using network analysis to infer microbial interactions we

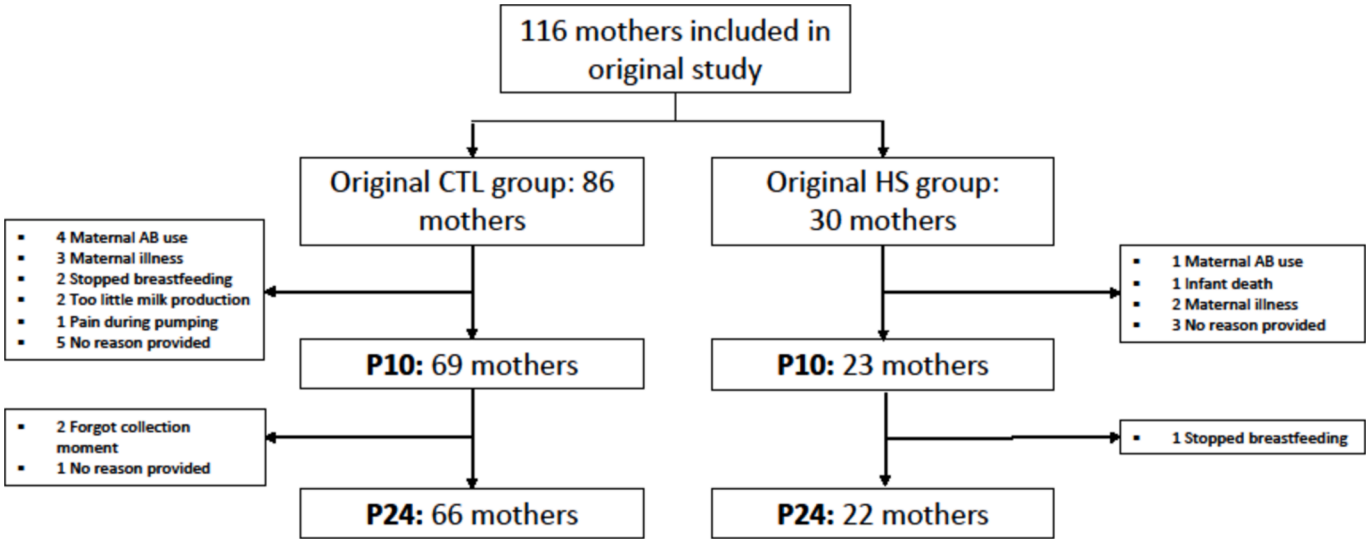

Fig. 2. Flowchart of study population.

Table 1
Maternal-infant characteristics.

	Maternal characteristics		
	Control	High stress	*p*-value
Maternal age in years *mean (SD)*	32.3 (3.4)	32.4 (3.5)	0.94
Maternal ethnicity *%*			0.06
Dutch	82.1 %	57.0 %	
Turkey	1.5 %	0.0 %	
Morocco	0.0 %	0.0 %	
Surinam	1.5 %	13.0 %	
Netherlands Antilles	1.5 %	4.3 %	
Other Western[1]	10.4 %	13.0 %	
Other non-Western[2]	3.0 %	13.0 %	
Maternal education *%*			0.10
Low[3]	6.1 %	26.1 %	
Medium[4]	3.0 %	4.3 %	
High[5]	90.9 %	70.0 %	
Maternal BMI (kg/m^2) *mean (SD)*	22.9 (3.6)	24.5 (5.6)	0.22
Mode of delivery *% of caesarean*	29.9 %	17.4 %	0.32
Smoking *% of*			0.38
Non smoker	68.3 %	54.5 %	
Past smoker	30.2 %	45.5 %	
Current smoker	3.0 %	0.0 %	
Parity *% of primiparous*	60.6 %	72.7 %	0.31
Season of human milk collection *%*			0.08
Winter	25.4 %	14.3 %	
Spring	23.9 %	9.5 %	
Summer	17.9 %	42.9 %	
Fall	32.8 %	33.3 %	
Maternal hospitalization after birth (days) *median (IQR)*	2 (2)	3 (3)	0.06
	Infant characteristics		
Birth weight child (gr) *mean (SD)*	3529 (493)	3421 (588)	0.39
Infant sex *% of male*	47.8 %	73.9 %	0.03*
Infant hospitalization after birth (days) *median (IQR)*	0 (0)	7 (7)	N.A.

1. Includes: Europe, North America, Oceania, Japan, Indonesia.
2. Includes: Latin-America, Africa, Asia, other.
3. Low education (ISCED 2011): Levels 0–2.
4. Medium education (ISCED 2011): Levels 3–4.
5. High education (ISCED 2011): Levels 5–8.
Abbreviations: SD = standard deviation, ISCE = International Standard Classification of Education, BMI = body mass index, IQR = interquartile range.
*statistically significant ($p < 0.05$).

Table 2
Maternal psychological and biological stress measures at baseline and during the study.

	CTL group (n = 69)	HS group (n = 23)	p-value
Baseline stress			
Perceived psychological Life-time stress (test score)			
JTV *median (IQR)*	27.0 (10.3)	29.0 (7.5))	0.35
LSC-r *median (IQR)*	7.0 (7.0)	6.0 (8.0)	0.56
Biological stress (cortisol) over last 3 months of pregnancy			
Hair cortisol *median (IQR)*	5.6 (9.8)	6.6 (9.6)	0.34
Stress during the study period			
Perceived stress (test score)			
PSS *mean (SD)*	15.8 (6.1)	21.5 (5.5)	<0.001***
EPDS *median (IQR)*	4.0 (5.0)	7.0 (6.0)	<0.01**
STAI-s *median (IQR)*	25.5 (12.0)	36.0 (15.0)	<0.01**
STAI-t *median (IQR)*	28.5 (9.0)	37.0 (9.0)	<0.01**
Biological stress (cortisol) on collection days			
Saliva cortisol (morning peak) *median (IQR)*	14.6 (10.6)	16.9 (12.1)	0.18[1]
Human milk cortisol AUC *median (IQR)*	52.7 (31.3)	72.8 (36.2)	0.19[1]

Abbreviations: CTL group = control group, HS group = high stress group, JTV = Dutch version of the Youth Trauma Questionnaire, LSC-r = Life stressor checklist revised, PSS = perceived stress scale, EPDS = Edinburgh Postnatal Depression Scale, STAI-s = State and Trait Anxiety Inventory (state), STAI-t = State and Trait Anxiety Inventory (trait), IQR = interquartile range, SD = standard deviation, HM = human milk, AUC = Area under the curve.
[1] = based on cortisol values of the two collection days together, difference statistically tested using Linear Mixed Models to control for within-person repeated measures.
** = $p < 0.01$, *** = $p < 0.001$.

examined whether inter-bacterial interactions in the HM communities differed between the HS and control HM samples. While in the CTL HM the majority of interactions were identified between *Streptococci*, *Staphylococci*, *Acinetobacter* and *Gemella* species (Fig. 4**A**), the HS HM samples the majority of interactions detected involved *Gemella* ASVs, including *Klebsiella-Gemella* interactions, while fewer *Acinetobacter* interactions were detected (Fig. 4**B**).

4.4. Correlations between microbiome and clinical features

Because it is likely that other factors than stress could also affect HM properties, we examined the relationship of the bacterial community (β-diversity) with maternal clinical features as well with infant sex. We found that the largest correlation with β-diversity was with stress group, followed by maternal education level, and Perceived Stress Scale (PSS) stress score ($p < 0.05$) (Fig. 5**A**). Maternal BMI and Life Stressor Checklist-Revised (LSCr) stress score had weaker correlations and were borderline significant ($p < 0.1$). Because child sex was one feature that was not balanced between study groups (Table 1), β-diversity correlations were adjusted for child sex. Since maternal ethnicity, maternal hospitalization, and season of milk collection were also not balanced between study groups (Table 1), we re-ran the β-diversity correlations while adjusting for all of these. Adjusting for these features did not affect which correlations were significant, and none of them correlated significantly with HM β-diversity. No significant correlations were detected for HM bacterial β-diversity between infant outcomes (temperament and weight) (**Figure S3A**). Cortisol measurements (in hair, HM and saliva) did not correlate significantly with β-diversity (**Figure S3B**). When correlating individual bacterial abundances directly with the various cortisol measurements, the abundance of *Paracoccus* exhibited a significant positive correlation with saliva morning cortisol (**Figure S3C**).

We then used redundancy analysis (RDA) to visualize which bacterial community drove the correlations with each clinical feature (Fig. 5**B**). While HS study group correlated mainly with the abundance of the *Klebsiella* genus and partially with *Gemella, Veillonella, Haemophilus* and *Staphylococcus,* these these genera correlated negatively with LSCr stress score and infant sex which were correlated with *Acinetobacter* and *Stenotrophomonas* (Fig. 5**B**).

5. Discussion

We here examined the relationship between maternal stress in the first month postpartum and the human milk (HM) microbiome using the Amsterdam Mother's milk observational cohort study. We reveal significant differences in HM bacterial β-diversity between the HS and control group. HM of women from the HS group had an overall lower relative abundance of *Streptococcus*, *Gemella* and *Veillonella* and higher abundances of *Staphylococcus*, *Corynebacterium* and *Acinetobacter*. While the HS group had the strongest correlation with HM bacterial in

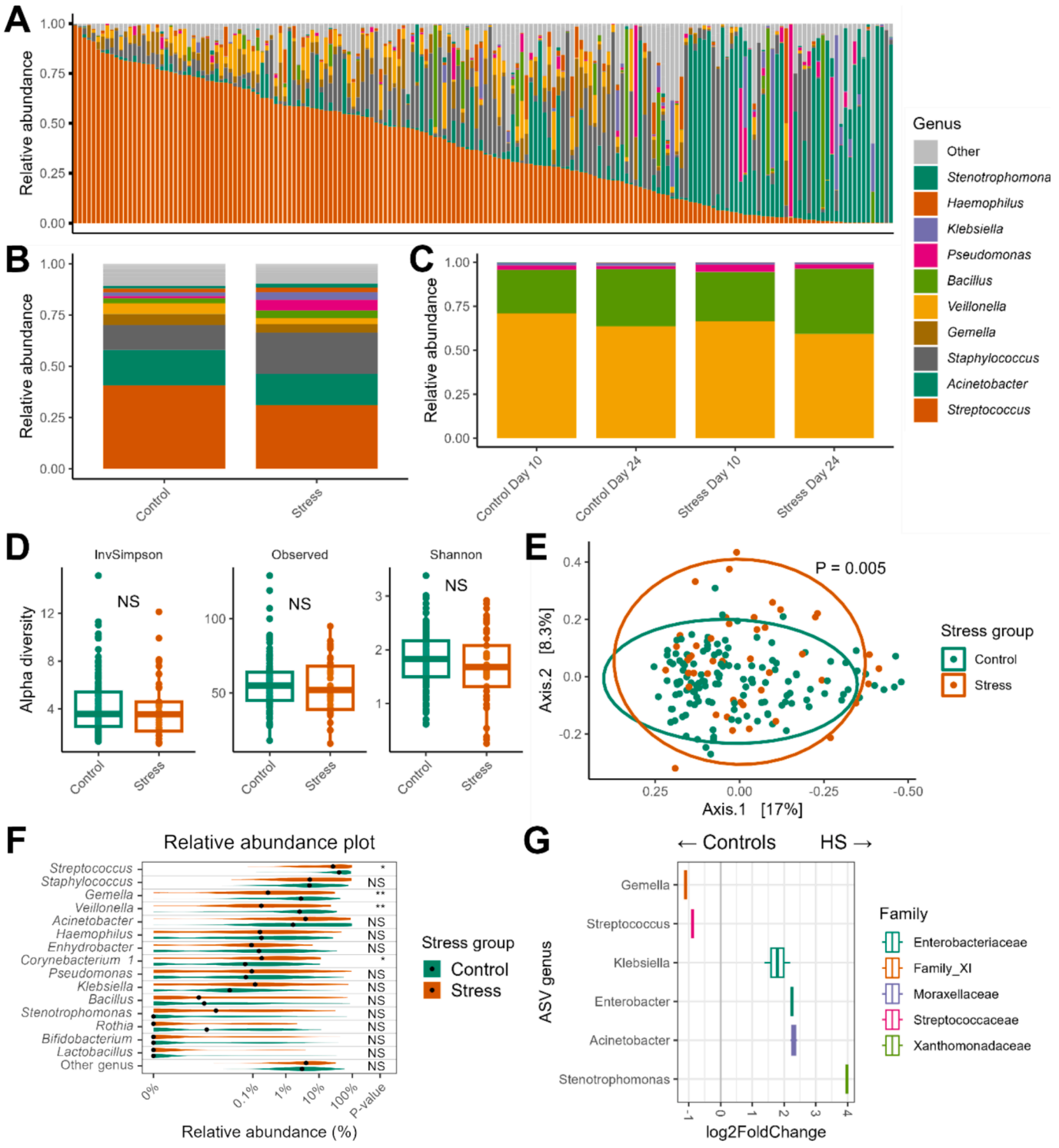

Fig. 3. Milk bacterial composition and comparison by stress groups (A) Relative abundance of bacterial composition summarized at genus level for all samples and collapsed by (B) stress group and (C) stress group and time point. (D) Within-sample diversity by stress group, using multiple α-diversity metrics. No significant differences detected with Analysis of Variance (ANOVA). (E) PCoA plot of Bray-Curtis dissimilarities between samples by stress group, p-value calculated using distance-based Permutational Multivariate Analysis of Variance (db-PERMANOVA). (F) Genus level differences in relative abundances by stress group showing the 15 most abundant genera, p-values denote Pearson correlation after FDR-correction. (G) Log fold change of differentially abundant ASVs between stress groups, showing ASVs with significance ($p < 0.05$) detected by DESeq-2 analysis with relative abundance > 0.5 % in at ≥ 2 samples.

β-diversity, we also observed significant correlations with maternal educational level and PSS scores as well as with maternal BMI and lifetime stress scores. Infant sex also exhibited a significant correlation with bacterial β-diversity, but did not confound the previous associations. No significant associations with HM cortisol concentrations were observed.

5.1. Comparison with previous literature

The HM microbiome was dominated by *Streptococcus, Acinetobacter*

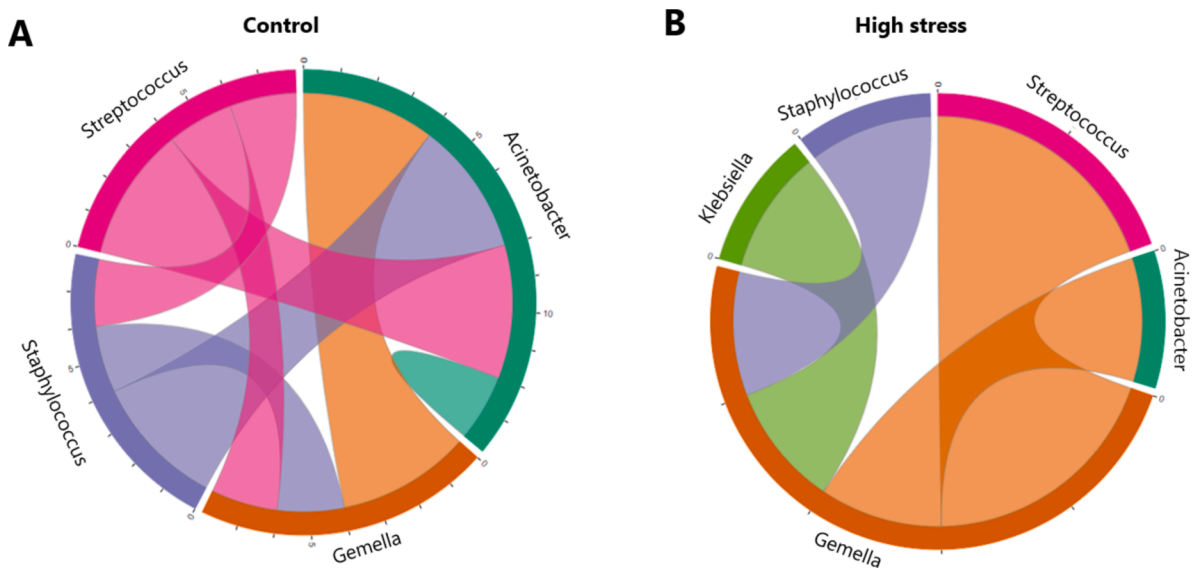

Fig. 4. Inter-bacterial interactions. Bacterial abundance correlation networks from control (A) and high stress (B) samples summarized at genus-level. Summary of the number of detected edges between ASVs in BEEM-Static interaction network analysis, coloured by genus.

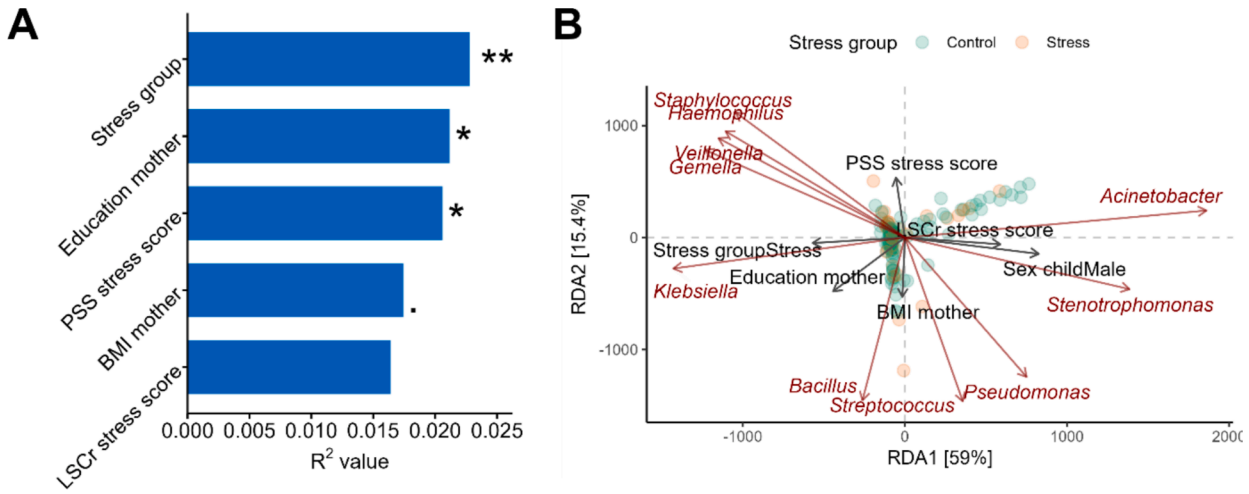

Fig. 5. Microbiome correlation with clinical factors. Effect-size plot of the strength of correlation between clinical phenotypes and the composition of the bacterial community. dbPERMANOVA adjusted for for child sexwas run individually for each variable using CSS-normalized Bray-Curtis dissimilarity matrices and FDR correction performed. (B) Redundancy analysis (RDA) ordination plot showing the correlation of the clinical variables on the milk bacterial composition and individual bacterial genera using CSS-normalized bacterial counts.

and *Staphylococcus*, with the latter decreasing over time, corroborating previous characterizations of the HM microbiome (Browne et al., 2019; Singh et al., 2023; Lyons et al., 2022). To our knowledge, only two previous studies have examined the association between maternal stress and HM microbiome to date (Browne et al., 2019; Fernández-Tuñas et al., 2023). The first study, focusing on the early postpartum period studied the impact of stress on HM microbiome in HM of mothers who delivered preterm. This study found no differences HM bacterial β-diversity, but did observe that HM samples of mothers with high stress levels trended towards decreasing proportions of *Firmicutes* and increasing proportions of *Proteobacteria* over the first 2 weeks of lactation compared to mothers with low stress (Fernández-Tuñas et al., 2023). These trends are opposite to what we observed in our current study. This discrepancy can likely be attributed to the substantial differences in the HM microbiome profiles of preterm versus term born infants (Singh et al., 2023; Urbaniak et al., 2016). The second study investigated term births and described an negative association between postpartum stress and α-diversity of the HM microbiome at 12 weeks postpartum (Browne et al., 2019). We did not detect differences in α-diversity, but we focused only on the first postpartum month, suggesting that the impact of stress on HM microbial diversity might be delayed and or be lactation stage dependent. In addition, the previous study described a positive correlation between stress and *Acinetobacter*, *Flavobacterium*, and *Lactobacillus* at the genus level (Browne et al., 2019). We also found several *Acinetobacter* ASV to be stress associated, suggesting that these changes might be of relevance.

5.2. Clinical implications of the stress-associated HM microbiome alterations

While the function of *Acinetobacter* in HM is still unclear, it was previously found to be negatively correlated with *Staphylococcus, Streptococcus* and other Gram-negatives (Li et al., 2017), and was described to be increased in milk from women with subacute mastitis (Patel et al., 2017). We can thus speculate that a pro-inflammatory maternal state, which is also commonly present during stress (Dragos

and Tanasescu, 2010), could contribute to the observed increase in HM *Acinetobacter*. The role of *Acinetobacter* spp. in human health is only emerging, thus we can only speculate on the potential implications of the observed higher amount of *Acinetobacter* in HM for infant health. *Acinetobacter* in HM is transferred to the infant where subsequently colonizes the breastfed infant's gut. Higher *Acinetobacter* concentrations have been shown to be related to more food allergies in infants whereas another study shows that higher concentrations in the gut and skin microbiome protects against allergic sensitization and inflammation (Wang et al., 2022; Wypych et al., 2019). We have currently no information on the gut microbiome composition, thus the implication of the altered *Acinetobacter* for infant gut and health remains to be studied.

Gemella abundance was reduced in HS samples compared to CTLs. Interestingly, Gemella has been described to be increased in HM from women with obesity and gestational diabetes mellitus (GDM) or impaired glucose tolerance (LeMay-Nedjelski et al., 2021; LeMay-Nedjelski et al., 2020). The pathogenesis of GDM and the stress system are interwoven and influence each other bi-directionally (OuYang et al., 2021), so modulation of *Gemella* could be a converging mechanism. The functional implication of such modulation will need to be further investigated, especially considering that we here observed an inverse relationship. *Veillonella*, an early coloniser of the infant gut, was reduced in HS samples. Lower infant gut levels of *Veillonella* have been associated with higher asthma risk (Arrieta et al., 2015). Interestingly, maternal stress in the perinatal period has also been associated with higher asthma risk in children (Douros et al., 2017), suggesting a possible role of *Veillonella* levels in linking maternal stress and increased asthma risk. *Corynebacterium* and several *Klebsiella* species at the ASV level were increased in HS samples. *Corynebacterium* are typically skin-associated and non-pathogenic, containing species that are opportunistic pathogens (Bernard, 2012). *Klebsiella* are also early HM-to-gut colonizers (Wang et al., 2023) and has been positively correlated with infant intestinal inflammation and necrotizing enterocolitis (NEC) (McCartney and Hoyles, 2023), two conditions that have also been linked to maternal perinatal stress (Cheddadi et al., 2023; Nelson et al., 2020). When we examined inter-bacterial interactions, we found that HS samples exhibited increased interaction between the *Gemella* and *Klebsiella* genera (Fig. 4). This could be a result of general increased presence of disease-associated HM bacteria in the stress group, and/or could indicate that these bacteria are interacting in a shared response to risk-factors. The majority of detected interactions in control samples were between Gram-negative *Acinetobacter* and the Gram-positive *Gemella*, *Staphylococcus* and *Streptococcus* genera. Since *Acinetobacter* was increased in HS samples, this could indicate that stress samples have less regulation by gram-positive commensals that outcompete or inhibit gram-negative opportunistic pathogens under healthy conditions. Similar negative correlations between gram-positive versus gram-negative genera were reported in a study investigating HM microbiomes from healthy mothers from China (Li et al., 2017).

To date there is very limited evidence concerning if and how HM microbiome relates to infant gut microbiome and mental health outcomes, making it difficult to speculate on the potential implications of the observed stress-associated alterations in HM microbiome on infant mental health. However, both Streptococcus and Veillonella that we found to be decreased in HS-samples, have associations between their presence in the infant GM and healthy neurodevelopmental outcomes (Bonham et al., 2023) possibly by aiding in the production of short chain fatty acids (SCFAs) in the gut (Zhang and Huang, 2023). SCFAs are known to cross the blood–brain barrier and have been described to be critical for brain health (Bonham et al., 2023). When speculating, this could mean that the stress-induced lower abundances of these bacteria in HM could lead to lower abundances in the infant gut and thereby contribute to the detrimental effects of maternal stress on her child.

5.3. Correlations with other maternal and infant factors

Maternal acute stress (PSS) scores significantly correlated with HM bacterial β-diversity, mostly explained by differences in *Streptococcus*. In contrast, the correlation with life-time stress (LSC-r) scores were mainly explained by increased *Acinetobacter* relative abundances. These differential effects suggests a different impact of acute and chronic stress on the HM microbiome, which in turn might contribute to different offspring outcomes as previously reported (Champagne et al., 2008; Zavala et al., 2011). Infant sex has been found by several studies to correlate with infant gut microbiota (Kim, 2022; Cong et al., 2016), but to our knowledge this is the first time it has been shown to be the case for HM as well. Unfortunately, we do not have sufficient power to further untangle the effects of sex and stress in this study. Stress during pregnancy has been shown to impact the infant gut microbiome in sex-specific manner (Jasarevic et al., 2017). While the HM microbiome could similarly be a vector of sex-specific impact of stress and bacterial transfer, further analyses are needed to explore this in detail (Li et al., 2017).

We did not detect differences in HM cortisol between study groups. In response to stress, cortisol is released in the maternal circulation and transferred to HM (Djurhuus et al., 2002; Motil et al., 1994), however, during breastfeeding, oxytocin is released, counteracting the release of cortisol (Uvnas Moberg et al., 2020; Brown et al., 2016), which could explain the lack of elevated cortisol concentrations in the HS group. It may also imply bias and that the differences in the HM microbiome are not due to stress, but rather other group characteristics. Given that the HM microbiome is thought to predominantly originate from the maternal intestinal/oral microbiome, we can speculate that the observed differences in HM derive from transfer of a stress-associated maternal microbiome phenotype, for example due to stress-induced changes to maternal diet, which we did not detect to be different in this study, or on the release of other stress hormones such as epinephrine or adrenaline (Molina-Torres et al., 2019). Despite the lack of group differences in cortisol, we explored if and how HM cortisol and microbiome relate to one another as it remains important to understand if cortisol might play a role in the modulation of HM microbiome. We did not detect a correlation between the two, suggesting that other biological substrates might be rather involved in HM microbiome modulation. It will be interesting to explore this further in future research.

5.4. Strengths and limitations

Strengths of this study are 1) the study period; the first month postpartum has been frequently missed in earlier HM research, but since breastfed infants depend on HM during this period as their only source of nutrition, these first weeks after birth represent a very sensitive time window. 2) the frequency of HM sample collection (three times a day). 3) the fact that mothers were exposed to a stressor (i.e. infant hospitalization) ensured that the study population contained participants with established high levels of perceived stress. 4) extensive information on additional maternal and infant characteristics and on both maternal psychological and biological stress, was collected. 5) A relatively large sample size compared to previous studies addressing stress and HM microbiome composition. This is an explorative study, and a limitation of this study is the imbalance of stress group sizes (23 HS, 69 controls) and the relatively low sample size, especially in the HS group, therefore results of the study should be interpreted with caution. In addition, all infants in the HS group were hospitalized for at least two days after birth, and even though the mothers did not stay at the hospital, the effect of frequent hospital visits on the HM microbiome cannot be ruled out or corrected for and, might cause bias. It should be considered that there are multiple baseline factors or unmeasured confounders could have influenced our results, for example paternal mental health, infant antibiotics use, breast or bottle feeding, freezing temperature/time of the samples or maternal genetic profile (Stinson et al., 2023; Golan and

Assaraf, 2020; Ryoo and Kang, 2022). The relatively high drop-out from the original study groups and the fact that this was higher in the HS group compared to the CTL group (23 % versus 26 % respectively) may have caused selection bias. Drop out of women who may have been more stressed, especially in the HS group, may have led to underestimation of associations between maternal stress and the HM microbiome. The impact of maternal stress on maternal and infant gut microbiome was beyond the scope of the current study, however the relationship between the altered HM microbiome and the maternal and infant gut microbiome will be of interest for future studies to gain further insights into how maternal gut and HM microbiome relate to one another and into the transmission to her infant. As our cohort mostly consisted of women in good health and with a high educational level, selection bias cannot be excluded and our findings might be hard to generalize to the population. Lastly, the majority of our participants were of a Western ethnic background and this may also limit generalizability of the results.

6. Conclusion

Results from this prospective observational cohort study suggest that maternal stress in the early postpartum period is associated with altered microbiome composition of HM. As the HM microbiome colonizes the infant gut and its composition has been associated with infant (mental) health outcomes, future preclinical research should aim at uncovering causality in the relationship between stress and milk, but also in the transfer from mother to pup and later life health outcomes. This will be important to allow for clinical translation and the opportunity to design clinical trials with the aim to investigate to what extent stress-associated changes in milk are of physiological importance for infant development and health.

CRediT authorship contribution statement

Hannah G. Juncker: Writing – original draft, Investigation, Formal analysis. **Rasmus R. Jakobsen:** . **Eva F.G. Naninck:** Writing – review & editing, Investigation, Conceptualization. **Mark Davids:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Hilde Herrema:** Writing – review & editing, Methodology, Formal analysis. **Johannes B. van Goudoever:** Writing – review & editing, Conceptualization. **Susanne R. de Rooij:** Writing – review & editing, Formal analysis, Conceptualization. **Aniko Korosi:** Writing – original draft, Supervision, Resources, Investigation, Conceptualization.

3. Data availability

Raw sequencing data is available at the European nucleotide archive at with project ID PRJEB74246 (https://www.ebi.ac.uk/ena/browser/view/PRJEB74246). All R code used for the statistical analysis is available online at (https://github.com/RasmusRiemer/ AMSMilkMaternalStress).

3.1. Statistical analysis

3.1.1. Baseline characteristics and stress measurements

Sample characteristics, questionnaire scores and cortisol concentrations were described as mean with standard deviation (SD), median with interquartile range (IQR) or frequencies. To test differences in maternal and infant characteristics and stress measurements between study groups, unpaired student T-tests, Chi-square tests, Mann-Whitney U tests or Linear Mixed Models were used as appropriate.

The area under the curve (AUC) for HM cortisol concentrations was calculated to provide a value that better reflects HM cortisol throughout the day, as cortisol is known to follow a circadian rhythm (van der Voorn et al., 2016). To this end, all HM cortisol values were standardized to 7:00 AM, 14:00 PM and 22:00 PM, using the following formula: HM cortisol in mmol/L −/+ unstandardized regression coefficient of all HM cortisol values * (new (standardized)) time point – real time point). Subsequently, the HM cortisol AUC for each collection day was calculated as described by Pruessner et al. (Pruessner et al., 2003). The cortisol value at 7:00 AM was considered the HM cortisol morning peak (van der Voorn et al., 2016). The highest cortisol value of the two morning saliva samples was considered the saliva cortisol morning peak. When time of S1 collection was > 30 min after waking up, saliva values were excluded.

3.1.2. Bioinformatics analysis

Within sample diversity (α-diversity) metrics were calculated based on raw reads rarefied to mean relative abundance. Between-sample comparisons (β-diversity) was obtained using Bray-Curtis dissimilarities calculated after cumulative sum scaling (CSS) of sample relative abundances.. Between-sample group comparisons and effect-size estimations for clinical characteristics were performed using permutational multivariate analysis of variance (PERMANOVA) (nperm = 9999) to ensure robustness for heterogeneous dispersions (Anderson and Walsh, 2013). Abundance comparisons at genus level used spearman correlations, and ASV-level comparisons used raw read data as input for DESeq2 differential abundance analysis (Love et al., 2014). Bacterial correlations used the BEEM-static expectation–maximization algorithm to infer interaction networks based on an ecological model (generalized Lotka-Volterra) (Li et al., 2021). P-values were adjusted for multiple comparisons using false discovery rate using false discovery rate (FDR) correction unless otherwise stated. Statistical significance is annotated in figures and tables as follows: $p > 0.05$: "ns" or not annotated, $p \leq 0.05$: *, $p \leq 0.01$: **, $p \leq 0.001$: *** and $p \leq 0.0001$: ****.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

EFGN, JBvG, SRdR and AK designed research. HGJ, RRJ and EFGN conducted research. JBvG and AK provided essential materials. MD and HH preformed laboratory analysis and data generation. HGJ, RRJ and MD analyzed data and performed statistical analysis. HGJ, RRJ and MD accessed and verified data. HGJ, RRJ, AK and SRdR interpreted the data. HGJ, RRJ and AK wrote the first version of the paper. All authors critically read and contributed to finalizing the manuscript. AK had primary responsibility for final content. All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

We want to thank the Dutch Human Milk Bank for helping with the transport of study samples and all the parents who participated in the Amsterdam Mother's Milk study. This research was funded by the Dutch Research Council (NWO) Food and Cognition and Behavior program (057-14-003) and by Amsterdam Brain & Cognition, The Netherlands.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2024.11.020.

Data availability

All raw data and R code for analysis is available online

References

Anderson, M.J., Walsh, D.C.I., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol. Monogr. 83 (4), 557–574.

Aparicio, M., Browne, P.D., Hechler, C., Beijers, R., Rodríguez, J.M., de Weerth, C., Fernández, L., 2020. Human milk cortisol and immune factors over the first three postnatal months: Relations to maternal psychosocial distress. Plos. One. 15 (5).

Arrieta, M.C., Stiemsma, L.T., Dimitriu, P.A., Thorson, L., Russell, S., Yurist-Doutsch, S., et al., 2015. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci. Transl. Med. 7 (307).

Bernard, K., 2012. The genus corynebacterium and other medically relevant coryneform-like bacteria. J. Clin. Microbiol. 50 (10), 3152–3158.

Bernstein, D.P., Stein, J.A., Newcomb, M.D., Walker, E., Pogge, D., Ahluvalia, T., et al., 2003. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child. Abuse. Negl. 27 (2), 169–190.

Boix-Amoros, A., Collado, M.C., Mira, A., 2016. Relationship between milk microbiota, bacterial load, macronutrients, and human cells during lactation. Front. Microbiol. 7.

Bonham, K.S., Bottino, G.F., McCann, S.H., Beauchemin, J., Weisse, E., Barry, F., et al., 2023. Gut-resident microorganisms and their genes are associated with cognition and neuroanatomy in children. Sci. Adv. 9 (51).

Braig, S., Grabher, F., Ntomchukwu, C., Reister, F., Stalder, T., Kirschbaum, C., et al., 2015. Determinants of maternal hair cortisol concentrations at delivery reflecting the last trimester of pregnancy. Psychoneuroendocrino. 52, 289–296.

Braig, S., Grabher, F., Ntomchukwu, C., Reister, F., Stalder, T., Kirschbaum, C., et al., 2016. The Association of Hair Cortisol with Self-Reported Chronic Psychosocial Stress and Symptoms of Anxiety and Depression in Women Shortly after Delivery. Paediatr. Perinat. Ep. 30 (2), 97–104.

Brown, C.A., Cardoso, C., Ellenbogen, M.A., 2016. A meta-analytic review of the correlation between peripheral oxytocin and cortisol concentrations. Front. Neuroendocrinol. 43, 19–27.

Browne, P.D., Aparicio, M., Alba, C., Hechler, C., Beijers, R., Rodriguez, J.M., et al., 2019. Human Milk Microbiome and Maternal Postnatal Psychosocial Distress. Front. Microbiol. 10, 2333.

Cabrera-Rubio, R., Collado, M.C., Laitinen, K., Salminen, S., Isolauri, E., Mira, A., 2012. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am. J. Clin. Nutr. 96 (3), 544–551.

Callahan, B.J., Wong, J., Heiner, C., Oh, S., Theriot, C.M., Gulati, A.S., et al., 2019. High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. Nucleic. Acids. Res. 47 (18).

Casalin, S., Luyten, P., Vliegen, N., Meurs, P., 2012. The structure and stability of temperament from infancy to toddlerhood: A one-year prospective study. Infant. Behav. Dev. 35 (1), 94–108.

Champagne, D.L., Bagot, R.C., van Hasselt, F., Ramakers, G., Meaney, M.J., de Kloet, E. R., et al., 2008. Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. J. Neurosci. 28 (23), 6037–6045.

Chan, J.C., Nugent, B.M., Bale, T.L., 2018. Parental advisory: maternal and paternal stress can impact offspring neurodevelopment. Biol. Psychiatry. 83 (10), 886–894.

Cheddadi, R., Khandekar, N.N., Yeramilli, V., Martin, C., 2023. The impact of maternal stress on the development of necrotizing enterocolitis: A comprehensive review. Semin. Pediatr. Surg. 32 (3).

Choi, K.R., Kim, D., Jang, E.Y., Bae, H., Kim, S.H., 2017. Reliability and Validity of the Korean Version of the Lifetime Stressor Checklist-Revised in Psychiatric Outpatients with Anxiety or Depressive Disorders. Yonsei. Med. J. 58 (1), 226–233.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health. Soc. Behav. 24 (4), 385–396.

Cong, X.M., Xu, W.L., Janton, S., Henderson, W.A., Matson, A., McGrath, J.M., et al., 2016. Gut Microbiome Developmental Patterns in Early Life of Preterm Infants: Impacts of Feeding and Gender. Plos. One. 11 (4).

Costea, P.I., Zeller, G., Sunagawa, S., Pelletier, E., Alberti, A., Levenez, F., et al., 2017. Towards standards for human fecal sample processing in metagenomic studies. Nat. Biotechnol. 35 (11), 1069-+.

Cox, J.L., Holden, J.M., Sagovsky, R., 1987. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. Br. J. Psychiatry. 150, 782–786.

Daiy, K., Harries, V., Nyhan, K., Marcinkowska, U.M., 2022. Maternal weight status and the composition of the human milk microbiome: A scoping review. Plos. One. 17 (10).

de Weerth, C., Aatsinki, A.K., Azad, M.B., Bartol, F.F., Bode, L., Collado, M.C., et al., 2023. Human milk: From complex tailored nutrition to bioactive impact on child cognition and behavior. Crit. Rev. Food. Sci. Nutr. 63 (26), 7945–7982.

Demmelmair, H., Jiménez, E., Collado, M.C., Salminen, S., McGuire, M.K., 2020. Maternal and Perinatal Factors Associated with the Human Milk Microbiome. Curr. Dev. Nutr. 4 (4).

Di Benedetto, M.G., Bottanelli, C., Cattaneo, A., Pariante, C.M., Borsini, A., 2020. Nutritional and immunological factors in breast milk: A role in the intergenerational transmission from maternal psychopathology to child development. Brain. Behav. Immun. 85, 57–68.

Djurhuus, C.B., Gravholt, C.H., Nielsen, S., Mengel, A., Christiansen, J.S., Schmitz, O.E., Moller, N., 2002. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am. J. Physiol. Endocrinol. Metab. 283 (1), E172–E177.

Douros, K., Moustaki, M., Tsabouri, S., Papadopoulou, A., Papadopoulos, M., Priftis, K. N., 2017. Prenatal maternal stress and the risk of asthma in children. Front. Pediatr. 5.

Dragos, D., Tanasescu, M.D., 2010. The effect of stress on the defense systems. J. Med. Life. 3 (1), 10–18.

Enlow, M.B., White, M.T., Hails, K., Cabrera, I., Wright, R.J., 2016. The Infant Behavior Questionnaire-Revised: Factor structure in a culturally and sociodemographically diverse sample in the United States. Infant. Behav. Dev. 43, 24–35.

Fernandez, L., Langa, S., Martin, V., Maldonado, A., Jimenez, E., Martin, R., Rodriguez, J. M., 2013. The human milk microbiota: origin and potential roles in health and disease. Pharmacol. Res. 69 (1), 1–10.

Fernández-Tuñas, M.D., Pérez-Muñuzuri, A., Trastoy-Pena, R., del Molino, M.L.P., Couce, M.L., 2023. Effects of Maternal Stress on Breast Milk Production and the Microbiota of Very Premature Infants. Nutrients. 15 (18).

Fitzstevens, J.L., Smith, K.C., Hagadorn, J.I., Caimano, M.J., Matson, A.P., Brownell, E. A., 2017. Systematic Review of the Human Milk Microbiota. Nutr. Clin. Pract. 32 (3), 354–364.

Galley, J.D., Mashburn-Warren, L., Blalock, L.C., Lauber, C.L., Carroll, J.E., Ross, K.M., et al., 2023. Maternal anxiety, depression and stress affects offspring gut microbiome diversity and bifidobacterial abundances. Brain. Behav. Immun. 107, 253–264.

Gartstein, M.A., Knyazev, G.G., Slobodskaya, H.R., 2005. Cross-cultural differences in the structure of infant temperament: United States of America (US) and Russia. Infant. Behav. Dev. 28 (1), 54–61.

Golan, Y., Assaraf, Y.G., 2020. Genetic and Physiological Factors Affecting Human Milk Production and Composition. Nutrients. 12 (5).

Guillen-Riquelme, A., Buela-Casal, G., 2014. Meta-analysis of group comparison and meta-analysis of reliability generalization of the State-Trait Anxiety Inventory Questionnaire (STAI). Rev. Esp. Salud. Publica. 88 (1), 101–112.

Gur, T.L., Hsiao, E.Y., 2024. The Microbiome at the Interface of the Exposome and Risk for Psychiatric Disorders. Biol. Psychiatry. 95 (4), 298–299.

Jasarevic, E., Howard, C.D., Misic, A.M., Beiting, D.P., Bale, T.L., 2017. Stress during pregnancy alters temporal and spatial dynamics of the maternal and offspring microbiome in a sex-specific manner. Sci. Rep-Uk. 7.

Jenmalm, M.C., 2018. The mother-offspring dyad, microbial transmission, immune interactions and allergy development. J. Reprod. Immunol. 128, 44 -.

Juncker, H.G., van Keulen, B.J., Finken, M.J.J., de Rooij, S.R., van Goudoever, J.B., Korosi, A., 2021. The Potential Role of Nutrition in Modulating the Long-Term Consequences of Early-Life Stress. Nestle. Nutr. Inst. Workshop. Ser. 96, 116–129.

Juncker, H.G., Naninck, E.F.G., Schipper, L., Lucassen, P.J., van Goudoever, J.B., de Rooij, S.R., Korosi, A., 2022. Maternal stress in the postpartum period is associated with altered human milk fatty acid composition. Clin. Nutr. 41 (11), 2517–2528.

Juncker, H.G., Naninck, E.F.G., van Keulen, B.J., Harinck, J.E., Schipper, L., Lucassen, P. J., et al., 2023. Maternal stress is associated with higher protein-bound amino acid concentrations in human milk. Front. Nutr. 10.

Kim N. Sex Difference of Gut Microbiota. Sex/Gender-Specific Medicine in the Gastrointestinal Diseases. p. 363-77.

Kimmel, M.C., Verosky, B., Chen, H.J., Davis, O., Gur, T.L., 2024. The maternal microbiome as a map to understanding the impact of prenatal stress on offspring psychiatric health. Biol. Psychiatry. 95 (4), 300–309.

Kingston, D., Tough, S., Whitfield, H., 2012. Prenatal and postpartum maternal psychological distress and infant development: a systematic review. Child. Psychiatry. Hum. Dev. 43 (5), 683–714.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Appl. Environ. Microb. 79 (17), 5112–5120.

LeMay-Nedjelski, L., Butcher, J., Ley, S.H., Asbury, M.R., Hanley, A.J., Kiss, A., et al., 2020. Examining the relationship between maternal body size, gestational glucose tolerance status, mode of delivery and ethnicity on human milk microbiota at three months post-partum. Bmc. Microbiol. 20 (1).

LeMay-Nedjelski, L., Yonemitsu, C., Asbury, M.R., Butcher, J., Ley, S.H., Hanley, A.J., et al., 2021. Oligosaccharides and microbiota in human milk are interrelated at 3 months postpartum in a cohort of women with a high prevalence of gestational impaired glucose tolerance. J. Nutr. 151 (11), 3431–3441.

Li, C., Av-Shalom, T.V., Tan, J.W.G., Kwah, J.S., Chng, K.R., Nagarajan, N., 2021. BEEM-Static: Accurate inference of ecological interactions from cross-sectional microbiome data. PLoS. Comput. Biol. 17 (9), e1009343.

Li, S.W., Watanabe, K., Hsu, C.C., Chao, S.H., Yang, Z.H., Lin, Y.J., et al., 2017. Bacterial Composition and Diversity in Breast Milk Samples from Mothers Living in Taiwan and Mainland China. Front. Microbiol. 8, 965.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome. Biol. 15 (12), 550.

Lyons, K.E., Shea, C.O., Grimaud, G., Ryan, C.A., Dempsey, E., Kelly, A.L., et al., 2022. The human milk microbiome aligns with lactation stage and not birth mode. Sci. Rep. 12 (1), 5598.

Macpherson, A.J., de Agüero, M.G., Ganal-Vonarburg, S.C., 2017. How nutrition and the maternal microbiota shape the neonatal immune system. Nat. Rev. Immunol. 17 (8), 508–517.

McCartney, A.L., Hoyles, L., 2023. The role of Klebsiella populations in preterm infants. Biochem. Soc. Trans. 51 (2), 887–896.

Moirasgenti, M., Doulougeri, K., Panagopoulou, E., Theodoridis, T., 2019. Psychological stress reduces the immunological benefits of breast milk. Stress. Health. 35 (5), 681–685.

Molina-Torres, G., Rodriguez-Arrastia, M., Roman, P., Sanchez-Labraca, N., Cardona, D., 2019. Stress and the gut microbiota-brain axis. Behav. Pharmacol. 30 (2–3), 187–200.

Moossavi, S., Sepehri, S., Robertson, B., Bode, L., Goruk, S., Field, C.J., et al., 2019. Composition and variation of the human milk microbiota are influenced by maternal and early-life factors. Cell. Host. Microbe. 25 (2), 324-+.

Motil, K.J., Thotathuchery, M., Montandon, C.M., Hachey, D.L., Boutton, T.W., Klein, P. D., Garza, C., 1994. Insulin, cortisol and thyroid hormones modulate maternal protein status and milk production and composition in humans. J. Nutr. 124 (8), 1248–1257.
Nelson, B.W., Wright, D.B., Allen, N.B., Laurent, H.K., 2020. Maternal stress and social support prospectively predict infant inflammation. Brain. Behav. Immun. 86, 14–21.
OuYang H, Chen B, Abdulrahman AM, Li L, Wu N. Associations between Gestational Diabetes and Anxiety or Depression: A Systematic Review. J Diabetes Res. 2021; 2021.
Oyetunji, A., Chandra, P., 2020. Postpartum stress and infant outcome: A review of current literature. Psychiatry. Res. 284, 112769.
Pannaraj, P.S., Li, F., Cerini, C., Bender, J., Yang, S.X., Rollie, A., et al., 2017. Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. Jama. Pediatrics. 171 (7), 647–654.
Patel, S.H., Vaidya, Y.H., Patel, R.J., Pandit, R.J., Joshi, C.G., Kunjadiya, A.P., 2017. Culture independent assessment of human milk microbial community in lactational mastitis. Sci. Rep. 7 (1), 7804.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology. 28 (7), 916–931.
Pundir, S., Wall, C.R., Mitchell, C.J., Thorstensen, E.B., Lai, C.T., Geddes, D.T., Cameron-Smith, D., 2017. Variation of human milk glucocorticoids over 24 hour period. J. Mammary. Gland. Biol. Neoplasia. 22 (1), 85–92.
Rautava, S., 2016. Early microbial contact, the breast milk microbiome and child health. J. Dev. Orig. Hlth. Dis. 7 (1), 5–14.
Reemst, K., Ruigrok, S.R., Bleker, L., Naninck, E.F.G., Ernst, T., Kotah, J.M., et al., 2022. Sex-dependence and comorbidities of the early-life adversity induced mental and metabolic disease risks: Where are we at? Neurosci. Biobehav. Rev. 138, 104627.
Rodríguez, J.M., 2014. The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? Advances. in. Nutrition. 5 (6), 779–784.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. Peerj. 4.
Ryoo, C.J., Kang, N.M., 2022. Maternal Factors Affecting the Macronutrient Composition of Transitional Human Milk. Int. J. Environ. Res. Public. Health. 19 (6).
Simons, S.S.H., Zijlmans, M.A.C., Cillessen, A.H.N., de Weerth, C., 2019. Maternal prenatal and early postnatal distress and child stress responses at age 6. Stress. 22 (6), 654–663.
Singh, P., Al Mohannadi, N., Murugesan, S., Almarzooqi, F., Kabeer, B.S.A., Marr, A.K., et al., 2023. Unveiling the dynamics of the breast milk microbiome: impact of lactation stage and gestational age. J. Transl. Med. 21 (1), 784.
Stalder, T., Kirschbaum, C., Alexander, N., Bornstein, S.R., Gao, W., Miller, R., et al., 2013. Cortisol in hair and the metabolic syndrome. J. Clin. Endocrinol. Metab. 98 (6), 2573–2580.
Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wust, S., et al., 2016. Assessment of the cortisol awakening response: Expert consensus guidelines. Psychoneuroendocrinology. 63, 414–432.
Stinson, L.F., Ma, J., Sindi, A.S., Geddes, D.T., 2023. Methodological approaches for studying the human milk microbiome. Nutrition. Reviews. 81 (6), 705–715.
Urbaniak, C., Angelini, M., Gloor, G.B., Reid, G., 2016. Human milk microbiota profiles in relation to birthing method, gestation and infant gender. Microbiome. 4.
Uvnas Moberg, K., Ekstrom-Bergstrom, A., Buckley, S., Massarotti, C., Pajalic, Z., Luegmair, K., et al., 2020. Maternal plasma levels of oxytocin during breastfeeding-A systematic review. PLoS. One. 15 (8), e0235806.
van der Voorn, B., Martens, F., Peppelman, N.S., Rotteveel, J., Blankenstein, M.A., Finken, M.J., Heijboer, A.C., 2015. Determination of cortisol and cortisone in human mother's milk. Clin. Chim. Acta. 444, 154–155.
van der Voorn, B., de Waard, M., van Goudoever, J.B., Rotteveel, J., Heijboer, A.C., Finken, M.J., 2016. Breast-milk cortisol and cortisone concentrations follow the diurnal rhythm of maternal hypothalamus-pituitary-adrenal axis activity. J. Nutr. 146 (11), 2174–2179.
Wang, S., Wei, Y., Liu, L.Y., Li, Z.L., 2022. Association Between Breastmilk Microbiota and Food Allergy in Infants. Front. Cell. Infect. Mi. 11.
Wang, K., Xia, X., Sun, L., Wang, H., Li, Q., Yang, Z., Ren, J., 2023. Microbial Diversity and Correlation between Breast Milk and the Infant Gut. Foods. 12 (9).
Wypych, T.P., Wickramasinghe, L.C., Marsland, B.J., 2019. The influence of the microbiome on respiratory health. Nat. Immunol. 20 (10), 1279–1290.
Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., et al., 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. Nucleic. Acids. Res. 42 (D1), D643–D648.
Yokokura, A., Silva, A., Fernandes, J.K.B., Del-Ben, C.M., Figueiredo, F.P., Barbieri, M.A., Bettiol, H., 2017. Perceived Stress Scale: confirmatory factor analysis of the PSS14 and PSS10 versions in two samples of pregnant women from the BRISA cohort. Cad. Saude. Publica. 33 (12), e00184615.
Zavala, J.K., Fernandez, A.A., Gosselink, K.L., 2011. Female responses to acute and repeated restraint stress differ from those in males. Physiol. Behav. 104 (2), 215–221.
Zhang, S.M., Huang, S.L., 2023. The commensal anaerobe veillonella dispar reprograms its lactate metabolism and short-chain fatty acid production during the stationary phase. Microbiol. Spectr. 11 (2).